World Health Organization

World Organisation for Animal Health

WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern

Edited by

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- Aetiology
- Echinococcosis in humans
- Echinococcosis in animals
- Diagnosis
- Treatment
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- Geographic distribution
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- Methods

Cover image: *Echinococcus granulosus*
Courtesy of the Institute of Parasitology, University of Zurich

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Foreword

Echinococcosis is one of the parasitic diseases that has been recognised since time immemorial. Over four centuries BC, Hippocratus reported that hydatid cysts in humans could sometimes burst open into the abdominal cavity. Furthermore, he compared these cysts to 'water-filled tumours' which he observed on post-mortem examination of cattle and pigs.

Since this time, echinococcosis has been described in a multitude of research papers, as it is not only one of the most widespread parastic diseases, but also one of the most costly to treat and prevent in terms of public health. Consequently, it is approriate to provide regular updates on the disease.

As is the case with all zoonoses, the control of echinococccosis, or more precisely the control of animal reservoirs of the parasite, is the result of very close collaboration between health authorities and, in particular, between both the Veterinary Services and Public Health Services at a national level. It was with the aim of assisting those responsible for echinococcosis control and prevention that this book was prepared and jointly published by the World Health Organization and the World Organisation for Animal Health (OIE – Office International des Epizooties). This joint publication is another example of the common objective of these two organisations to work together to assist their Member Countries in designing, implementing and standardising control strategies against zoonoses on both national and international levels.

This is the result of years of efforts expended by both the OIE and WHO to organise the surveillance and control of a parasitic disease which is present world-wide.

This WHO/OIE Manual on echinococcosis in humans and animals: a zoonosis of global concern *is a compilation of the knowledge and valuable experience of over fifty experts of international renown. We would like to express our profound gratitude to them for their contributions and for sharing their expertise, and hope that the book achieves the success that it deserves.*

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Preface

The second edition of the WHO Guidelines for Surveillance, Prevention and Control of Echinococcosis/Hydatidosis*, published in 1984, was focused on diagnostic methods and control measures available to combat this disease in humans and animals. These guidelines were very well received throughout the world and represented a valuable source of information for medical and Veterinary Services of many countries. Since then the understanding of the epidemiology of echinococcosis has been greatly improved, new diagnostic techniques for both humans and animals have been developed, progress has been made in the treatment of human echinococcosis, and new prevention strategies have emerged with the development of a vaccine against* Echinococcus granulosus *in intermediate hosts.*

In spite of significant progress achieved in the field of research and control, human cystic echinococcosis, caused by Echinococcus granulosus*, remains a considerable public health problem in many regions of the world. Ultrasound surveys of populations at risk have shown that cystic echinococcosis is more prevalent than previously anticipated in many endemic regions. To date, disease transmission has been reduced or interrupted in some limited areas only, especially on islands, such as Cyprus, New Zealand and Tasmania. In continental situations, however,* E. granulosus *control is more difficult, often less effective, is costly and requires sustained efforts over many decades.*

Recent studies in Europe, Asia (i.e. People's Republic of China and Japan) and North America have shown that E. multilocularis*, the causative agent of human alveolar echinococcosis, is more widely distributed in the northern hemisphere than previously understood. Alveolar echinococcosis, although rare, represents a considerable public health burden as the infection is lethal in most untreated patients and treatment is very costly. In addition, in Central and South America, cases of polycystic echinococcosis in humans, caused by* E. vogeli *and* E. oligarthrus*, occur in apparently increasing numbers.*

Therefore, the World Health Organization in close collaboration with the WHO Collaborating Centres and specialists of the WHO Informal Working Group on Echinococcosis and the International Association of Hydatidology felt it necessary to update and revise the existing document by preparing a Manual on echinococcosis in humans and animals. *Over fifty international experts contributed to the development of this document. This* Manual *covers all important aspects of echinococcosis, including parasite biology and life-cycles, geographic distribution and prevalence, epidemiology, clinical presentation in humans and animals, diagnosis and treatment, as well as control and prevention using newly developed tools and methods. It also provides descriptions of important techniques and a large number of bibliographical references. The* Manual *should help personnel from the medical and veterinary sectors involved in surveillance, prevention and control of echinococcosis to develop effective programmes based on current knowledge and modern techniques.*

The Editors wish to thank all the scientists for their contributions, the Animal Health Services of the Food and Agriculture Organization of the United Nations for the support given to the development of an early draft of this document and all other persons who could not be mentioned in the list of authors and contributors. The administrative support during the initial phase of the project provided by Dr T. Fujikura, formerly of the WHO Unit of Veterinary Public Health in Geneva, is gratefully acknowledged.

The Editors express special thanks to the Office International des Epizooties for its support, without which this Manual *could not have been published, and to Gill Dilmitis, Head of the Publications Department, for her excellent editorial work.*

The Editors

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Glossary of Terms

The terms given below have been restricted to those that are likely to be useful in this *Manual*.

A. Biology

Strain of *Echinococcus*: Group of individuals which differs statistically from groups of the same species in gene frequencies, and in one or more characters of actual potential significance to epidemiology and control of hydatid disease (4).

B. Diagnostics (3)

Cut-off point: The result of a diagnostic test selected for distinguishing between positive and negative results.

Predictive value: The predictive value (PV) can be expressed as positive (PV+) or negative (PV-) value. The PV+ is an indicator of the probability that individuals with positive test results do have the disease, whereas the PV-expresses the probability that individuals with negative testing results do not have the disease.

Repeatability: Agreement between replicates within and between runs of the same assay.

Reproducibility: The ability of a test to provide consistent results when applied to aliquots of the same sample at different laboratories.

Sensitivity (diagnostic): Proportion of known infected individuals that test positive in an assay. Infected individuals that test negative are considered as false negatives.

Sensitivity (analytical): Smallest amount of the analyte (for example, antigen) which is detectable.

Specificity (diagnostic): Proportion of uninfected reference individuals that test negative in an assay. Uninfected individuals that test positive are regarded as false positives. This type of specificity can be denominated as specificity 1. Specificity which refers to reference individuals that are not infected with a specific agent (for example *Echinococcus*) but harbour other parasites is denominated as specificity 2.

C. Epidemiology and control (1, 2, 5)

Aberrant host: Host which does not play a role in epidemiology and is a 'blind end' for the parasite (same as accidental host).

Accidental host: see aberrant host.

Carrier: Host that is infected without displaying clinical symptoms and signs, and that can be a source of infection to other individuals.

Case fatality: Proportion of individuals that die of a disease in a population of **affected** individuals (=number of deaths/number of diseased individuals) (e.g. if 10 individuals of 100 patients with echinococcosis die, then case fatality is 10%).

Control: Active implementation of a programme to limit the prevalence of a specific disease.

Cost-benefit ratio: Ratio of costs of a disease in relation to the benefits that accrue from their control.

Endemic occurrence: Constant occurrence of a disease in a population.

Epidemic occurrence: Occurrence of a disease to a level in excess of the expected (i.e., endemic) level.

Eradication: Reduction of a specific disease prevalence to the point of continued absence of transmission (or absence of a parasite) within a specific area.

Hyperendemic occurrence: Constant occurrence of a disease at a high level (see also steady state)

Incidence: Number of new cases of a disease that occur in a population in a particular geographic area within a defined period, (e.g. 10 new cases per 100,000 population per year).

Infection: Infection is not synonymous with an infectious disease, but in this text, the term is used frequently to denote both an infection and a disease (5).

Life-cycle: Biological cycle of a parasite.

Domestic cycle: Cycle involves only domestic animals.

Mixed (intermediate) cycle: Cycle involves domestic and wild animals.

Sylvatic cycle: Cycle involves only wild animals.

Synanthropic cycle: Cycle associated with human habitats (syn: with *anthropos*: man), often used as a synonym for domestic cycle.

Monitoring: Routine collection of information on disease and parameters possibly related to them in a population.

Morbidity: Proportion of diseased individuals in a population.

Mortality: Number of deaths due to a disease that occur in a population in a particular geographic area within a defined period (e.g. 10 deaths per 100,000 population per year).

Odds ratio: Ratio of probability of an event occurring to that of it not occurring.

Pandemic occurrence: Widespread epidemic that usually affects a large proportion of the population.

Prevalence:

Point prevalence: Number of cases of an infection or related attributes (e.g. presence of antibodies) in a population at one given time without distinction of old and new cases (example: 10 cases per 100,000 in May 1998).

Period prevalence: Number of cases of an infection or related attributes (e.g. presence of antibodies) in a population during a specified period of time without distinction of old and new cases (example: 10 cases per year).

Group prevalence: prevalence in a smaller group of subjects which may not be representative for a larger population.

Rate: a ratio that indicates changes of disease occurrence over time (e.g. rate of new cases of a disease occurring in a population during a defined period of time). 'Rate' is often used to describe the proportion of infected/diseased individuals in a population, independent of changes over time.

Reproduction (basic) ratio (R_0): In helminths, the ratio of the number of adult parasites in the following generation to the number of adult parasites in the present generation defines the basic reproduction ratio of the parasite population, and is usually denoted by R_0. By definition R_0 is the reproduction ratio in the absence of density-dependent constraints (1).

Sample: Selected part of a population.

Screening: Procedure for identification of an unrecognised infection or disease or defect using tests or other procedures that can be applied to a population or a selected subset.

Spatial distribution: Distribution of organisms within an area.

Sporadic occurrence: Occurring irregularly, from time to time and generally infrequently (5).

Steady state: Parasite population is neither increasing nor decreasing over time (1). The following definitions are modified after Gemmell and Roberts (1):

Endemic steady state: Parasite population size is constant (with effective reproduction ratio R=1) at a low or moderate level.

Extinction steady state: No parasite present (see also eradication).

Hyperendemic steady state: Parasite population size is constant at a high level and strongly regulated by density-dependent constraints.

Surveillance: Intensive form of monitoring designed so that action can be taken to improve the health status of a population.

Survey: Investigation for collecting information on a disease.

Transhumance: Seasonal moving of livestock to regions of different climate.

Zoonosis: Infection shared in nature by humans and other vertebrates.

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Abbreviations

ABZ	albendazole
Arc5	antigen-antibody precipitation line detected by double diffusion (DD) or IEP
AE	alveolar echinococcosis
bw	body weight
CA-ELISA	coproantigen-ELISA
CE	cystic echinococcosis
CIOMS	Council for International Organizations of Medical Sciences
CT	computed (computer assisted) tomography
Da	dalton
DD	double diffusion test
DNA	deoxyribonucelic acid
EITB	enzyme-linked immunoelectro transfer blot
ELISA	enzyme-linked immunosorbent assay
ERC	endoscopic retrograde cholangiography
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
G	genotype
IB	immunoblot
IEP	immunoelectrophoresis
IFAT	indirect fluorescent antibody test
IHA	indirect haemagglutination assay
kDa	kilodalton
LAT	latex agglutination test
MBZ	mebendazole
MRI	magnetic resonance imaging
n or N	number
OD	optical density
OIE	Office International des Epizooties (World Organisation for Animal Health)
PAHO	Pan American Health Organization
PAIR	puncture, aspiration, injection, reaspiration
PAP	peroxidase anti-peroxidase
PAS	periodic acid-Schiff
PCR	polymerase chain reaction
PE	polycystic echinococcosis
RAPD	random amplified polymorphic DNA
p.i.	post infection
RH	relative humidity
RFLP	restriction fragment length polymorphism
SSCP	single-stranded confirmation polymorphism
sp., spp.	species (singular and plural)
US	ultrasonography
WHO	World Health Organization
WHO/IWGE	World Health Organization Informal Working Group on Echinococcosis
X-ray	radiography

Chapter 1

Aetiology: parasites and life-cycles

R.C.A. Thompson and D.P. McManus

Summary

The control of any infectious agent requires a sound knowledge of the taxonomy and transmission cycles which perpetuate the agent in nature. This is essential for surveillance and predictive epidemiology, and in determining the aetiology and appropriate treatment regimes in cases of disease. In this chapter, the biology of the causative agents of various forms of echinococcosis are described and details provided of the major cycles of transmission which are known to maintain the parasites in different geographic areas. Emphasis is given to the extent and nature of variability within the genus Echinococcus *which reflects considerable inter- and intraspecific heterogeneity which has a profound influence on the epidemiology of echinococcosis. The identification of species and strains within the genus is an essential prerequisite to the establishment of local control programmes and appropriate molecular biological tools are now available for this.*

1.1. Introduction and terminology

Echinococcosis is a zoonotic infection caused by adult or larval (metacestode) stages of cestodes belonging to the genus *Echinococcus* and the family *Taeniidae*.

At present, four species of *Echinococcus* are recognised, namely *Echinococcus granulosus*, *E. multilocularis*, *E. oligarthrus* and *E. vogeli* (Table 1.1.). The parasites are perpetuated in life-cycles with carnivores as definitive hosts, which harbour the adult egg-producing stage in the intestine, and intermediate host animals, in which the infective metacestode stage develops after peroral infection with eggs. Metacestodes may incidentally also develop in humans causing various forms of echinococcosis (Chapter 2, Table 2.1.), and this may also occur in various animals species, which do not play a role in the developmental cycle of the parasite (= aberrant or accidental hosts; see below) (Chapter 3, Table 3.1.).

Within the species *E. granulosus*, genetic heterogeneity is common resulting in a number of intraspecific variants or 'strains'. However, some of the forms which have been recognised as distinct strains were, in fact, described many years ago as species or subspecies. The reinstatement of their formal taxonomic status has recently been proposed following a reappraisal of the taxonomy of *Echinococcus* in light of phylogenetic analyses of deoxyribonucleic acid (DNA) sequence data (29).

1.2. General morphology

General features

Echinococcus exhibits certain unique characteristics that set it apart from the other major genus in the family, *Taenia.* An adult *Echinococcus* is only a few millimetres long (rarely more than 7 mm) and usually has no more than six segments, whereas species of *Taenia* can grow to several metres in length and consist of several thousand segments. Like all tapeworms, *Echinococcus* has no gut and all metabolic interchange takes place across the syncytial outer covering, the tegument.

Scolex and strobila

Anteriorly, the adult *Echinococcus* possesses a specialised attachment organ, the scolex, which has four muscular suckers and two rows of hooks, one large and one small, on the rostellum (Fig. 1.1.). The body, or

Table 1.1.
Characteristics of the four species currently recognised within the genus *Echinococcus*

Character	***Echinococcus granulosus*** **(Batsch, 1786)**	***E. multilocularis*** **Leuckart, 1863**	***E. oligarthrus*** **(Diesing, 1863)**	***E. vogeli*** **Rausch and Bernstein, 1972**
Geographic distribution	Cosmopolitan	Central and northern Eurasia, northern North America	Central and South America	Central and South America
Host range				
Definitive hosts	Primarily dogs and other canids	Primarily foxes, also other canids and cats	Wild felids	Bush dog
Intermediate and aberrant hosts	Primarily ungulates, also marsupials and primates, humans	Primarily arvicolid rodents, also other small mammals, humans	Rodents; agoutis, paca, spiny rats, humans	Primarily agoutis, also other rodents, humans
Metacestode				
Nature of cyst	Unilocular, endogenous proliferation, no infiltration or metastasis	Multivesicular, endogenous proliferation, infiltration and metastasis	Polycystic, endogenous and exogenous proliferation, no infiltration or metastasis	Polycystic, endogenous and exogenous proliferation, no infiltration or metastasis
Location of cyst	Visceral, primarily liver and lungs	Visceral, primarily liver	Peripheral, primarily muscles	Visceral, primarily liver
Protoscoleces				
Mean length (μm) of large hooks (range)	25.9-35.0 (19.4-44.0)	26.7-28.5 (25.0-29.7)	30.5-33.4 (29.1-37.9)	39.3-41.6 (38.2-45.6)
Mean length (μm) of small hooks (range)	22.6-27.8 (17.0-31.0)	23.1-25.4 (21.8-27.0)	25.4-27.3 (22.6-29.2)	32.5-34.0 (30.4-36.9)
Adult				
Mean length (μm) of large hooks (range)	32.0-42.0 (25.0-49.0)	31.0 (24.9-34.0)	52.0 (43.0-60.0)	53.0 (49.0-57.0)
Mean length (μm) of small hooks (range)	22.6-27.8 (17.0-31.0)	27.0 (20.4-31.0)	39.0 (28.0-45.0)	42.6 (30.0-47.0)
Mean number of segments (range)	3 (2-6)	4-5 (2-6)	3	3
Total length of strobila (mm)	2.0-7.0	1.2-4.5	2.2-2.9	3.9-5.6
Ratio of length of anterior part of strobila to length of gravid segment	1:0.86-1.30	1:0.31-0.80	1:0.96-1.10	1:2.2-3.1
Position of genital pore				
Mature segment	Near (usually posterior) to middle	Anterior to middle	Anterior to middle	Posterior to middle
Gravid segment	Posterior to middle	Anterior to middle	Near to middle	Posterior to middle
Mean number of testes (range)	32-68 (25-80)	18-26 (16-35)	29 (15-46)	56 (50-67)
Form of uterus	Lateral sacculations	Sac-like	Sac-like	Long, tubular and sac-like
Onset of egg production (days)	34-53	28-35	80?	?

Source: Thompson (24)

strobila, is segmented and consists of a number of reproductive units (proglottids), which may vary in number from two to six. The adult worm is hermaphrodite with reproductive ducts opening at a common, lateral, genital pore, the position of which may vary depending on species and strain. There is a prominent cirrus sac, which may be horizontal or tilted anteriorly and the vitellarium is globular (Fig. 1.1.). The uterus dilates after fertilisation, eventually occupying most of the terminal segment when the eggs are fully developed.

Eggs

The eggs are ovoid (30 μm-40 μm diameter), consisting of a hexacanth embryo (oncosphere = first larval stage) surrounded by several envelopes, the most noticeable one being the highly resistant keratinised embryophore, which gives the egg a dark striated appearance (Fig. 1.2.). The outer capsule quickly disappears once the eggs are liberated from the host. The eggs of *Echinococcus* are morphologically indistinguishable to those of other tapeworms of the genus *Taenia.*

Metacestode

The metacestode (= second larval stage) basically consists of a bladder with an outer acellular laminated layer and an inner nucleated germinal layer, which may give rise by asexual budding to brood capsules. Protoscoleces arise from the inner wall of the brood capsules (Fig. 1.3.a.). The structure and development of the metacestode differs between the four species of *Echinococcus* (see paragraph 1.5. and Fig. 1.3.b.).

1.3. General life-cycles

Basic life-cycle pattern

Echinococcus spp. require two mammalian hosts for completion of their life-cycles (Fig. 1.4.). Segments containing eggs (gravid proglottids) or free eggs are passed in the faeces of the definitive host, a carnivore. The eggs are ingested by an intermediate host, in which the metacestode stage and protoscoleces develop. The cycle is completed if such an intermediate host in eaten by a suitable carnivore.

Eggs in the environment

The eggs are highly resistant to environmental factors and can remain infective for many months or up to about 1 year in a moist environment at lower ranges of temperatures (about +4°C to +15°C). Eggs of *Echinococcus* are sensitive to desiccation. At a relative humidity of 25%, eggs of *E. granulosus* were killed within 4 days and at 0% within 1 day. Heating to 60°C-80°C killed eggs of *E. granulosus* in less than 5 min. On the other hand, *Echinococcus* eggs can survive freezing temperatures (8, 12, 30) (Chapter 7).

Intermediate and aberrant (= accidental) hosts

The intermediate hosts, represented by a wide range of mammals, acquire the infection by the ingestion of eggs. Following the action of enzymes in the stomach and small intestine, the oncosphere is released from the keratinised embryophore (24) (Fig. 1.4.). Bile assists in activating the oncosphere, which penetrates the wall of the small intestine. Penetration is then aided by the hook movements, and possibly by secretions, of the oncosphere. Upon gaining access to a venule or lacteal, the oncosphere is passively transported to the liver, where some are retained. Others reach the lungs, and a few may be transported further to the kidneys, spleen, muscles, brain or other organs (24). All mammals (including man) in which metacestodes of *Echinococcus* species develop after infection with eggs, may be referred to as 'intermediate hosts'. From the epidemiological point of view, it might be useful to differentiate between 'intermediate hosts', which play a role in the perpetuation of the cycle, and 'aberrant or accidental hosts' which represent a 'blind alley' for the parasite as the latter are not involved in disease transmission. This may be because metacestode stages do not become fertile (see below) in these hosts or because such hosts do not interact in the transmission cycle. With a few rare exceptions, humans belong to the group of 'aberrant hosts'.

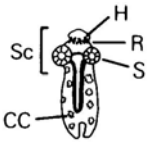
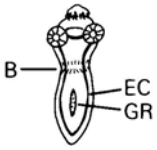
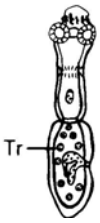
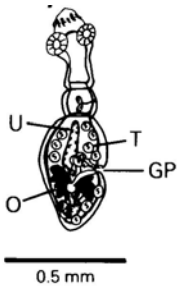
Days and stages		Description of development
Day 1 	Sc : scolex CC : calcareous corpuscles H : hooks R : rostellum S : sucker	Protoscolex has evaginated and elongated; contains numerous calcareous corpuscles
Days 11-14 	B : band EC : excretory canal GR : genital rudiment	Calcareous corpuscles have disappeared; lateral excretory canals are conspicious; genital rudiment present denoting formation of first proglottid; constriction and clear area below the neck ('banding') marks the site of the first segment
Days 14-17 	Sg : segment	Genital rudiment has divided into two and extends unilaterally; first segment fully formed
Days 17-20 	Tr : rudimentary testes	Rudimentary testes appear in the first proglottid; initial stages in formation of second proglottid
Days 20-28 	U : uterus O : ovarium T : testes GP : genital pore	Two-segmented worm; male genitalia – testes, cirrus and vas deferens – have developed; female genitalia – ovary, Mehlis' gland and vitelline gland – still developing; uterus appears as a streak; both cirrus and vagina open to exterior via lateral genital pore

Fig. 1.1.
Stages of development of *Echinococcus granulosus* to the adult form in the definitive host
The period at which various stages appear may vary and are dependent on strain of parasite and various host factors
Reproduced from (24) with permission from CABI *Publishing*

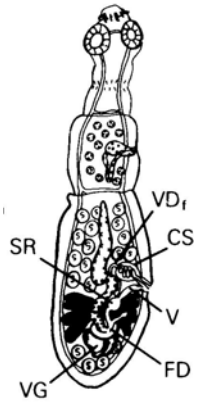
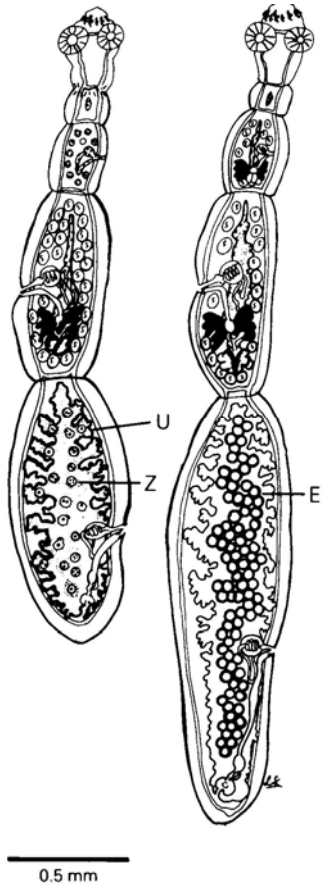
Days and stages		Description of development
Days 28-33  	SR : seminal receptacle VG : vitelline gland VD_f : vas deferens CS : cirrus sack V : vagina FD : female reproductive ducts	Male and female genitalia in terminal proglottid fully mature; uterus still dilating; penultimate proglottid has developing genitalia; either a band or third segment appear
Days 33-37 **Days 37-45**  	U : uterus Z : zygotes E : embryonated eggs	**Days 33-37** Ovulation and fertilisation in terminal proglottid; fully dilated uterus contains dividing zygotes; male and female genitalia degenerating in terminal proglottid; mature genitalia in penultimate proglottid and developing genitalia in ante-penultimate proglottid; strobila divided by three or four segments **Days 37-45** Gravid with embryonated eggs in uterus of terminal proglottid – embryo (oncosphere); zygotes in uterus of penultimate proglottid; strobila divided by three, four or five segments

Fig. 1.1. (contd)
Stages of development of *Echinococcus granulosus* to the adult form in the definitive host
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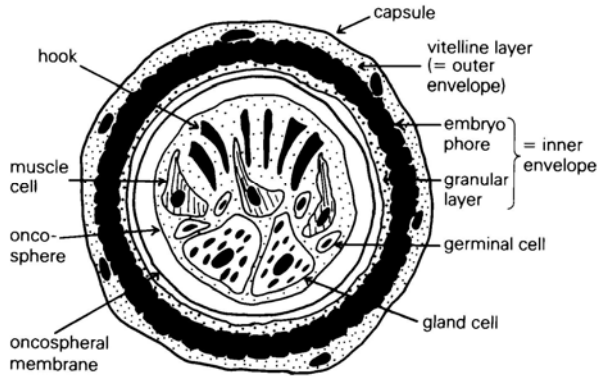


Fig. 1.2.
Diagram of the egg of *Echinococcus* (24)

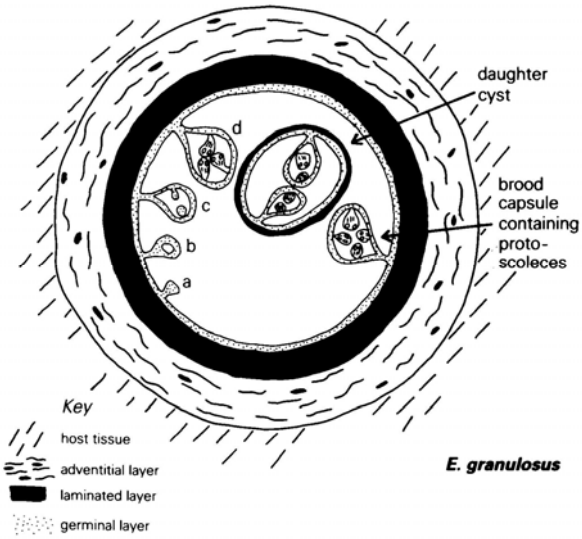


Fig. 1.3.a.
Diagrammatic representation of the metacestode of *Echinococcus granulosus* (24)

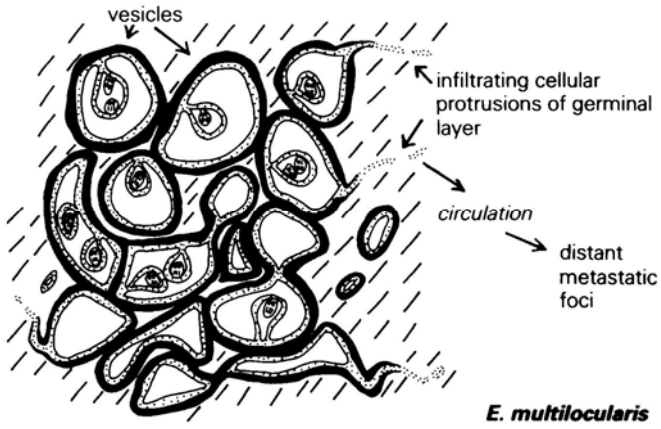
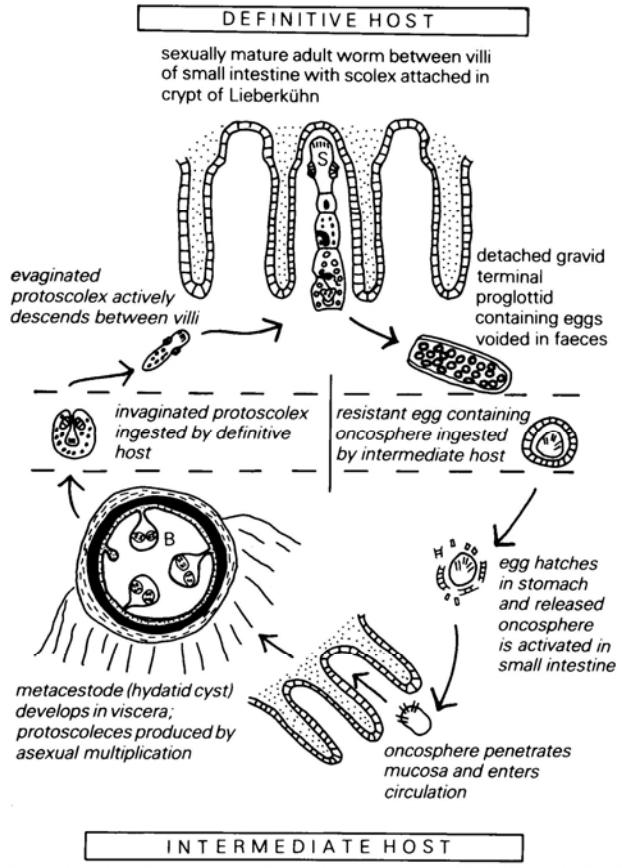


Fig. 1.3.b.
Diagrammatic representation of the metacestode of *Echinococcus multilocularis* (24)
Reproduced from (24) with permission from CABI *Publishing*



S : scolex

B : cyst with brood capsule containing protoscoleces

Fig. 1.4.
Basic life-cycle of *Echinococcus* (24)
Reproduced from (24) with permission from CABI *Publishing*

The metacestode stage

Once the oncosphere has reached its final location, it develops into the metacestode stage. Time of development is variable and it may take several months before protoscoleces are produced (fertile metacestode). There may be several thousand protoscoleces within a single cyst of *E. granulosus* or an aggregation of vesicles of *E. multilocularis*. Each single protoscolex is capable of developing into a sexually mature adult worm. Not all metacestodes produce protoscoleces (sterile metacestode). When protoscoleces are ingested by a suitable definitive host, following the action of pepsin in the stomach, they evaginate in the upper duodenum in response to a change in pH, exposure to bile and to increased temperature. They then develop to the sexually mature adult tapeworm (Fig. 1.4.), approximately four to six weeks after infection, depending on the species and strain, and on the susceptibility of the host. Morphological details of this development are shown in Figure 1.1.

1.4. Specific life-cycle patterns

The basic life-cycle patterns of the two major species, *E. granulosus* and *E. multilocularis,* are illustrated in Figure 1.5. These may be considered to be natural cycles and, in the case of *E. granulosus*, is thought to be ancestral (16, 28). However, the public health and economic significance of echinococcosis as the most important of the cestode zoonoses, is directly attributable to human factors, which have allowed interaction between natural (sylvatic) and domestic cycles and have resulted, particularly in the case of *E. granulosus,* in the widespread global perpetuation of *Echinococcus* in a variety of domestic, man-made life-cycle patterns (Fig. 1.5.) (6, 20, 21, 23, 26).

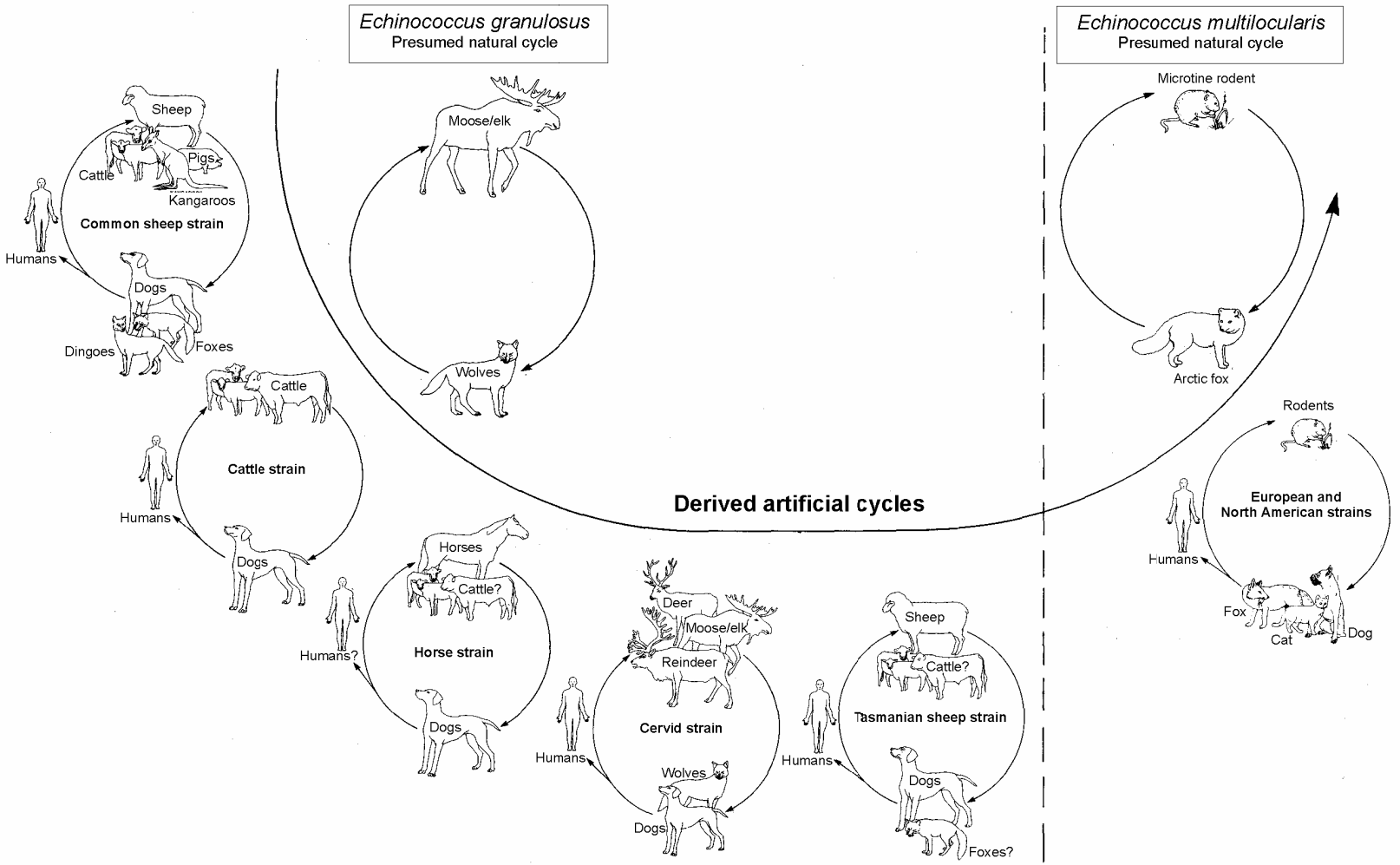


Fig. 1.5.
Life-cycle patterns of *Echinococcus granulosus* and *Echinococcus multilocularis* showing presumed natural (sylvatic) cycles and some derived artificial cycles
Source: R.C.A. Thompson and A.J. Lymbery (28)
Reproduced with permission from Elsevier Science

1.4.1. *Echinococcus granulosus*

This species has a low intermediate host specificity and has been recorded from domestic and wild ungulates belonging to eight families, particularly bovids, as well as primates, leporids and macropod marsupials (24, 26) (Chapter 3).

Sylvatic cycle

The ancestral form of *E. granulosus* is thought to be represented in a sylvatic cycle involving wolves and cervids, such as moose and reindeer, in northern North America and Eurasia. This cycle is primarily perpetuated by a predator-prey relationship, although domestic cycles involving dogs and domesticated reindeer operate in parts of Canada, Alaska, Scandinavia and the Russian Federation.

Domestic cycle

The most important cycles for perpetuating *E. granulosus* involve domestic ungulates, of which representatives from every species are reportedly susceptible. The domestic form of *E. granulosus* is believed to have evolved from that in cervids, and to have become adapted to domestic ungulates with the development of animal husbandry. Today, there are several different life-cycle patterns involving domestic ungulates and dogs, all of which are perpetuated by man's irresponsibility and/or ignorance. Undoubtedly, the most important cycle is that involving domestic dogs and sheep.

Wild animals as hosts

Wild animals are also involved in cycles in different parts of the world, although the zoonotic importance of such cycles is minimal compared to domestic cycles. Wild ungulates of several species have been found infected, principally in Africa, where wild canids, such as hunting dogs (*Lycaon pictus*), jackals (*Canis mesomelas* and *C. aureus*) and hyaenas (*Crocuta crocuta*), as well as occasionally domestic dogs, act as definitive hosts. The lion has also been recorded as a definitive host of *E. granulosus* in Africa and this is the only record for a felid; the domestic cat is not a suitable host for adult *E. granulosus*.

The red fox, *Vulpes vulpes*, is susceptible to certain domestic forms of *E. granulosus,* and may play an increasing role in the epidemiology of cystic echinococcosis (CE) in countries such as Australia (20). In South America, species of fox in the genus *Dusicyon* appear to be important definitive hosts in certain areas and in particular, are involved in cycles in which European hares (*Lepus europaeus*) act as intermediate hosts.

A significant sylvatic cycle operates on the Australian mainland between dingoes (and feral dogs) and macropod marsupials such as wallabies. The practical significance of this cycle is the possibility of overlap and interaction with the domestic cycle, thus impeding control efforts directed at the latter cycle.

1.4.2. *Echinococcus multilocularis*

The typical cycle for this species is sylvatic and involves foxes of the genera *Vulpes* and *Alopex* and rodents, particularly those of the family *Arvicolidae*. Rodents in the families *Soricidae*, *Talpidae*, *Sciuridae*, *Cricetidae* and *Dipodidae*, and pikas (*Ochotonidae*) may also be involved (6, 16, 24, 26) (Chapters 3 and 5.3.).

Domestic dogs and cats are also susceptible definitive hosts and may become infected by predating wild intermediate hosts. Such is the case in the Arctic, where a cycle involving dogs and voles occurs. Such cycles may also operate in any other area, where dogs and cats may capture and eat infected rodents; they have been observed in central Europe, Japan and other regions. Cycles involving cats and house mice may also exist in certain areas, although such partially domestic cycles may be of minimal significance in the overall perpetuation of *E. multilocularis*.

1.4.3. *Echinococcus oligarthrus*

Only felids are capable of acting as definitive hosts of this species. With the larval stage occurring in large South American rodents such as agoutis (*Dasyprocta* spp.) and pacas (*Cuniculus paca*) (6, 16, 24, 26). The

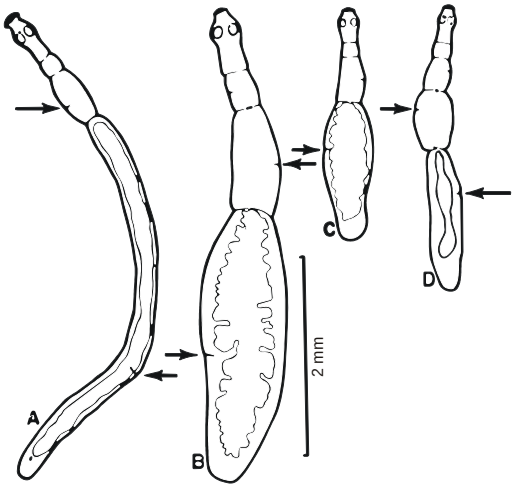
principal definitive hosts are the cougar (*Felis concolor*), jaguar (*Panthera onca*), ocelot (*F. pardalis*), jaguarundi (*F. yaguaroundi*) and Geoffroyi's cat (*F. geoffroyi*). The cycle is thus sylvatic, although domestic cats are known to be suitable hosts experimentally and establishment of a partially domestic cycle is therefore possible.

1.4.4. *Echinococcus vogeli*

As with *E. oligarthrus*, *E. vogeli* is maintained primarily in a sylvatic predator/prey cycle between the bush dog (*Speothos venaticus*) and pacas, although other rodents such as agoutis and spiny rats (*Proechimys* spp.) are susceptible (6, 16, 24, 26). Domestic dogs are also suitable definitive hosts and may be involved in cycles in endemic rural areas of South America and would appear to be the only likely source of infection to humans.

1.5. Species of the genus *Echinococcus*

The four currently recognised species of the genus *Echinococcus* (Table 1.1.) which are regarded as valid taxonomically are *Echinococcus granulosus* (Batsch, 1786), *Echinococcus multilocularis* Leuckart, 1863, *Echinococcus oligarthrus* (Diesing, 1863) and *Echinococcus vogeli* Rausch and Bernstein, 1972 (15, 16, 24). These four species are morphologically distinct in both adult and larval stages. Specific morphological characters that are valuable for taxonomic discrimination of the adult stage of each species are indicated in Table 1.1. and Figure 1.6.



A : *Echinococcus vogeli*
B : *Echinococcus granulosus*
C : *Echinococcus oligarthrus*
D : *Echinococcus multilocularis*
→ genital pore
← genital pore

Fig. 1.6.
Comparative general morphology of adult *Echinococcus* species
Source: adapted from R.L. Rausch (16)

1.5.1. *Echinococcus granulosus*

Adult stage

The adult worm varies between 2 mm-7 mm in length (rarely up to 11 mm) and usually possesses three or four segments (rarely up to six). The penultimate segment is mature, and the genital pore normally opens posterior to the middle of both mature and gravid segments. The gravid uterus is characterised by well-developed lateral sacculations (Table 1.1. and Fig. 1.6. B).

Metacestode

The metacestode stage is a fluid-filled bladder usually unilocular but communicating chambers also occur (24). The cyst consists of an inner germinal or nucleated layer supported externally by a tough, elastic, acellular laminated layer of variable thickness, surrounded by a host-produced fibrous adventitial layer (Fig. 1.3.a.). Typically, *E. granulosus* produces a single-chambered unilocular cyst in which growth is expansive by

concentric enlargement. Asexual proliferation of the germinal layer and brood capsule formation takes place entirely endogenously. Pouching of the cyst walls may occur giving rise to secondary chambers communicating with the central cavity. Sometimes, the central cavity may be partly separated from the secondary chambers by incomplete septa. Occasionally, cysts may abut and coalesce, forming groups or clusters of small cysts of different size. In some hosts, particularly man, where unusually large cysts may develop, daughter cysts may form within the primary cyst.

1.5.2. *Echinococcus multilocularis*

Adult stage

The adult worm varies between 1.2 mm-4.5 mm in length and usually possesses four to five segments. The antepenultimate segment is characteristically mature and the genital pore is anterior to the middle of both mature and gravid segments. The gravid uterus is sac-like (Table 1.1. and Fig. 1.6.D).

Metacestode

The metacestode of *E. multilocularis* is a complex structure and develops quite differently to that of *E. granulosus*. It is a multivesicular, infiltrating structure consisting of numerous small vesicles embedded in a more or less dense stroma of connective tissue (Fig. 1.3.b.). The larval mass usually contains a semisolid matrix rather than fluid. Proliferation occurs both endogenously and exogenously and is attributable to the undifferentiated cells of the germinal layer. The metacestode consists of a network of filamentous solid cellular protrusions of the germinal layer which are responsible for infiltrating growth (Fig. 1.3.b.) transforming into tube-like and cystic structures. Furthermore, the detachment of germinal cells from infiltrating cellular protrusions and their subsequent distribution via the lymph or blood can give rise to the distant metastatic foci characteristic of *E. multilocularis* (1, 6).

In contrast to *E. granulosus*, in which growth is slow and variable, *E. multilocularis* develops rapidly in its natural intermediate host, producing protoscoleces in only 2-4 months, an adaptation to the short-lived arvicoline rodents it utilises (15, 16). Thereafter, proliferation of vesicles is curtailed, and there is little if any further increase in size. In man, growth is very different. Proliferation is progressive but slow, and only a few, if any, protoscoleces are produced (1, 6, 17). The larval mass proliferates peripherally and, at the same time, regressive changes occur centrally. Thus, a progressively enlarging mass of necrotic tissue with a relatively thin zone of viable proliferating parasite may be produced. The term 'alveolar echinococcosis' (Chapter 2, Table 2.1.) refers to the alveolar structure of the metacestode tissue which consists of agglomerates of small vesicles up to about 3 cm in diameter. In recent years, cases of self-cure have been observed in humans connected with limited proliferation and final death of the metacestode.

1.5.3. *Echinococcus vogeli*

Adult stage

The adult worm varies between 3.9 mm-5.6 mm in length, and usually has three segments. The penultimate segment is mature and the genital pore is situated posterior to the middle of both the mature and gravid segment. The gravid uterus has no lateral branches or sacculations, and is characterised by being relatively long and tubular in form (6, 15) (Table 1.1. and Fig. 1.6.A).

Metacestode

The metacestode is polycystic and fluid-filled with a tendency to become septate and multi-chambered (24). The cysts vary greatly in size from 2 mm-80 mm and may occur singly, in small groups, or occasionally in dense aggregations, in which each cyst is enclosed by its separate adventitia. In *E. vogeli*, endogenous proliferation and convolution of both germinal and laminated layers leads to the formation of secondary subdivisions of the primary vesicle with production of brood capsules and protoscoleces in the resultant chambers, which are often interconnected. Exogenous proliferation occurs, but appears to be abnormal and does not occur in the natural intermediate host.

1.5.4. *Echinococcus oligarthrus*

Adult stage

The adult worm varies between 2.2 mm-2.9 mm in length and normally possesses three segments, the penultimate of which is mature. The genital pore is anterior to the middle in mature segments and approximately at the middle in gravid segments. The gravid uterus is sac-like (Table 1.1. and Fig. 1.6.C).

Metacestode

The metacestode is, like *E. vogeli,* polycystic and fluid-filled with a tendency to become septate and multi-chambered (6, 24). In *E. oligarthrus,* there is less subdivision into secondary chambers and the laminated layer is much thinner than that of *E. vogeli.* Exogenous proliferation has been reported.

1.6. Variation in *Echinococcus*

General aspects

A number of intraspecific variants or strains are known to occur within the species *E. granulosus* (4, 7, 23, 24, 27, 28, 29). The term 'strain' is used to describe variants which differ statistically from other groups of the same species in gene frequencies, and in one or more characters of actual or potential significance to the epidemiology and control of echinococcosis (28). This variability may be reflected in characters which affect the life-cycle pattern, host specificity, development rate, pathogenicity, antigenicity and sensitivity to chemotherapeutic agents, transmission dynamics, epidemiology and control of echinococcosis.

1.6.1. Variation in *Echinococcus granulosus*

General aspects

In many cases, these variable forms of *E. granulosus* have been studied in detail and shown to differ in a variety of morphological features and life-cycle characters (28). As such, a number of well characterised strains of *E. granulosus* are recognised which all appear to be adapted to particular life-cycle patterns and host assemblages (Table 1.2. and Fig. 1.5.), some of which clearly warrant species status (Table 1.2.) (29). Analysis of DNA has been used to categorise variants of *E. granulosus* into distinct genotypic strain groups; to date, 9 genotypes (G1-9) have been identified (32, 33) and this categorisation follows very closely the pattern of strain variation emerging based on biological characteristics (Table 1.2.). The notion of a series of host-adapted species in the genus *Echinococcus* is not new. It is a situation that was recognised by many of the early descriptive parasitologists whose published observations provide a logical nomenclature for the 'new' species that have been proposed on the basis of molecular phylogeny. Consequently, a revised nomenclature for species within the genus *Echinococcus* should not be a contentious issue since we can find taxonomic designations for all the putative species in the literature, supported by appropriate ecological information (25).

Strain identification

All four species of *Echinococcus* are clearly distinguishable using morphological and biological features and/or molecular techniques, such as sequence comparison of a 366 bp-fragment of the mitochondrial cytochrome oxidase subunit 1 DNA (CO1) and a 471 bp-region in the mitochondrial NADH dehydrogenase gene 1 (ND1), by analysis of a ribosomal (r)DNA fragment (1ST2) or by the random amplified polymorphic DNA-PCR (RAPD-PCR) (2, 4, 7, 10). Recent genetic studies have principally confirmed the concept of strain diversity within the species *E. granulosus*, previously based on morphological and biological features. Several molecular techniques are now available which would quite easily allow the identification of certain *E. granulosus* strains using genetic markers. Such studies could contribute to the rapid clarification of the epidemiological situation in a given area, but they have to be carried out by an experienced reference laboratory (Annex 1.1.).

Table 1.2.
Strains and isolates of *Echinococcus* species

Strain/isolate (G: genotype)	Intermediate hosts and aberrant hosts	Definitive hosts	Probable geographic distribution[a]
Echinococcus granulosus			
Sheep strain (G1)	Sheep, cattle, pigs, camels, goats, macropods, man	Dog, fox, dingo, jackal, hyena	Australian mainland, Europe, United States of America, New Zealand, Africa, People's Republic of China, Middle East, South America, Russian Federation
Tasmanian sheep strain (G2)	Sheep, cattle?, man	Dog (fox)	Tasmania, Argentina
Buffalo strain (?) (G3)	Buffalo (cattle?) (man?)	Dog (fox?)	Asia
Horse strain (G4)	Horses and other equines	Dog	Europe, Middle East, South Africa (New Zealand?, United States of America?)
Cattle strain (G5)	Cattle, man	Dog	Europe, South Africa, India, Sri Lanka, Russian Federation
Camel strain (G6)	Camels, goats, cattle? man?	Dog	Middle East, Africa, People's Republic of China, Argentina
Pig strain (G7)	Pigs, man?	Dog	Europe, Russian Federation, South America
Cervid strain[a] (G8)	Cervids, man	Wolf, dog	North America, Eurasia
Lion strain[b]	Zebra, wildebeest, warthog, bushpig, buffalo, various antelope, giraffe? Hippopotamus?	Lion	Africa
Echinococcus multilocularis			
European isolate	Rodents, domestic and wild pig, dog, monkey, man	Fox, dog, cat, wolf	Europe, People's Republic of China (?)
Alaskan isolate	Rodents, man	Fox, dog, cat	Alaska
North American isolate	Rodents, man	Fox, dog, cat, coyote	North America
Hokkaido isolate	Rodents, pig, monkey, horse, man	Fox, dog, cat, raccoon-dog	Japan
Echinococcus vogeli			
None reported	Rodents	Bush dog	Central and South America
Echinococcus oligarthrus			
None reported	Rodents	Wild felids	Central and South America

? : unclear status

a) with some strains, the geographic range of isolates which have been characterised simultaneously using morphological and genetic criteria is limited (see text)

b) no detailed genetic characterisation; at present separated on the basis of morphological, biological and epidemiological features

Material collection for strain identification and techniques

Identification of *E. granulosus* using morphological and biological features is very difficult and labour-intensive. Therefore, strain identification using molecular techniques is the preferred method today. For this purpose, protoscoleces should be collected from *E. granulosus* cysts, washed several times in physiological saline

solution and preserved in 70% ethanol. Adult stages can also be used, but they should be purified as much as possible from contaminating intestinal material before preservation. The material should be sent to an experienced laboratory (Annex 1.1.).

From a practical point of view, studies on a range of parasites, including *Echinococcus*, have shown that the ITS region (internal transcribed spacer) of rDNA can not only give an overall picture of the extent of genetic variation but can also provide a useful diagnostic marker for taxonomic purposes (2, 4). The rDNA ITS1 region has been shown to be a potentially very useful genetic marker for distinguishing strains and species of *Echinococcus* and small quantities of *Echinococcus* material can be characterised using a PCR-RFLP 'fingerprinting' technique (2). This technique, which may be modified in the future once additional restriction enzymes have been evaluated, offers a most reliable and technically reproducible procedure for the routine laboratory identification of species and strains of *Echinococcus*, particularly when corroboration is obtained by mitochondrial DNA sequencing. This is exemplified by a recent study (19), where the ITS1-PCR-RFLP fingerprinting technique and sequencing of the mitochondrial COI and NDI genes were used to characterise 33 *E. granulosus* isolates collected from different regions and hosts in Argentina, and to determine which genotypes occurred in humans with cystic hydatid disease. A new method, single strand conformation polymorphism (SSCP), has been developed, which is technically relatively simple, has a high resolution capacity under optimised conditions, and is well suited for screening large samples sizes for nucleotide variations in small gene fragments. The utility of SSCP was recently established for the categorisation of *Echinococcus* genotypes (11), and the method has been applied for the genetic analysis of a large number of isolates of *E. granulosus* collected from the People's Republic of China and Argentina (34). The principles of some of these techniques are explained in Annex 1.1. At the present time, the PCR-RFLP fingerprinting technique and/or the determination of CO1/ND1 gene sequences by PCR/direct sequencing probably represent the best methods available for the molecular identification of *Echinococcus* species and strains.

1.6.2. Epidemiological significance of *Echinococcus granulosus* strains

Variation in the pathogenicity of strains/species of *Echinococcus* will influence the prognosis in patients with echinococcosis. Epidemiological evidence suggests that the sylvatic strain of *E. granulosus* in northern North America is infective to humans causing a benign infection of low pathogenicity, with predominant localisation of cysts in the lungs (25). Epidemiological observations in the People's Republic of China suggest that strains of *E. granulosus* in certain regions may have lower pathogenicity. In contrast, in parts of Kenya and Libya, it has been suggested that there are local virulent strains of *E. granulosus* (24).

There is also increasing epidemiological evidence that certain strains of *E. granulosus* may be of no or low infectivity to humans, such as the form adapted to horses (24). In contrast, recent isoenzyme and molecular studies have confirmed what has long been presumed on the basis of epidemiological data, that the sheep strain is infective to humans (2, 3). Indeed, until recently, most *E. granulosus* material obtained from human patients by surgery conformed to the sheep strain (2), except one case from the Netherlands, in which the cattle strain was typed by PCR-based molecular characterisation procedures (5). A study of genetic variation and epidemiology of *E. granulosus* in Argentina has reported for the first time the presence in humans of the Tasmanian sheep strain (G2 genotype) and the same genotypic strain (G6) previously identified in camels (19); these findings may have important consequences for human health.

It had been suspected, on circumstantial grounds, that *E. granulosus* from pigs has a low infectivity for humans (9, 14). Indeed, recent investigations of endemic foci in the Ukraine and Poland demonstrated the common occurrence of *E. granulosus* infections in dogs and pigs, but little evidence of the disease in humans. Nevertheless, molecular genetic analysis of human cystic hydatid cases from Poland has identified a new genotypic group (G9) of *E. granulosus* (22). The molecular analysis indicated that these patients were clearly not infected with the common sheep strain. Instead, the hydatid parasite shared molecular affinity with the previously characterised pig strain, but exhibited some genetic differences as well. The major question arising from this study, still unanswered, concerns the reservoir(s) of human hydatid disease in Poland. The national figures for cystic hydatidosis in slaughtered animals indicate five times the prevalence in pigs compared with sheep (22), and it is likely that pigs naturally harbour the newly identified genotype of *E. granulous* present in humans there although this has not yet been definitively proven. Similarly, whether the common sheep strain occurs in Poland remains to be determined.

Although camels are commonly infected in the Middle East and Africa, opinions have differed regarding the infectivity of *E. granulosus* of camel origin to humans. As referred to above, however, recent molecular genetic studies of isolates collected from Argentina have indicated for the first time that the camel strain genotype (G6) can infect humans (19). There are no camels in Argentina, but other American camelids, including the Guanaco, Llama and Alpaca can be found. Attempts are in progress to analyse isolates of *E. granulosus* from these animals, though they are not easy to obtain, and also from goats, since the G6 genotype has also previously been found in goats (31). In areas where there are several intermediate host species, it is important to know whether each harbours a different strain and whether there is the possibility of interaction between cycles. For example, in Great Britain, *E. granulosus* is perpetuated in two distinct cycles of transmission, sheep/dog and horse/dog, and interaction is unlikely since each cycle is associated with the perpetuation of a distinct strain/species exhibiting different intermediate host specificity characteristics. Molecular characterisation of isolates of the parasite from horses and sheep has shown them to be genetically distinct thus supporting the epidemiological observations (24).

Developmental differences between species and strains of *Echinococcus*, and in particular variation in the onset of egg production, is likely to be a limiting factor in control programmes which employ regular, adult cestocidal treatment of definitive hosts for breaking the cycle of transmission. This has been demonstrated in several strains of *E. granulosus*. For example, with the cattle strain, the adult parasite exhibits a precocious development in the definitive host with a short prepatent period of only 33-35 days, nearly a week earlier than that of the common sheep strain (24).

1.6.3. Variation in *Echinococcus multilocularis*

There is some morphological and biological variation between *E. multilocularis* isolates from North America and Eurasia (Table 1.2.). However, the situation with *E. multilocularis* is not as clear-cut as with *E. granulosus* and, although there is some variability in a range of behavioural and other phenotypic characteristics between geographically separated populations, compared to *E. granulosus*, there is little evidence of genetic distinctness between populations of *E. multilocularis* (13, 27). However, both mitochondrial and rDNA sequencing of isolates of *E. multilocularis* from Europe, North America and Japan have confirmed the genetic distinctness of Eurasian and North American isolates of *E. multilocularis* (13, 18).

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Annex 1.1.

Principles of molecular techniques for the identification of *Echinococcus* species and strains

1.1.1. Material collection

Echinococcus granulosus

As mentioned in Chapter 1, protoscoleces should be collected from *E. granulosus* cysts, washed several times in physiological saline solution and preserved in 70% ethanol. Adult stages can also be used but they should be purified as much as possible from contaminating intestinal material before preservation.

Echinococcus multilocularis

Metacestode tissue (with or without protoscoleces) isolated from naturally or experimentally infected rodents (preserved in 70% ethanol or by deep-freezing) is a suitable source of material. Intestinal smears from foxes containing adult stages of *E. multilocularis* have also been used (9). Deoxyribonucleic acid from tissue samples was prepared by proteinase K digestion and phenol/chloroform extraction. The DNA isolation from intestinal smears requires an alkaline lysis method (9).

Other species

Material can be collected according to the recommendations for *E. granulosus* or *E. multilocularis*.

1.1.2. Principles of methods

Molecular studies on identification of *Echinococcus* species and strains have involved several techniques (5, 7):

Restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP) of ribosomal DNA (rDNA) or other genomic regions. The DNA is digested by restriction enzymes, the resulting fragments are electrophoretically separated on an agarose gel, transferred to a nitrocellulose or nylon filter and hybridised with a specific DNA probe that has been radioactively or otherwise labelled in a Southern Blot approach (RFLP-SB) (8, 11).

The rDNA RFLP technique has been linked with the polymerase chain reaction (RFLP-PCR or PCR-linked RFLP) to provide a greatly simplified procedure, without loss of resolution or accuracy (2). During the PCR, a fragment of DNA, defined by oligonucleotide primers at either end, is amplified several million fold using a thermostable Taq polymerase. Ribosomal RNA genes are organised into rDNA units with the very highly conserved coding regions separated by relatively poorly conserved non-coding spacer regions. Internal transcribed spacer 1 (ITS1) was chosen as the sequence for PCR amplification and primers were designed based on highly conserved regions at the 3' end of the 18S rRNA gene (forward primer BD1) and within the 5.8S rRNA gene (reverse primer 4S). The PCR product, which spans ITS1 of the rDNA repeat unit and includes most of the 5.8S gene, has been amplified from various *Echinococcus* isolates and digested with one of a number of 4-base cutting restriction enzymes. Characteristic RFLP patterns are produced when samples within the various species and strain groups are analysed by agarose gel electrophoresis.

Comparison of polymerase chain reaction-amplified deoxyribonucleic acid sequences

The nucleotide sequences (and inferred amino acid sequences) of fragments of the mitochondrial cytochrome c oxidase subunit I (COI) and of the NADH dehydrogenase 1 (ND1) genes are determined using two conserved PCR primers. The variable segment between the primers is PCR-amplified for a particular *Echinococcus* isolate and then directly sequenced manually or by automatic means (1, 3, 4, 13). The sequences obtained can then be directly compared with sequences already published for the four *Echinococcus* species and the different genotypes of *E. granulosus* and the genotypic identity of a particular isolate thus determined.

Random amplified polymorphic deoxyribonucleic acid-polymerase chain reaction

Random amplified polymorphic DNA-PCR (RAPD-PCR) is a technique by which genomic DNA is amplified by PCR using a single oligonucleotide primer of arbitrary nucleotide sequence (10, 11). This technique is relatively simple, it requires only small amounts of DNA (approximately 25 ng) and is rapid. However, reliable results are only obtained under carefully controlled conditions, especially with regard to the quantity and quality of template DNA. Therefore, it is recommended that RAPD-PCR should be used simultaneously with one or other of the DNA techniques available (10).

Single-strand conformation polymorphism

Single-strand conformation polymorphism (SSCP) is a simple mutation scanning method with the potential to discriminate DNA sequence differing by a single nucleotide. The method is based on the principle that the electrophoretic mobility of a single-stranded DNA molecule in a non-denaturing gel is dependent on its size and structure. A mutation or base change at a particular site in the primary sequence can modify the conformation of the molecule which alters its electrophoretic mobility. SSCP has been used for the direct visual display of sequence variation in PCR-amplified fragments of the mitochondrial COI and NDI genes of *Echinococcus* species and *E. granulosus* genotypes (6). Although, the technique has to be very carefully controlled, it has the advantage that there is no need for DNA sequencing or restriction analysis and large numbers of samples can be analysed in a short period.

1.1.3. Selected addresses of laboratories experienced in using deoxyribonucleic acid techniques for the identification of *Echinococcus* isolates

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Chapter 2

Echinococcosis in humans: clinical aspects, diagnosis and treatment

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Summary

In humans, three forms of echinococcosis are known to occur: cystic echinococcosis (CE), caused by Echinococcus granulosus*, alveolar echinococcosis (AE), caused by* E. multilocularis*, and polycystic echinococcosis (PE), due to* E. vogeli *or* E. oligarthrus*. In this Chapter, the natural history, clinical presentation, diagnosis and treatment of these diseases are described. The diagnostic repertoire includes imaging techniques, mainly ultrasound (US) and computed tomography (CT) examination for abdominal echinococcosis and X-ray for lung echinococcosis, and immunodiagnostic tests. The US examination can be used under field conditions for population screening. Today, treatment options for CE include: surgery, PAIR (puncture, aspiration, injection, reaspiration) and chemotherapy. For AE, the first choice of treatment in all operable cases is radical surgical resection of the entire parasitic lesion from the liver and all affected organs. After radical surgery, chemotherapy is indicated for at least two years. Long-term chemotherapy is mandatory after incomplete resection of lesions, in inoperable patients (including patients after interventional procedures) and in AE patients after liver transplantation. Ethical aspects related to research, novel diagnostic or therapeutic approaches and population-based studies are discussed.*

2.1. Forms of echinococcosis in humans

Echinococcosis in humans is an infection which is caused by a larval stage, the metacestode, of *Echinococcus* species and may result in asymptomatic infection to severe disease; it may even be fatal. The metacestodes of all four recognised *Echinococcus* species can infect humans and cause various forms of echinococcosis (Table 2.1.). Among these forms cystic and alveolar echinococcosis are of special medical importance.

Table 2.1.
Forms of echinococcosis in humans (3, 84)

Form of echinococcosis	Causative agent	Disease synonyms
Cystic echinococcosis	*Echinococcus granulosus*	Hydatid disease, hydatidosis, *E. granulosus* echinococcosis
Alveolar echinococcosis	*Echinococcus multilocularis*	Alveolar hydatid disease, *E. multilocularis* echinococcosis
Polycystic echinococcosis	*Echinococcus vogeli*	*E. vogeli* echinococcosis
Polycystic echinococcosis	*Echinococcus oligarthrus*	*E. oligarthrus* echinococcosis

Although *Echinococcus granulosus* and *E. multilocularis* occur simultaneously in large endemic areas, mixed infections of cystic echinococcosis (CE) and alveolar echinococcosis (AE) in humans are apparently rare (125).

With regard to the mode of infection the following two entities have to be distinguished.

Primary echinococcosis

Metacestodes develop in various sites of the human body from oncospheres liberated from ingested eggs of *Echinococcus* spp. In CE, parasite cysts may establish in virtually all anatomic sites, but the liver and the lung are the most frequently affected organs. In AE, the liver is involved in 98% to 100% of the cases as primary site of metacestode development, but in later phases metastases may establish in other organs (see below).

Secondary echinococcosis

Metacestode material spreads from the primary site to adjacent or distant organs and proliferates. In CE, this form occurs after release of viable parasite material (protoscoleces, small daughter cysts) during invasive treatment procedures or after spontaneous or trauma-induced cyst rupture (129). Secondary echinococcosis in AE is caused by the tumour-like proliferation of the metacestode with direct infiltration of adjacent organs or by metastasis formation in distant organs due to spreading of parasite cells via lymph and blood vessels (3, 32, 69).

A uniform terminology related to *Echinococcus* and echinococcosis has been recently proposed and is used in this document (84).

2.2. Cystic echinococcosis

Several review papers or monographs on human CE have been published in recent years (2, 3, 5, 6, 45, 74, 83, 85, 129). For further references of original papers the reader is referred to these sources.

2.2.1. Causative agent and course of infection

Causative agent

The causative agent of CE is the metacestode of *Echinococcus granulosus.* The metacestode develops from the oncosphere and is a cystic structure typically filled with a clear fluid (hydatid fluid) (Chapter 1). The post-oncospheral development takes 10-14 days. By this time, the bladder (measuring 60 μm-70 μm in diameter) consists of a nucleated germinal layer and a thin laminated layer which lacks nuclei. Most of the cysts grow slowly in size and become surrounded by host tissue (pericyst) encompassing the endocyst of metacestode origin. The endocyst consists of the outer laminated layer and the inner cellular germinal layer, which may form brood capsules and protoscoleces. The minimum time required for the development of protoscoleces in cysts in humans is not exactly known, but based on data from animals, it is expected to be 10 months or longer after infection (2, 85). Protoscoleces can be already formed in small cysts of 0.5 cm-2.0 cm diameter. In the same patient, fertile (with protoscoleces) and sterile (without protoscoleces) cysts may coexist. Quite frequently, smaller daughter cysts are formed within a larger mother cyst (see below). Several small single cysts growing in close proximity to each other may form clusters, thus presenting a 'polycystic' or 'multivesicular' appearance which has to be distinguished from AE and PE.

Echinococcus granulosus cysts have a variable natural course of development. According to an ultrasound study in 66 human patients in Turkana area of Kenya, about 30% of cysts grew slowly (1 mm to 5 mm per year), 43% showed a moderate growth (6 mm to 15 mm per year), 11% exhibited a more rapid increase (average: 31 mm, maximum: 160 mm per year), and 16% of cysts did not expand or had collapsed (96, 97). Partially or totally calcified cysts are not uncommon. The size of cysts is variable and ranges usually between 1 cm and 15 cm, but much larger cysts containing 48 l of cyst fluid have been noted (2). Spillage of viable protoscoleces or small daughter cysts after cyst rupture may result in secondary echinococcosis.

Course of infection

The natural history of *E. granulosus* cysts and its clinical implications are presented in Figure 2.1. The initial phase of primary infection is always asymptomatic, and small (<5 cm) well-encapsulated cysts located in organ sites, where they do not induce major pathology, may remain asymptomatic for many years or permanently (3,

83, 85). In two Italian series with 420 and 424 patients, 38% and 60% of all CE cases were asymptomatic (14, 50), but this rate may be lower in other regions.

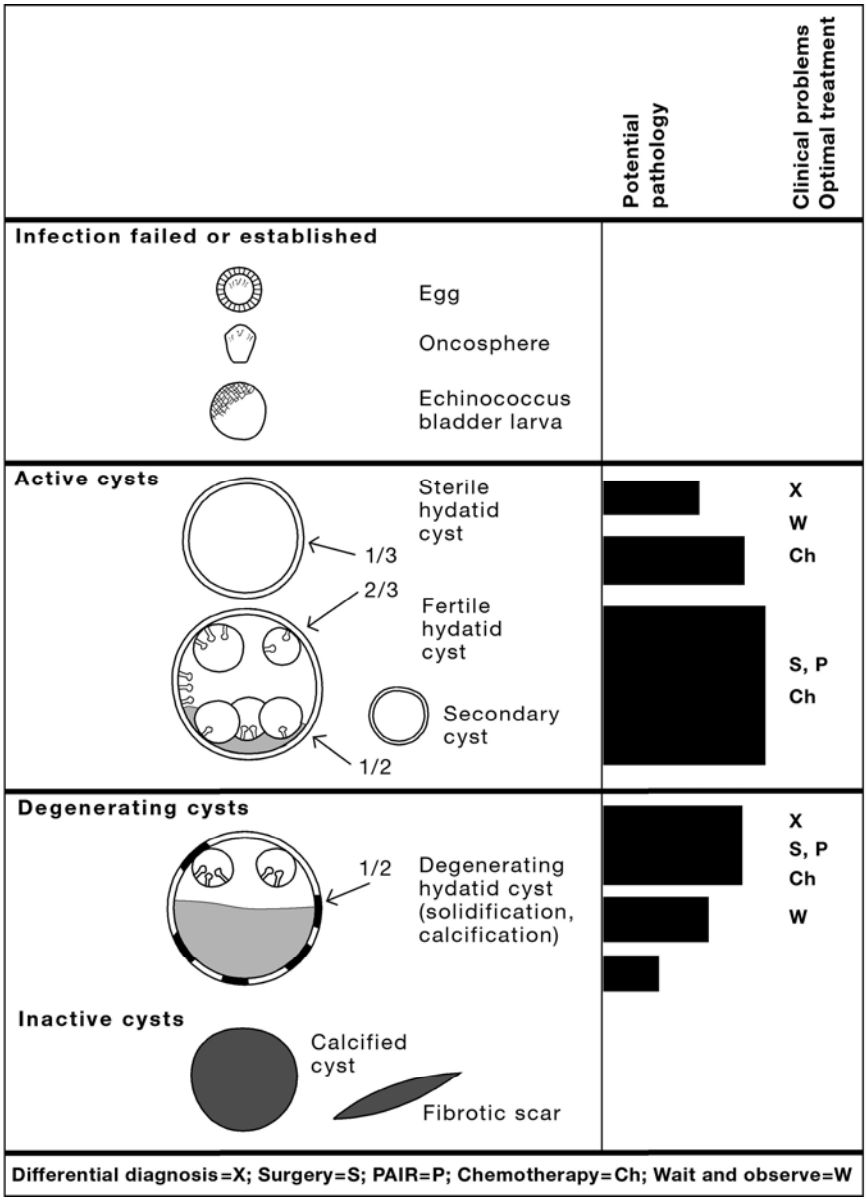


Fig. 2.1.
Natural history of *Echinococcus granulosus* liver cysts
The numbers (1/3 etc.) indicate approximate frequencies of cyst types
Reproduced from (85) with permission from F.L. Andersen (ed.)

After an undefined incubation period of several months or years, the infection may become symptomatic if cysts exert pressure on adjacent tissue and induce other pathological events. Sudden symptomatology may be due to spontaneous or traumatic cyst rupture. Spontaneous cure is possible, due to collapse and resolution of cysts, cyst calcification or cyst rupture into the bile duct or the bronchial tree with discharge of the cyst content. Recurrence of the disease may occur after operation on primary cysts (see surgery).

It is difficult to present exact data from recent years on the rates of morbidity, mortality and fatality. One of several reasons is that the terms mortality (= rate of fatal cases per 100,000 of the total population in a defined area) and fatality (= fatal cases related to the number of confirmed CE cases) are often not clearly differentiated. Therefore, only some examples are given here.

Up to 60% of the CE cases may be asymptomatic (see above), but it is assumed that some may become symptomatic with the time. In the Regional Hospital of Valdivia, Chile, a total of 137 new cases of CE was registered in 1987-1991; the mortality rate was 0.2 per 100,000 population, and the fatality rate 2.2% (34). The fatality rate is highly dependent on the severity of the infection and on facilities for treatment. For example, the fatality rate in 98 cases of CE of the heart was 23% (33), whereas this rate is around 2% (3) or less in cases of uncomplicated CE of the liver if adequate surgical facilities are available.

2.2.2. Clinical presentation

Age and sex of patients

Cystic echinococcosis may reach medical attention in almost all ages, from below 1 year of age to over 75 years old, and in both sexes. Among 1,473 patients admitted to a children's hospital in Madrid (Spain), 2% were <1 year old, 21% between 1 and 4 years and 77% between 5 and 14 years (116). In a Chinese series of 15,289 surgical cases, 49% were in males and 51% in females (70). In both sexes, case numbers reached a peak between 6 and 15 years and then decreased with successive age (Fig. 2.2.).

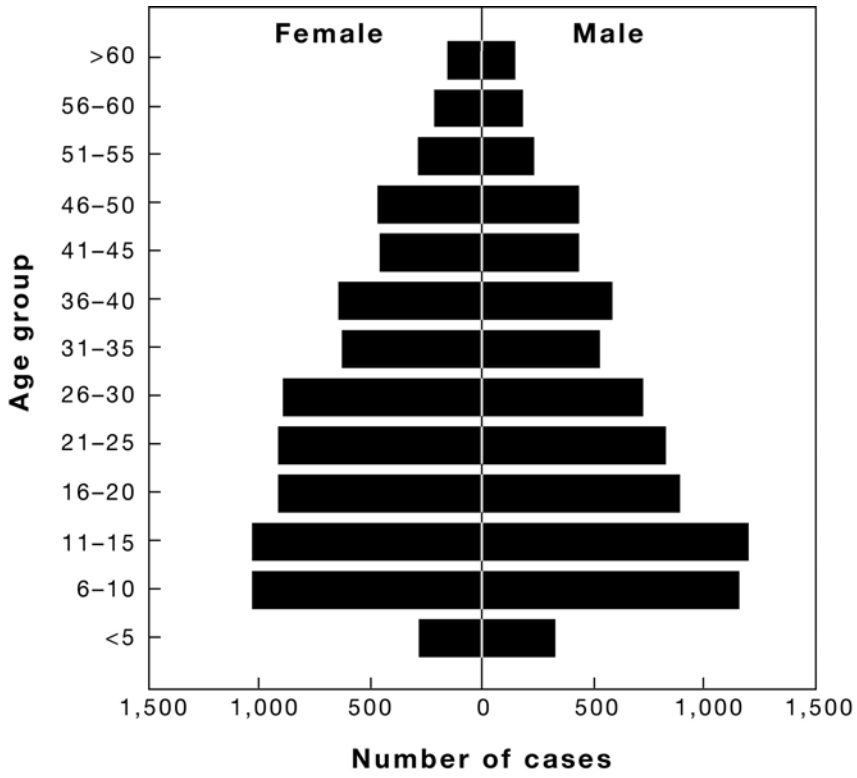


Fig. 2.2.
Age and sex distribution of surgical cases of cystic echinococcosis in Xinjiang, People's Republic of China, 1951-1990
Reproduced from (70) with permission from F.L. Andersen (ed.)

In other regions, the highest numbers of CE cases were recorded in older age groups, e.g. between 21 and 30 years (Kenya) or 21 to 40 years (Libya) (17). Further, it should be noted that the patterns of age distribution of the cases may vary with the mode of selection of patients and the technique of examination. In series of surgical patients, the frequency of interventions declines in older age groups, but it increases with age when populations are screened by ultrasound (17).

Occupation of patients

The occupational distribution of patients may vary widely from country to country depending on epidemiological and socio-economic circumstances. One example from the People's Republic of China is given in Figure 2.3.

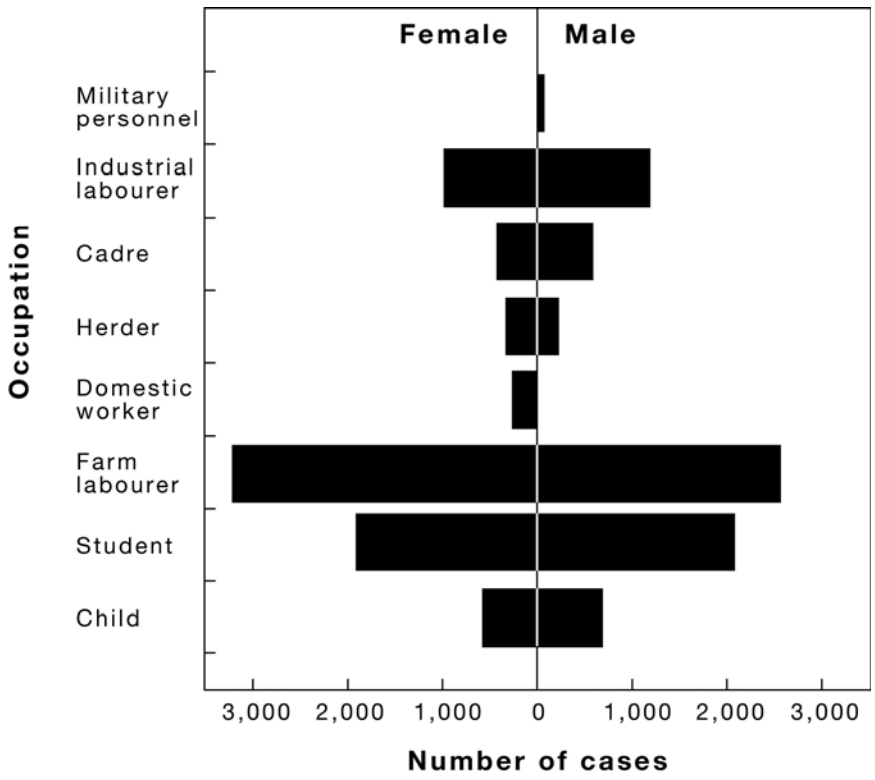


Fig. 2.3.
Occupational distribution of surgical cases of cystic echinococcosis in Xinjiang, People's Republic of China, 1951-1990
Reproduced from (70) with permission from F.L. Andersen (ed.)

Organ sites of cysts

Many patients (about 40% up to 80%) with CE have a single organ involved and harbour a solitary cyst. Examples of sites of the cysts in cases with single organ involvement and with single and multiple organ sites are presented in Table 2.2. Relative percentages of liver and lung locations, which together account for at least 90% of the cysts, may vary depending on the country.

Symptoms

The clinical symptomatology of CE is variable and never pathognomonic (2, 3, 74, 83, 85). The spectrum depends primarily on:

a) the organ(s) involved
b) the size of the cysts and their site within the affected organ
c) the interaction between the expanding cysts and the adjacent organ structures
d) the complications related to cyst rupture, spread of protoscoleces, and bacterial infection.

In addition, systemic immunological reactions may be observed like urticaria, asthma, anaphylaxis or membranous nephropathy (3). Presenting symptoms and signs are listed in Table 2.3. Asymptomatic liver CE is quite common and may remain symptom-free for more than ten years (37).

The course of CE may be associated with a wide spectrum of complications. Some examples of complications which may occur in cases of liver echinococcosis are presented in Table 2.4.

Table 2.2.
Organ sites of *Echinococcus granulosus* cysts in humans
A Single organ involvement in 459 patients[a] (*Source*: 30, 42)
B Single and multiple organ involvement in 15,289 Chinese surgical cases (modified from 70)

Organ	A		B	
	Number of cases	Percentage of cases	Number of cases[b]	Percentage of cases
Liver	316	68.8	11,499	75.2
Lung	79	17.2	3,432	22.4
Kidney	17	3.7	68	0.4
Spleen	15	3.3	160	1.0
Muscles and skin	10	2.2	29	0.2
Abdominal and pelvic cavity	9	2.0	794	5.2
Mediastinum, heart	5	1.1	4	0.03
Brain	4	0.9	61	0.4
Bones	3	0.6	30	0.2
Ovarium	1	0.2	9	0.06
Other organs: skin, eye, spinal cord, pancreas, urinary bladder, testis, etc.	–	–	–	Each <0.1

a) single organ involvement is indicative for cyst development after primary infection
b) the number of cases in this column exceeds the total of 15,289 since many patients had multiple organ involvement. The same applies to the percentages

2.2.3. Diagnosis

2.2.3.1. General aspects

The process of diagnosis of CE in individual patients goes through various steps, as follows:

- suspicion on clinical grounds or upon screening
- confirmation by imaging (US, CT, X-ray, etc.) and identification of characteristic or suspicious cyst structures
- confirmation by detection of specific antibodies with immunodiagnostic tests (ELISA, IFAT, immunoblot, detection of arc 5 antibodies, etc.) (Chapter 2.2.3.8.)
- in doubtful cases diagnostic puncture may be considered, if it is not contraindicated (Chapter 2.2.3.6.)
- material obtained by biopsy puncture or surgery can be examined: hydatid fluid for *Echinococcus* protoscoleces or hooks; protoscoleces for DNA by PCR; antigen from sterile cysts, and cyst wall material for characteristic structures by histology.

In many cases, a diagnosis can be made by detecting the characteristic structure and size of *E. granulosus* cysts visualised by various imaging techniques, including ultrasonography (US), computed tomography (CT) standard radiology (X-ray), and magnetic resonance imaging (MRI) in specialised centres. Introduction of US has improved both the diagnosis of CE and the understanding of the natural history of the disease (2, 3, 13, 14, 67, 68, 74, 83, 88, 93, 120, 121). The US examination is a suitable technique for population studies aimed at detecting cases and determining prevalence of CE. In this indication, US has achieved great significance in recent years since portable US units allow the application of this technique in field situations (67, 68). Immunodiagnostic tests for detecting specific antibodies are commonly used for the aetiological confirmation of the findings of imaging examinations (Chapter 2.2.3.8.).

Table 2.3.
Presenting symptoms and signs of cystic echinococcosis (3)

Organ	Symptoms and signs
Liver	'Tumour' – hepatomegaly, ± cholestasis ± jaundice Secondary biliary cirrhosis Biliary colic-like symptoms ± cholangitis or pancreatitis (elimination of fragments of the cyst via biliary tract) Liver abscess Calcified lesions in liver or spleen Portal hypertension ± ascites Inferior vena cava compression or thrombosis Budd-Chiari syndrome Cyst rupture, peritoneal spread, biliary peritonitis Haemobilia Biliary fistula to skin, bronchial system or gastrointestinal tract
Lung	Lung 'tumour' ± chest pain Chronic cough, expectoration, dyspnea Haemoptysis Biliptysis Pneumothorax Pleuritis Lung abscess Eosinophilic pneumonitis Parasitic lung embolism
Cyst rupture into biliary tree	Biliary colic Cholestatic jaundice Cholangitis Symptoms of pancreatitis Symptoms of anaphylaxis Fever
Cyst rupture into bronchial tree	Asthma-like symptoms Cough, expectoration, dyspnea Haemoptysis Symptoms of anaphylaxis Fever
Heart	Pain 'Tumour' Cardiac insufficiency Embolism Pericardial effusion
Bone and muscles	Pain Bone 'outgrowth' Bone fragility Disturbances of motility Muscle cyst
Brain and spine	Headache 'Tumour' with neurological symptoms Back pain
Eyes	Pain *Protrusio bulbi* Ptosis Visual disturbances

± : with or without

Table 2.4.
Complications in 221 patients with cystic echinococcosis

Complication and site of involvement	Number of cases	Percentage of total[a]
Biliary tract	47	21.3
Cystic rupture into bile ducts	36	
Gallbladder or common duct obstruction	9	
Fibrosis of the papilla	4	
External bile fistulas	4	
Bacterial infection	27	12.2
Intracystic	26	
Subphrenic	21	
Intraperitoneal rupture	23	10.4
Acute (anaphylactic shock)	2	
Multiple intraperitoneal cysts	21	
Hepatopulmonary cysts	20	9.0
Lung involvement, intact cyst	11	
Pericystobronchial fistula[b]	3	
Biliptysis[c]	4	
Rupture into pleural cavity	2	
Portal hypertension and gastro-intestinal bleeding	1	0.5

a) percentages refer to total number of cases; many patients had more than one complication
b) expectoration of cysts
c) vomiting of bile, in two cases in association with haemoptysis

Source: Barros, cited in (3)

A direct method of diagnosis is finding characteristic protoscoleces or hooks of *E. granulosus* in aspirated hydatid fluid specimens (Chapter 1). The method requires only a simple microscope and very basic laboratory training. This examination is not performed frequently as the material for such a direct examination can only be available after a surgical intervention, therapeutic puncture (PAIR) or diagnostic puncture. Rarely characteristic hooks or protoscoleces may be found in sputum, bile, stool or urine after a spontaneous rupture of the cysts in lungs, liver or kidneys.

The direct diagnosis can also be made by macroscopic identification of the structure and size of *E. granulosus* cysts obtained by surgery and/or by histological examination of the parasite tissue, available after surgery or biopsy (Figs 2.4. and 2.5.). More sophisticated techniques in direct diagnosis include finding of specific *E. granulosus* antigen (antigen 5) in the fluid from sterile cysts (79, 103) or DNA markers in the cysts fluid or parasite tissue (e.g. by PCR).

In view of the availability of different diagnostic methods, it is necessary to proceed rationally in selecting techniques, taking their contribution to diagnosis into account. In some cases, performing an additional imaging examination adds nothing to the diagnosis, but may well provide guidance concerning surgical procedure. It is important to select the simplest and most cost-effective method, and one that is most valid and least harmful.

Fig. 2.4.
Intraoperative situs of an opened large liver cyst with daughter cysts of *Echinococcus granulosus*
Photograph: courtesy of Professor R. Ammann, University Hospital, University of Zurich

2.2.3.2. Standard radiology

Chest radiography

This is still the technique of choice for the diagnosis of pulmonary cysts of *E. granulosus* which may display various features (2, 74, 93, 120, 121).

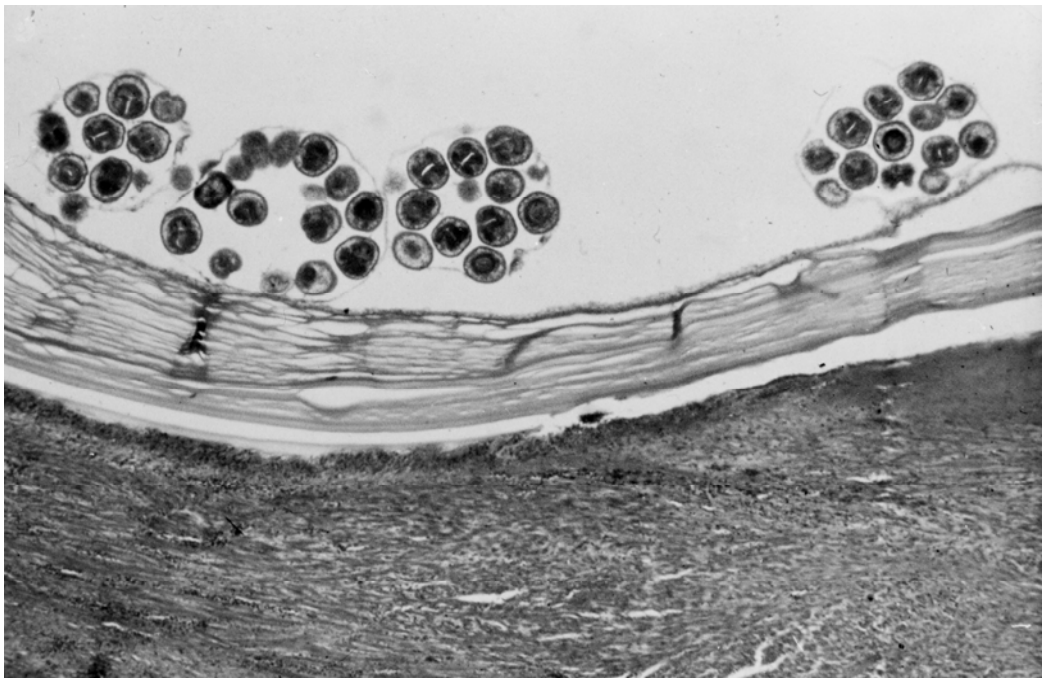


Fig. 2.5.
***Echinococcus granulosus*, histological section through cyst wall**
Reproduced from (120) with permission from Elsevier Science

- **Uncomplicated cysts**

Uncomplicated cysts are clearly defined, usually round or oval structures with diameters between 1 cm and >20 cm, displaying a homogeneous shadow indicating a fluid-filled space. They may also occur as thin-walled 'empty' cysts. The cysts may be located anywhere in the lung as solitary or multiple cysts. Pulmonary cysts usually do not calcify, and daughter cyst formation is rare.

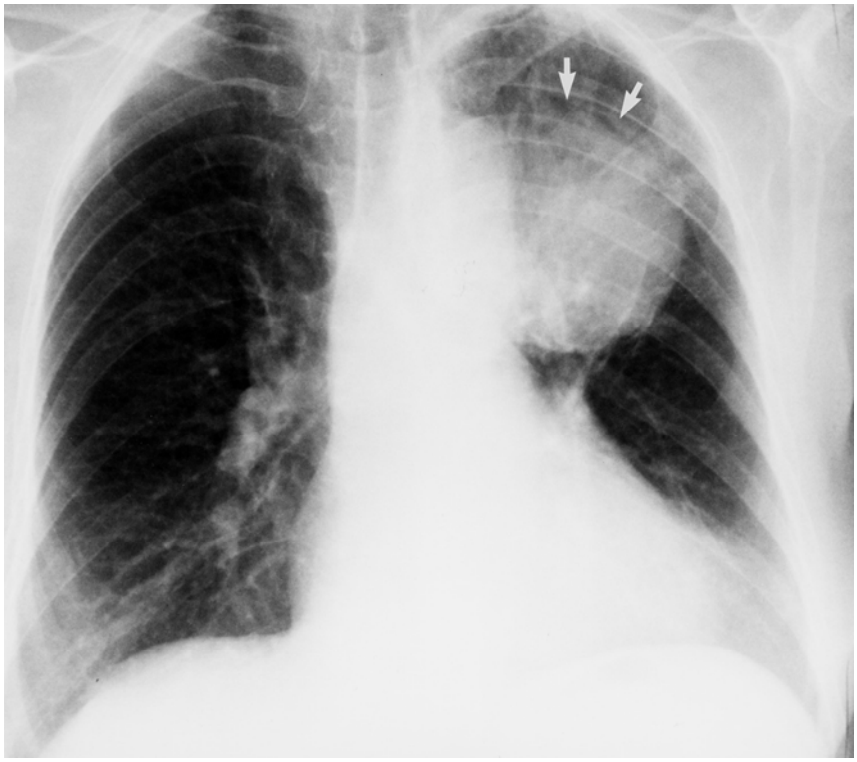


Fig. 2.6.
Radiograph of *Echinococcus granulosus* lung cyst (diameter: 6 cm)
Arrows indicate a small 'meniscus sign' suggesting the presence of a hydatid cyst
Reproduced from (120) with permission from Elsevier Science

- **Complicated cysts**

Complicated cysts may exhibit the following:

a) the 'air meniscus sign' caused by air entering the space between ecto- and endocyst producing a radioluscent shadow (= pneumocyst)

b) the 'double arch sign' caused by the ectocyst (outer arch) and the detached wall of the endocyst (inner arch); and

c) the 'water-lily sign' indicative of collapsed wavy endocyst membranes floating on top of the remaining cyst fluid.

Following rupture of the cyst, the endocyst may be ejected completely, leaving a cavity that may retract or become infected with bacteria. Radiography may also show lobar homogenous consolidation of lung parenchyma.

- **Other findings**

Chest X-ray images may also show upward displacement of the diaphragm, possibly indicative for a hydatid cyst of the liver; asymmetry of the heart outline, which may be a sign of a hydatid cyst of the heart to be confirmed by US or CT; pleurisy and pneumothorax in the event of rupture of a hydatid cyst into the pleura; a costal subpleural cyst.

- **Differential diagnosis**

Cysts filled with clear fluid, with an air shadow or with water-lily sign are pathognomonic. If a rounded parenchymatous opacity is seen, it is necessary to consider tuberculoma, a tumour or pulmonary sequestration. A fluid and air shadow will lead to consideration of a bacterial, fungal or amoebic abscess.

Plain abdomen radiography

In case of an abdominal cyst site, a fluid-type shadow may be seen, displacing the air-filled radiolucent areas of the digestive tube. The best indicator for a hydatid origin is the presence of calcifications, which may be crescent-shaped, or like homogenous or heterogenous globules, or ring-shaped. This examination should be supplemented by US or CT.

Bone radiography

Bone localisations of cysts are not common (<1% of CE cases). In about 50% of such cases, the site is the spine. At the initial stage, one or more lacunae are seen in the body or posterior arch of the vertebra. At a more advanced stage, an extension is seen to the adjacent vertebral bodies, with involvement of the neighbouring bones (ribs and iliac bone) (93).

2.2.3.3. Ultrasonography

General aspects

Abdominal US has overturned the hierarchy of diagnostic methods. It can be used not only to detect abdominal cysts and determine their number, site and dimensions (cyst >1 cm), but also to identify whether they are hydatid in nature and their relationships with other organs. Schemes for classification of *E. granulosus* cysts have been proposed by various authors, including Gharbi *et al.* (40), Caremani *et al.* (13), and Perdomo *et al.* (88, 89). Recently, an expert committee of the WHO Working Group on echinococcosis has presented a proposal for an internationally agreed classification of US images in hepatic CE (Table 2.5.).

- **Hepatic cysts**

The classification system proposed by the WHO Informal Working Group on Echinococcosis is presented in Table 2.5. For more details see the Working Group document (130).

Cysts in other abdominal sites

Cysts in other abdominal sites are less common and are located in spleen, kidney, uterus and other organs. Their images are essentially similar to those observed at hepatic sites.

Differential diagnosis

Differential diagnosis poses various problems. It is difficult to differentiate simple hydatid cysts (Table 2.5., Type CL) from simple hepatic cysts, renal, ovarian, mesenteric or pancreatic cysts, from a non-organised haematoma, amoebic liver abscess or necrotic tumour. In such cases, serological examination for specific *E. granulosus* antibodies may bring an important hint to verification or exclusion of CE. Type CE 4 cysts may be difficult to distinguish from abscesses, neoplasms, AE lesions, cavernous haemangiomas and other structures. On the other hand, cysts of Types 1, 2 and 3 are usually pathognomonic and can be diagnosed with a high degree of accuracy.

Pathognomonic features of *Echinococcus granulosus* cysts

The following US images of space occupying lesions in the liver are considered to be pathognomonic for cysts of *E. granulosus*:

a) unilocular anechoic lesions which are round or oval with a clearly visible cyst wall (laminated layer) with snowflake-like inclusions or floating laminated membranes

b) multivesicular or multiseptate cysts with a wheel-like appearance

c) unilocular cysts with daughter cysts with honeycomb appearance.

- **Pulmonary cysts**

For pulmonary sites, US examination is unhelpful in most cases, but it can sometimes confirm the cystic nature of a parenchymatous mass that is juxtaparietal. It will display an anechoic area with posterior strengthening.

- **Cardiac cysts**

In the cardiac site, two-dimensional US displays a mass that is echo-free or has a mixed echo structure.

- **Ultrasonography for field use**

Ultrasonography is the only imaging technique that can be used in the field. It has a number of characteristics that make it an excellent screening tool, as follows:

a) acceptability by the population

b) can explore the abdominal sites, which are most commonly infected

c) can evaluate a broad spectrum of the disease, i.e. number and location of the parasite, its stage (active, degenerating and inactive), some complications

d) can be performed by less qualified but easily trainable staff

e) it is easy to be performed in the field at low cost (Chapter 6.1.2.).

2.2.3.4. Computed tomography

General aspects

Computed tomography (CT) can detect small cysts (≥1 cm in diameter), it has the potential to inspect any organ, it allows the measurement of cysts and facilitates differential diagnosis of lesions caused by *Echinococcus* metacestodes from non-parasitic lesions (2, 3, 28, 83, 119, 120, 121). It also allows the determination of the liver volume from CT transverse sections by the point-integrating method (3).

On CT, round or spherical cysts with contents near water density are easily recognised. Measurement of the cyst density is a useful diagnostic parameter, particularly for follow-up examinations during and after chemotherapy. The information obtained from CT varies depending on the organ systems to be inspected.

In one study, CT findings alone allowed a correct diagnosis in 61% of 120 patients with CE of the liver, lung, kidney, spleen and some other sites, and in 94% if CT was combined with serology (28). In another study, CT provided a correct diagnosis in 96% of 157 patients with CE of the liver and other visceral organs (3).

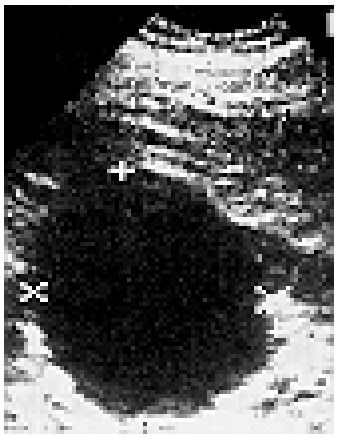
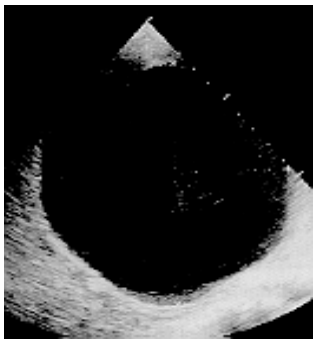
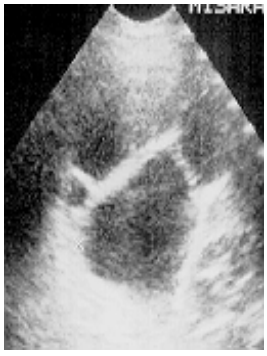
Hepatic cysts (Figs 2.7. and 2.8.)

Hepatic cysts can be diagnosed by US in the majority of cases, but CT is indicated when US diagnosis is uncertain, mainly in cysts of types CE 4 and CE 5 (Table 2.5.). Differential diagnosis of CL lesions is, however, not made easier by the use of CT. CT can detect small-sized cysts, study their content (univesicular or multivesicular), visualise membrane detachment, and provide information on the condition of the liver parenchyma and bile ducts. Pathognomonic images are membrane detachment and daughter cysts (spherical formations within a larger 'mother cyst' scattered or located at the periphery of the cyst). Completely calcified cysts that are difficult to explore by US can be studied by CT or X-ray, which usually reveal the typical 'egg-shell' pattern of calcification (1).

Pulmonary cysts

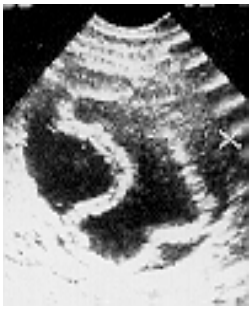
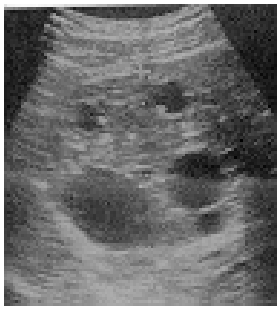
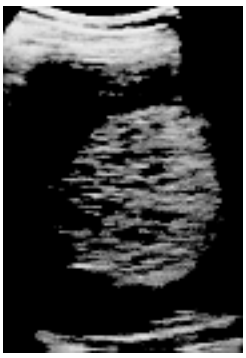
In the case of pulmonary sites, CT may add some additional information to plain X-ray examinations. It can confirm the liquid nature of a 'shadow' and visualise signs of the onset of complications, such as incipient membrane detachment or small bubbles located in the cyst wall.

Table 2.5.
Types of cystic lesions (CL) and *E. granulosus* cysts (CE) which may be found on ultrasound (US) examination of the liver *
Classification proposed by the WHO Informal Working Group on Echinococcosis (130)

Type of cyst	Imaging features and remarks (s): small; (m): medium; (l): large
Type CL 	• Status: active (if CE) • Unilocular, cystic lesion(s) (CL) with uniform anechoic content, not clearly delimited by an hyperechoic rim (=cyst wall not visible) • Normally round but may be oval • Size variable: but usually small. CL(s): <5.0 cm CL(m): 5-10 cm, CL(l): >10 cm **Remarks** If these cystic lesions are due to CE then these cysts are usually at an early stage of development and are not fertile US does not detect any pathognomonic signs Differential diagnosis of these cystic lesions require further diagnostic techniques
Type CE 1  	• Status: active • Unilocular, simple cyst with uniform anechoic content. Cyst may exhibit fine echoes due to shifting of brood capsules which is often called hydatid sand ('snowflake sign') • Cyst wall is visible • Normally round or oval • Size variable: Type CE1(s): <5.0 cm Type CE1(m): 5-10 cm, Type CE1(l): >10 cm **Remarks** Usually fertile Pathognomonic signs include visible cyst wall and snowflake sign
Type CE 2  	• Status: active • Multivesicular, multiseptated cysts; cysts septations produce 'wheel-like' structures, and presence of daughter cysts is indicated by 'rosette-like' or 'honeycomb-like' structures. Daughter cysts may partly or completely fill the unilocular mother cyst • Cyst wall normally visible • Normally round or oval • Size variable: Type CE2(s): <5.0 cm Type CE2(m): 5-10 cm, Type CE2(l): >10 cm **Remarks** Usually fertile US features are pathognomonic

* **Important note:** Schemes of classification should be used with caution because of great variability of cyst appearance, and in cyst recognition by different observers

Table 2.5. (contd)
Types of cystic lesions (CL) and *E. granulosus* cysts (CE) which may be found on ultrasound (US) examination of the liver *
Classification proposed by the WHO Informal Working Group on Echinococcosis (130)

Type of cyst	Imaging features and remarks (s): small; (m): medium; (l): large
Type CE 3  	• Status: transitional • Unilocular cyst which may contain daughter cysts • Anechoic content with detachment of laminated membrane from the cyst wall visible as floating membrane or as 'water-lily sign' which is indicative of wavy membranes floating on top of remaining cyst fluid • Cyst form may be less rounded due to decrease of intra-cystic fluid pressure • Size variable: Type CE3(s): <5.0 cm Type CE3(m): 5-10 cm, Type CE3(l): >10 cm **Remarks** Transitional stage: cyst is usually starting to degenerate but may sometimes also produce daughter cysts US features are pathognomonic
Type CE 4 	• Status: inactive • Heterogenous hypoechoic or hyperechoic degenerative contents. No daughter cysts • May show a 'ball of wool' sign which is indicative of degenerating membranes • Size variable: Type CE4(s): <5.0 cm Type CE4(m): 5-10 cm, Type CE4(l): >10 cm **Remarks** Most cysts of this type do not contain living protoscoleces US features are not pathognomonic and further diagnostic tests are required to ascertain a diagnosis
Type CE 5 	• Status: inactive • Cysts characterised by thick calcified wall which is arch-shaped, producing a cone shaped shadow. Degree of calcification varies from partial to complete • Size variable: Type CE5(s): <5.0 cm Type CE5(m): 5-10 cm, Type CE5(l): >10 cm **Remarks** The majority of cysts does not contain living protoscoleces Diagnosis is uncertain. Features are not pathognomonic but highly suggestive of *E. granulosus*

* **Important note:** Schemes of classification should be used with caution because of great variability of cyst appearance, and in cyst recognition by different observers

Brain cysts

Computed tomography is the principal method for the diagnosis of cerebral cysts (120, 121). It shows a spherical cyst with a thin wall, not enhanced after injection of contrast medium, without perilesional oedema displacing the adjacent structures.

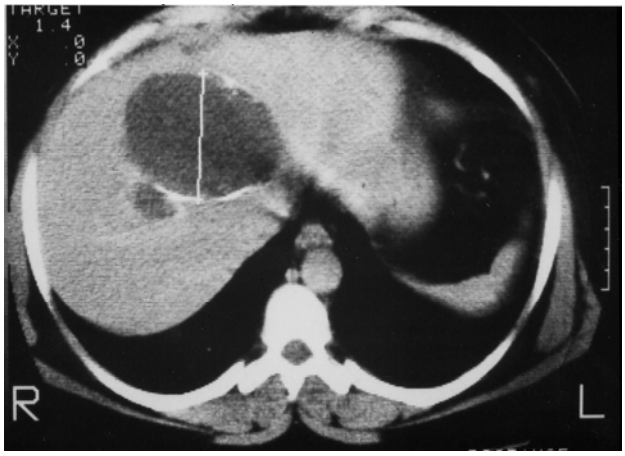


Fig. 2.7.
Computed tomography scan of a liver cyst of *Echinococcus granulosus* with partial wall calcification and a small bulging cyst
Photograph: courtesy of Professor R. Ammann, University Hospital, University of Zurich

Cysts in the other sites

Computed tomography is of little additional value at splenic and renal sites, except in doubtful cases, such as type CE 4 cysts (Table 2.5.). In the case of bone involvement, CT displays areas of osteolysis with localised bone expansion and fluid formations of cyst-like appearance developing in the soft tissue.

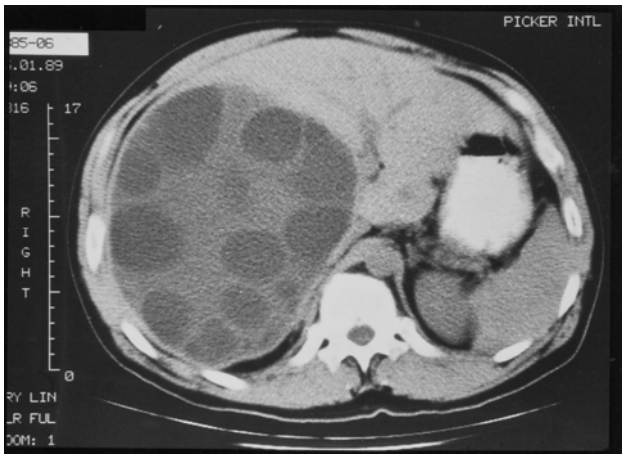


Fig. 2.8.
Computed tomography scan of the liver with a large *Echinococcus granulosus* cyst containing daughter cysts
Photograph: courtesy of Professor R. Ammann, University Hospital, University of Zurich

2.2.3.5. Other exploratory methods

Magnetic resonance imaging (MRI) is indicated only for certain sites, particularly the diagnosis of cerebral cysts (Fig. 2.9.). It supplies not much additional information for the pleuropulmonary and abdominal sites, but is useful in identifying changes of the intrahepatic and extrahepatic vascular system, due to intrinsic contrast of vascular structures (3, 120, 121).

Endoscopic retrograde (or percutaneous transhepatic) cholangiography (ERC) may be indicated in patients with cholestatic jaundice. This technique can be combined with therapeutic drainage procedures (2).

Angiography and scintigraphy have now been replaced by other imaging methods.

Intravenous urography may be useful in the case of renal cysts in order to assess the quality of the renal parenchyma, particularly if the excretory ducts are compressed.

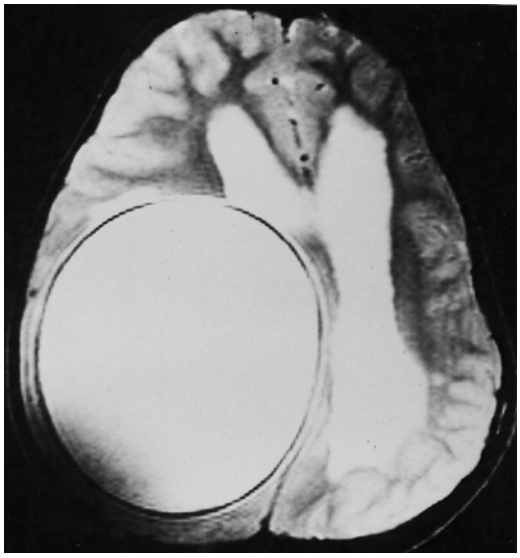


Fig. 2.9.
Magnetic resonance imaging of *Echinococcus granulosus* cyst in brain
Reproduced from (120) with permission from Elsevier Science

2.2.3.6. Diagnostic puncture

Traditionally the diagnostic puncture of *E. granulosus* cysts of the liver was discouraged, as it was regarded as carrying the risk of dangerous anaphylactic reactions or spillage of viable cyst material inducing secondary echinococcosis. Recently, some studies have shown that fine-needle puncture of cysts performed under US-guidance, by transhepatic routes and under anthelmintic cover can be regarded as a rather safe technique (107, 108, 122). Thus, ultrasound-guided fine-needle puncture has been used as a diagnostic procedure in doubtful cases, i.e. in absence of detectable anti-*Echinococcus* serum antibodies, with small lesions resembling simple hepatic cysts, and with lesions which cannot be distinguished from liver abscesses, neoplasms or other conditions by any of the non-invasive techniques (85, 107). Diagnostic puncture is the only technique which helps to diagnose pre-surgically sterile *E. granulosus* cysts by finding the specific antigen 5 in the aspirated hydatid fluid (82). However, the use of fine-needle biopsy is still controversial and it is definitely contraindicated, when diagnosis can be made by standard methods or when the risk of anaphylactic shock is high, i.e. in patients with a high level of total IgE antibodies and/or with allergy history, and patients with larger cysts superficially situated and/or under a high hydatid fluid pressure. In order to prevent secondary echinococcosis, chemotherapy with albendazole is recommended for four days before puncture and for at least one month after puncturing a lesion that was diagnosed as *E. granulosus* cyst (Chapter 2.2.4.3.). It has to be mentioned that puncture is now used as part of a new treatment procedure of CE (PAIR, Chapter 2.2.4.2.).

2.2.3.7. Laboratory findings

Haematology and blood chemistry

As a rule, the routine laboratory tests show non-specific results. The biochemical profile in patients with liver involvement may be normal or exhibit evidence of cholestasis with or without hyperbilirubinaemia and/or elevation of transaminases and/or gamma-glutamyl transferase (γ-GT). In patients with rupture of a cyst into the biliary tree, marked transient elevations of γ-GT and alkaline phosphatase concentrations may occur, often in association with hyperamylasaemia and eosinophilia (>500/µl). However, in most instances, eosinophilia is moderate (500/µl-1,000/µl) or absent. Hypergammaglobulinaemia is observed in about 30% of the CE cases. Marked eosinophilia usually occurs in cases of cyst rupture.

2.2.3.8. Immunodiagnosis

The current status of immunodiagnosis of human CE has been discussed in several review articles (17, 19, 51, 64, 105). A summary of practical aspects is presented in the following section. For determination of performance characteristics for immunodiagnostic assays see Annex 2.1.

2.2.3.8.1. Immunodiagnosis in individual patients

In the procedure for diagnosing human CE imaging methods for detecting space occupying lesions (US, CT, MRI, X-ray, etc.) are commonly the primary approaches. Immunodiagnostic procedures for serum antibody detection are used for the aetiological confirmation of imaging structures suggestive for CE or for diagnosis or differential diagnosis in cases of uncharacteristic imaging findings.

In clinical practice tests for detecting specific serum antibodies are of particular importance in the diagnosis of CE, whereas detection of circulating antigens is less relevant. Even if highly sensitive tests are used, such as the IgG-ELISA, antibodies may not be detectable in a certain proportion of patients with echinococcosis (false-negative results; see below). Cysts in the brain or eye and calcified cysts often induce low or no antibody titres. Antibody response may also be low in certain human population groups and in young children. False-positive results may also occur, especially in patients with other helminthic diseases.

The following approach can be used for immunodiagnosis of human CE:

Primary antibody test: test for serum antibody detection: IgG-ELISA with *E. granulosus* antigen or another adequate system (Table 2.6.).

Table 2.6.
Approaches for immunodiagnosis of cystic echinococcosis in humans

First step: Primary antibody test
Test for serum antibody detection: IgG-ELISA with *E. granulosus* antigen or another adequate system (Table 2.7.). A combination of two or more primary tests may increase sensitivity

Subsequent steps

↓	↓	↓
Seronegative samples	**Seronegative samples**	**Seropositive samples**
People without imaging structures or other signs suggestive for CE	**People with imaging structures suggestive for CE**	**People with or without imaging structures suggestive for CE**
No further serological follow-up or further steps of differential diagnosis	**Asymptomatic cases** Extended and/or advanced imaging and repeated serological examinations, including differential diagnosis for AE* 'Wait and observe' approach with repeated serological examinations **Symptomatic cases** Consideration of cyst puncture (Chapter 2.2.3.6.) Consideration of surgical intervention and/or chemotherapy without further serological examinations	**Asymptomatic and symptomatic cases** Secondary antibody test (Tables 2.8. and 2.9.) Arc 5 test IgG4-ELISA Immunoblot for antibodies reactive with subunits of *E. granulosus* antigens Serological differential diagnosis for AE (ELISA-Em2plus, immunoblot) (Chapter 2.3.3.4.)*

* differential diagnosis for AE and in certain cases (for example brain cyst) for cysticercosis may be necessary in patients from areas with endemic occurrence of these diseases

Primary tests for antibody detection

Of the serological tests for detecting anti-*Echinococcus* serum antibodies, the enzyme-linked immunosorbent assay (for detecting of IgG) (IgG-ELISA), the indirect hemagglutination antibody test (IHAT), and the latex agglutination test (LAT) are commonly used in laboratories (51); less frequently, the immunofluorescence antibody test (IFAT), immunoelectrophoresis (IEP) and some other tests are employed. In many countries, the materials, reagents and equipment to perform the IgG-ELISA are readily available, and this technique is probably the best overall choice for use in immunodiagnosis for human CE. However, there is still no standard, highly sensitive, and specific serological test for antibody detection in cases of human CE (17).

Therefore, for clinical practice, it should be noted that the results of serological tests depend on multiple factors, such as antigen quality, test system, organ site and number of hydatid cysts, individual variability of immune responses, etc. One example is presented in Table 2.7., which shows that in more than 20% of patients with hepatic cysts and more than 40% of patients with pulmonary cysts specific antibodies may not be detectable with some of the test systems. As shown in Table 2.7., the IgG-ELISA is one of the most sensitive tests presently available. The IFAT has a sensitivity similar to that of the ELISA-IgG. Because of the variable sensitivities of the various tests, many laboratories employ at least two different primary tests for routine diagnosis of CE which usually increases the sensitivity.

Table 2.7.
Sensitivities of various assays for antibody detection in patients with confirmed cystic echinococcosis*

Test	Organ sites of cysts and number of patients (N)			
	Liver (N: 41)	Lung (N: 79)	Liver and lung (N: 49)	Others (N: 7)
	Sensitivity (%)			
Latex agglutination (LA)	80	58	88	57
Indirect haemagglutination (IHA)	80	61	90	57
Immunoelectrophoresis (IEP)	68	51	71	50
IgG-ELISA	93	83	96	93

* of 165 patients 79 (48%) patients had one cyst and 86 (52%) had more than one cyst
Source: Orduna *et al.* (80)

Most of the routine laboratory test systems or commercialised test kits are based on crude or semi-purified preparations of *E. granulosus* antigens (i.e. hydatid fluid or protoscolex antigen for IFAT). The use of the two major hydatid cyst fluid antigens, antigen 5 (thermolabile) and antigen B (thermostable), is predominantly restricted to scientific applications, and these antigens are not generally available. Both antigens are lipoproteins which are composed of subunits. In antigen 5 subunits of 52 kDa-67 kDa have been identified under non-reducing conditions, while subunits of 20-24 and 38 kDa were detected under reducing conditions. Antigen B consists of 8-12, 16 and 24 kDa subunits detectable under both non-reducing and reducing conditions (27, 104). It has been shown that antigen B, purified from human hydatid cyst fluid by the method of Oriol *et al.* (81) exhibited a high sensitivity of 94% and a high specificity (excluding 60% cross-reactivity in AE cases) in the ELISA (17, 95). There are few reports on the use of antigen 5 in various types of ELISAs (17), and definite conclusions cannot be drawn. Antigen B is currently considered to be more specific to *E. granulosus* than antigen 5 (105). A recombinant antigen B had comparable diagnostic sensitivity and specificity compared to native antigen B (17). There is a need to provide purified native and recombinant antigens 5 and B in large quantities for further large-scale evaluation.

Secondary tests for antibody detection

Tests using crude *E. granulosus* antigens are reasonably sensitive (Table 2.7.) (51), but specificity is not always satisfactory. Specificity may be expressed as specificity 1 (Sp1) and specificity 2 (Sp2) indicating the percentage

of correct negative testing results in non-infected and in parasite-infected individuals, respectively (Annex 2.1.); both may be combined to overall specificity (Spo). In various studies, Sp1 in the IgG-ELISA was generally high at 96%-100%, while Sp 2 varied between 2% and 49% (49). Cross-reactivity (causing low Sp2) is especially high in cases of AE, PE, cysticercosis, fasciolosis, filariosis and other helminthic infections, whereas protozoan infections normally do not induce cross-reactions. Therefore, positive serological results should be confirmed by a more specific secondary test, except in cases in that imaging structures are clearly suggestive for CE.

In recent years, several secondary test systems have been used in specialised laboratories, such as the detection of a precipitation line designated as arc 5, the identification of IgG subclasses, and immunoblotting which demonstrates the reactivity of serum antibodies with subunits of *E. granulosus* antigens (17, 27, 54, 62, 63, 64, 91, 104, 105, 124). Generally, these tests are less sensitive, but more specific than primary test systems. Examples are presented in Tables 2.8. and 2.9.

Table 2.8.
Sensitivities of secondary tests for antibody detection in cases of human cystic echinococcosis (examples)

Antibody type detected (test system)	Number of CE cases tested	Percentage of sensitivity (= percentage of cases seropositive)	Ref.
Arc 5 (DD)	Not given	50-60	94
Arc 5 (DD and IEP with antigen 5)	166	78[a]	106
IgG4 (ELISA)	Symptomatic[b]: 58 Asymptomatic[c]: 133	71 31	102
IgG4 (IB with antigen B)	30	87	54
IgG4 (ELISA)	56	62	49
IgG1 (ELISA)	56	96	49
39 kDa (IB)[d]	65 166	94 90	17 106
10, 16, 20 kDa (IB)	65	57	17
16 kDa (IB)	166	46	106
16 kDa (IB with antigen B)	30	50	54
12 kDa (IB)	166	34	106
12 kDa (IB)	55	91	63
12 kDa (IB with antigen B)	30	80	54

DD : double diffusion
IB : immunoblot
IEP : immunoelectrophoresis
a) positive by one or both tests
b) clinical, hospitalised cases
c) asymptomatic cases diagnosed by ultrasound examination
d) reactivity of human sera to *E. granulosus* antigen subunits

Of the various secondary test systems, the arc 5 precipitation test has mostly a low sensitivity of 50%-60%, but it is taeniid specific, and this includes cross-reactivity in cases of AE and in approximately 15%-20% of cysticercosis cases (17). Detection of IgG4 is more sensitive, but can be low in asymptomatic cases of CE (Table 2.8.). Cross-reactivity occurs in cases of AE and in a low percentage of cysticercosis cases, but not in cases of schistosomosis, onchocercosis, and some other helminthic infections (Table 2.9.). Identification of specific IgE antibodies has a sensitivity of approximately 60%-80% and a Sp2 of 80%-100%. Immunoblotting for the detection of antibody reactivity with certain subunits of *E. granulosus* antigens, predominantly 39 kDa, 16 kDa, and 12 kDa subunits, is of diagnostic value as sensitivity and specificity are quite high (Tables 2.8. and

2.9.). However, cross-reactivity is not completely excluded. For example, cross-reactivity has been observed between the 12 kDa subunit and 40% of sera from AE patients and 5% of cysticercosis patients (73) (Table 2.9.). In some studies, a combination of subunit bands have been used for diagnosis of CE cases. Interpretation of immunoblots requires experience; therefore, such tests should be performed in specialised laboratories.

Table 2.9
Specificities of secondary tests for antibody detection using *Echinococcus granulosus* antigens

Antibody type detected (test system)	Percent specificity and number of cases tested (N)						
	Alveolar echinococcosis or polycystic echinococcosis	Cysticercosis	Schistosomosis	Onchocercosis /filariosis	Other helminthoses	Healthy controls or non-parasitic diseases	Ref.
IgG4 (ELISA)	–	95 (N: 38)	100 (N: 17)	100 (N: 28)	–	100 (N: 50)	102
IgG4 (ELISA)	48 (N: 54)	100 (N: 8)	100 (N: 8)	100 (N: 8)	100 (N: 32)[a]	99 (N: 253)	49
39 kDa (IB)	–	–	100 (N: 15)	–	100 (N: 7)[b]	100 (N: 20)[c]	106
16 kDa (IB)	–	–	100 (N: 15)	–	100 (N: 7)[b]	100 (N: 20)[c]	106
12 kDa (IB)	60 (N: 60)	95 (N: 55)	100 (N: 3)	–	–	100 (N: 15)	63

IB : immunoblot
a) each eight cases of fasciolosis, strongyloidosis, toxocarosis and trichinellosis
b) cases of trichinellosis
c) non-parasitic diseases

Antibody response and assessment of treatment

Antibody assays for IgG generally have poor value in assessment of the results of surgery or chemotherapy. Analysis of IgG subclasses may better reflect qualitative changes in serum parameters after surgery or chemotherapy (17). However, there are neither conclusive results nor reproducible tests system which could be generally recommended (17).

Antibody response and puncture – aspiration – injection – reaspiration (PAIR)

Antibody detection in serum samples is also used for confirmation of the ultrasound diagnosis during the PAIR procedure (35) (Chapter 2.2.4.2.). A new test, the hydatid antigen dot immunobinding assay (HA-DIA), was developed which allows a quick diagnosis and is particularly suited for application in medical units where laboratory facilities are not readily available (72). So far, follow-up of PAIR is based on ultrasound or other imaging techniques. Apparently, long-term observations on the course of antibody titres after PAIR have not yet been published.

Detection of circulating antigens

Detection of circulating *E. granulosus* antigens in serum samples is less sensitive than antibody detection and therefore, it is not recommended for routine purposes. The sensitivity of antigen detection was only 43% in 116 patients with confirmed CE (17).

Detection of antigens in cyst fluid

Putative hydatid cyst fluid samples obtained by puncture or after surgical intervention can be tested for the presence or absence of *Echinococcus* antigen through binding of enzyme-labelled anti-*Echinococcus* (hydatid cyst

fluid) antibodies in an ELISA (18). The sensitivity of this test was 100% in nine proven human hydatid cyst fluids (18). In a recent Polish study, an ELISA with a monoclonal antibody against antigen 5 (Ag5) was used for the same purpose (82). In all fluids of fertile liver cysts obtained by puncture from 6 CE patients Ag5 was detected, and also in the cyst fluids of 9 out of 81 patients harbouring sterile cysts. These data indicate that detection of Ag5 may be useful in confirmation of the *Echinococcus* nature of the fluid.

2.2.3.8.2. Immunodiagnosis of cystic echinococcosis in human populations

Mass-screening programmes for human CE have been conducted using serological tests in a number of endemic countries including Argentina, China, Israel, Kenya, Tunisia, Uruguay and others. To date, three approaches exist for mass-screening of human populations for CE (17, 19), as follows:

a) application of a sensitive serological test (for example ELISA) to blood samples from the target population as a primary test and follow-up of all seropositives by ultrasound screening and, if possible, by X-ray examination for CE of the lung

b) application of ultrasound screening to the population using portable units as a primary test and use of serology to confirm image positives

c) combination of approaches (*a*) and (*b*).

Ethical rules have to be followed in all mass-screening programmes (Chapter 2.5.).

In approach (*a*), the test should be highly sensitive and specific. Before seropositive individuals are examined clinically, they should be tested by a secondary serological test. The relative low specificity of most of the primary test systems (see above) can lead to a rather high number of false-positive reactors. For example, in a recent study in Libya, the population of a village was screened for CE using portable ultrasound equipment and IgG-ELISA for serum antibody detection (103). Abdominal CE was detected in 4.5% of 485 individuals, but 13.2% were seropositive. Part of the seropositives could have been attributed to extra-abdominal CE or to abortive *Echinococcus* infections, but a relatively high proportion had to be classified as false-positive. In a hypothetical mass-screening programme carried out under the same conditions with 40,000 individuals, 5,280 seropositives have to be expected and would need serological and/or clinical follow-up.

Using the IgG-ELISA or a similar test, the probability to obtain a correct positive result is relatively low (positive predictive value). This is illustrated by a hypothetical example published by Craig (17) in which an immunodiagnostic test with 70% sensitivity and 90% specificity was applied to a population with a CE prevalence of 2% (Table 2.10.). In this case, the positive predictive value is only 12.5%, whereas the negative predictive values is high.

Therefore, approach (*b*) might be more appropriate with application of serology as secondary test for confirmation of positive images.

Table 2.10.
Hypothetical data for predictive value calculation with a 2% cystic echinococcosis prevalence and an immunodiagnostic test with 70% sensitivity and 90% specificity (17)

Result of serological test	CE present	CE absent	Total number of cases
Positive	14	98	112
Negative	6	882	888
Total	20	980	1,000
Positive predictive value: 12.5%	Calculation: $14/(14 + 98) = 14/112 = 0.125 \times 100 = 12.5\%$*		
Negative predictive value: 99.3%	Calculation: $882/888 = 0.993 \times 100 = 99.3\%$*		

* for details for calculating predictive values, see Annex 2.1.

A combination of approaches (*a*) and (*b*) has been suggested, in which venous blood samples (3 ml-5 ml) be taken from ultrasound positive cases or cases with images suggestive for CE and finger (or ear) prick blood samples be collected onto filter paper from every individual examined. Filter paper blood samples are reliable for CE antibody testing if stored at –20°C. The sensitivity of ultrasound scanning of the liver for space occupying lesions (with minimum resolution around 1 cm-2 cm) is high (approximately 70%), but lower (approximately 30%) if only CE characteristic lesions are included. A small number of strongly seropositive, but ultrasound negative (and X-ray negative) will be identified. These individuals should be followed-up at 12-24 months intervals by clinical examination, preferably including CT examination.

It should be noted that the general level of antibody seroreactivity (IgG), and therefore, test sensitivity, is likely to be lower in CE cases identified by US from an endemic community. This is because most cases will be asymptomatic and with greater probability of presentation either with early pathology, i.e. small, unilocular, vesicular cysts, or with calcified cysts, that are known to be less seroreactive (19).

2.2.4. Treatment

The following chapter refers mainly to special guidelines published by the WHO Working Group on Echinococcosis (129).

General considerations

Currently, surgery is still the treatment that has the potential to remove *E. granulosus* cysts and lead to complete cure (129). It can be performed successfully in up to 90% of patients if a cyst does not have a risky localisation or if the disease is not too far advanced. However, surgery may be impractical in patients with multiple cysts localised in several organs and if surgical facilities are inadequate. The introduction of chemotherapy and of the PAIR technique (puncture – aspiration – injection – respiration) offers an alternative treatment, especially in inoperable patients and for cases with a high surgical risk (129). Cysts with homogeneously calcified cyst walls need probably no surgery, but only a 'wait and observe' approach (86, 89). The choice of an optimal treatment should be carefully assessed in each case.

Treatment options for CE are as follows (85, 86, 89, 129):

- surgery
- PAIR
- chemotherapy
- 'wait and observe' approach.

2.2.4.1. Surgery

Indications

Surgery is indicated for large liver cysts with multiple daughter cysts; single liver cysts, situated superficially that may rupture spontaneously or as a result of trauma; cysts that are infected; cysts communicating with biliary tree and/or exerting pressure on adjacent vital organs; cysts in the lung, brain and kidney, bones and other organs.

Contraindications

Surgery of CE is contraindicated as defined for surgical procedures in general, i.e. patients refusing surgery, patients at the extremes of age, pregnant women, patients with concomitant severe diseases (i.e. cardiac, renal or hepatic diseases, diabetes and hypertension). In addition, surgery is contraindicated in patients with multiple cysts or cysts difficult to access, dead cysts either partly or totally calcified, and in patients with very small cysts.

Choice of surgical technique

Surgical procedures include several main options that are summarised in Table 2.11. and described in more detail by Morris and Richards (74).

Table 2.11.
Surgical techniques for cystic echinococcosis (CE) of the liver and lung (74, 129)

Surgical techniques for CE of the liver	Surgical techniques for CE of the lung
Partial hepatectomy	Lobectomy
Pericystectomy	Extrusion of cysts (Barrett's technique)
Open cystectomy with or without omentoplasty	Pericystectomy
Palliative surgery (tube drainage of infected cysts)	

Usually, the more radical the intervention, the higher the intraoperative risk but the lower likelihood of relapses, and vice versa. With the inclusion of chemotherapy before surgery the aggressive surgical procedures are less commonly performed (see below).

Use of protoscolicides

The use of protoscolicidal substances for intraoperative killing of protoscoleces is questionable, as there is no ideal agent that is both effective and safe (129). The lethal action observed *in vitro* may be hampered *in vivo* by instability of the substance used (e.g. hydrogen peroxide), or by an unpredictable dilution by hydatid fluid, and difficulties in penetrating daughter cysts. Potential communication between the hydatid cyst and the biliary tree substantially increases the safety requirements for using protoscolicides, which can cause chemical cholangitis leading to frequently fatal subsequent sclerosing cholangitis. Therefore, formalin should never be used.

The following protoscolicides, which appear to be effective, have a relatively low risk of toxicity: 70%-95% ethanol (both protoscoleces and germinal layer of the cyst are damaged), 15%-20% hypertonic saline solution, and 0.5% cetrimide solution. For optimal efficacy, the substances have to be left in the cyst cavity for at least 15 min (129). More experimental studies and clinical observations are urgently needed in evaluating the efficacy and safety of protoscolicides.

Peri-interventional chemotherapy

Preoperative treatment with benzimidazoles has been reported to soften the cysts and to reduce intracystic pressure, enabling the surgeon to remove the endocyst more easily. However, neither the required duration of such treatment, nor its efficacy has been adequately determined. There are hints from several studies that postoperative treatment of patients can reduce the rate of recurrences (2). In rodents, the number of *E. granulosus* cysts developing from intraperitoneally inoculated protoscoleces could be reduced by 80%-90% if albendazole treatment (10 mg/kg body weight [bw] per day) for a duration of 1 week was initiated immediately after inoculation; when treatment was delayed for 15 days, it was ineffective (75). Based on these hints, it is recommended for cases in which spillage of protoscoleces may have occurred during surgery to initiate postoperative chemotherapy with albendazole (ABZ) or mebendazole (MBZ) (for dosages, see below) immediately after operation for at least 1 month (ABZ) or 3 months (MBZ).

Benefits

Radical surgery has the potential to cure completely the patient, but involves some perioperative risks.

Risks

The risks include those associated with any surgical intervention (anaesthesia, stress, infections including those transmitted by blood transfusion e.g. viral hepatitis, HIV). Despite progress in surgical techniques, secondary echinococcosis owing to spillage of viable parasite material during the intervention may occur. The prevalence of long-term recurrence is in the range of 2% to 25% (3). In a Chinese series (1950-1990), with 15,289 surgical cases, 92% of the patients had one operation, 7% two, 0.8% three and 0.2% four to eight operations (70). Recurrence can be due to incomplete cyst removal or to previously undetected cysts. Anaphylactic reactions represent a further risk on rare occasions. Postoperative fatality is about 2% or less, but may be higher in the second or further operations or if medical facilities are inadequate.

2.2.4.2. Puncture, aspiration, injection, reaspiration (PAIR)

General considerations and technique

Ultrasound-guided cyst puncture for treatment of CE was introduced in the mid-1980s (9, 35, 39) and includes the following steps:

- percutaneus puncture of cysts under ultrasonic guidance
- aspiration of a substantial amount of cyst fluid
- injection of protoscolicidal substance (preferably 95% ethanol)
- re-aspiration of the fluid cyst content after 15 min to 20 min.

Favourable results have been reported from PAIR interventions in several hundred patients with the follow-up periods of up to 5 years (35, 87, 129). However, the efficacy and potential risks have not yet been fully evaluated and require further properly controlled long-term studies. The PAIR should be accompanied by a chemotherapeutic coverage to minimise risks of secondary echinococcosis (see below).

This minimal-invasive technique should be reserved for use by skilled and well experienced physicians and with a surgical and intensive care back-up team well prepared to deal immediately with complications. Ultrasound-guided transhepatic puncture is the essential technique. The WHO scheme for US-classification of *E. granulosus* cysts (Table 2.5.) can be used as a rough guideline for judging their suitability for PAIR procedure. It is essential that aspirates of liver cysts are analysed immediately for traces of bilirubin and protoscoleces or hooks. PAIR should only be performed under chemotherapeutic coverage, except in early pregnant patients (35).

Indications

PAIR is indicated for inoperable patients with CE (see contraindications for surgery) and those who refuse surgery. It has been used in treatment of cysts in the liver, the abdominal cavity, spleen, kidney and bones, but it should not be used for lung cysts (129). Various types of liver cysts CL, CE 1, CE 2 and CE 3 may be selected for PAIR (Table 2.5.); especially anechoic lesions >5 cm in diameter; cysts with a regular double laminated layer; cysts of >5 cm diameter with multiple septal divisions; multiple cysts (>5 cm in diameter) in different liver segments. PAIR can also be used in cases of a relapse after surgery or in failure to respond to chemotherapy.

Experience using PAIR in pregnant women and children aged <3 years is still limited. The application of PAIR might be indicated in pregnant women with symptomatic CE, but the potential risk associated with peri-interventional chemotherapy (see below) has to be carefully assessed since the benzimidazoles are contraindicated during pregnancy, especially during the first 3 months.

Contraindications

PAIR is contraindicated for inaccessible or superficially located cysts in the liver (for the latter, there is a risk of spillage of cyst content into the abdominal cavity); for cysts with multiple septal divisions (honeycomb-like cysts); for cysts with hyperechogenic solid patterns or calcified cysts; cysts communicating with bile ducts, and cysts in the lung. In order to avoid the induction of chemical cholangitis, aspirates from liver cysts should be

analysed for traces of bilirubin prior to injection of protoscolicides. Contamination of cyst content with bilirubin indicates that there is a communication with biliary ducts.

Peri-interventional chemotherapy

Peri-interventional treatment with benzimidazoles is highly recommended for four days before PAIR and at least for 1 month (albendazole) or 3 months (mebendazole) thereafter (61, 129). The duration of chemotherapy should be adapted according to the cyst size and US appearance (35).

Benefits

PAIR is minimally invasive and less risky than surgery. It confirms the diagnosis and removes large numbers of protoscoleces and antigens with the aspirated cyst fluid. The costs of PAIR with concomitant chemotherapy is less than that of surgery. Fewer days of hospitalisation are needed (35). For example, in a series of 33 PAIR-treated patients in Argentina the mean hospitalisation time was 1.8 days (range: 0-15 days) (87).

Risks

Risks include those associated with any puncture (haemorrhage, mechanical damage of other tissues and infections); anaphylactic shock or allergic reaction caused by leakage of cyst fluid and secondary echinococcosis due to spillage. Transhepatic puncture is strongly advised, since puncture of superficially located cysts involves a higher risk of spillage. Other potential risks or failures are chemical sclerosing cholangitis, sudden intracystic decompression leading to biliary fistulas, and persistence of satellite daughter cysts.

2.2.4.3. Chemotherapy

General considerations

Over 2,000 well documented cases of CE have been treated with benzimidazoles, to date (2, 3, 22, 25, 41, 53, 110, 111, 112, 113, 119, 126, 129). When evaluated up to 12 months after initiation of chemotherapy, 10% to 30% of patients show cyst disappearance (cure), 50%-70% show degeneration of cysts and/or significant size reductions (improvement) (Fig. 2.10.), but 20%-30% exhibit no morphological changes in cysts (i.e., failure). Chemotherapy is apparently more effective among young rather than older patients. Small cysts that have thin walls without infection or communication, as well as secondary cysts (even when multiple) are most susceptible to chemotherapy. Chemotherapy may, however, be less effective for thin-walled daughter cysts within a mother cyst. Some of the treated patients exhibit relapses, but these are usually sensitive to retreatment in a high proportion of cases (up to 90%). The rate of relapses after chemotherapy is relatively high (14%-25%) (53, 111).

Indications

Chemotherapy is indicated for inoperable patients with primary liver echinococcosis and for patients with multiple cysts in two or more organs. Cysts localised in bones are less susceptible to chemotherapy. Since radial surgery is often impossible (e.g. cyst localisation in spine or pelvis), long-term chemotherapy may be needed. Another important indication for chemotherapy is the prevention of secondary echinococcosis. The pre-surgical use of benzimidazoles (ABZ or MBZ) may reduce the risk of recurrence of CE and/or facilitate the operation by reduction of intracystic pressure, but this is not well documented. Peri-interventional chemotherapy is also recommended for PAIR (Chapter 2.2.4.2.).

Contraindications

Chemotherapy is contraindicated for large cysts with a risk of rupture (notably superficially situated, infected cysts) or for inactive or calcified cysts. Patients with severe chronic hepatic diseases and with bone marrow depression should not be treated. Early pregnancy is a contraindication. Chemotherapy during later stages of pregnancy might better be postponed until after delivery.

Choice of drugs (see also alveolar echinococcosis and Annex 2.2.)

Two benzimidazoles have been extensively evaluated using animal models and used on over 2,000 patients:

- Albendazole (ABZ) (Eskazole®, Zentel®; 400 mg tablets and 4% suspension, SmithKline Beecham, England)
- Mebendazole (MBZ) (Vermox®; 500 mg tablets, Janssen Pharmaceutica, Belgium).

These drugs show definite efficacy against CE, and are generally well tolerated. Studies with different groups of CE patients, summarised by Horton (53), have shown that 48% of 665 cysts disappeared, and further 24% improved after chemotherapy with ABZ, compared to 28% of 516 cysts disappeared and 30% improved after treatment with MBZ. MBZ is apparently more effective against cysts in the lungs than in the liver, whereas such a difference was not observed for ABZ. Exact comparative efficacy of the drug is difficult to assess, as treatment protocols were variable in the different groups of patients.

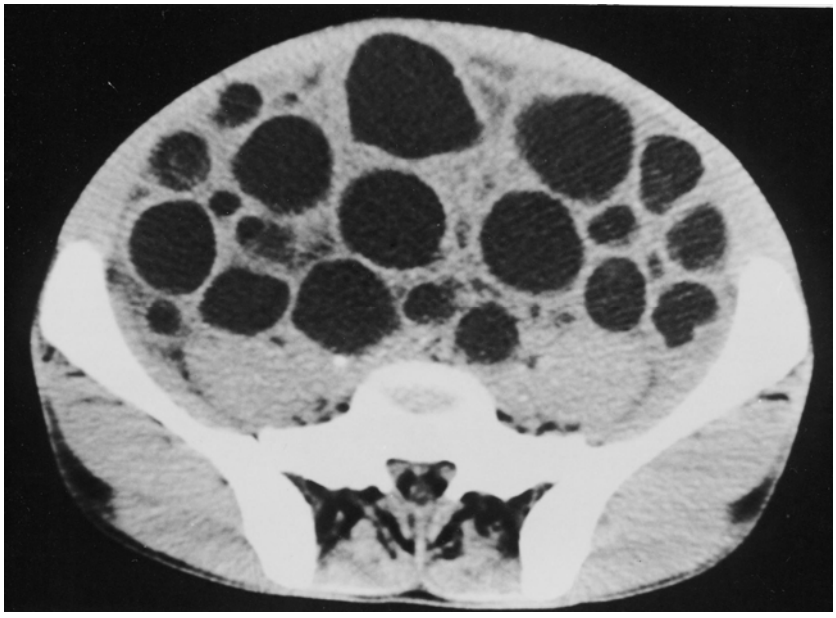


Fig. 2.10.a
Computed tomography scan of the pelvis of a patient with disseminated *Echinococcus granulosus* cysts: before treatment
Photograph: courtesy of Professor W. von Sinner, King Faisal Specialist Hospital and Research Centre, Riyadh

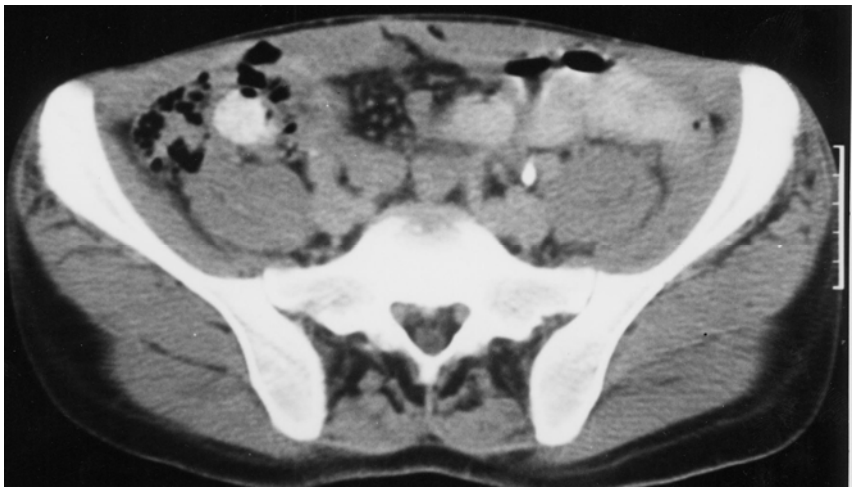


Fig. 2.10.b
Computed tomography scan of the pelvis of a patient with disseminated *Echinococcus granulosus* cysts: after 3 months of albendazole treatment
Photograph: courtesy of Professor W. von Sinner, King Faisal Specialist Hospital and Research Centre, Riyadh

For treatment of CE the following oral dosages are recommended:

- Albendazole: 10 mg/kg-15 mg/kg bw per day in two divided doses postprandially. In practice, adults receive 800 mg/day in two single doses of 400 mg each (53). The division of the daily dose is supported by pharmacokinetic data (58).

Cyclic treatment with intervals of 14 days was originally recommended by the manufacturer, and 3- to more than 6-monthly courses have been regarded as necessary for treating patients with single or multiple cysts (53, 126). However, recent data have shown equal or improved efficacy of continuous treatment for 3 to 6 months or longer without an increase of adverse effects (36, 65). In a recent comparative study, this type of treatment was more effective than chemotherapy with mebendazole (36). Therefore, cyclical albendazole treatment seems to be no longer advisable.

- Mebendazole: the usual oral dosage of mebendazole is 40 mg/kg-50 mg/kg bw per day in three divided doses for at least 3-6 months.

In animal experiments, it has been shown that efficacy of mebendazole against *Echinococcus* metacestodes was positively correlated with drug concentration in the serum and duration of treatment (31). In human patients, serum drug levels of MBZ and ABZ may vary widely in individual patients, and correlation with oral doses and drug efficacy is inconsistent. Drug dosing in conjunction with a fatty meal improves intestinal absorption of benzimidazoles (3, 53).

The use of praziquantel (PZQ) (Biltricide®, Bayer, Germany), a heterocyclic pyrazinoisoquinoline derivative, has been proposed at a dose of 40 mg/kg bw once a week concomitantly with benzimidazoles. The PZQ might also be useful in cases of cyst content spillage during surgery. A recent study has shown that a combined treatment with albendazole (10 mg/kg/day) and praziquantel (25 mg/kg/day) given during the month prior to surgery increased the number of patients with nonviable protoscoleces as compared to monotherapy with albendazole (16). However, further studies are needed for evaluating the efficacy of the combined treatment. According to the manufacturer, the plasma levels of albendazole metabolites (sulphoxide) are increased 4.5 times if praziquantel is given simultaneously, and this may increase the rate of side effects (133).

Benefits

Chemotherapy is a non-invasive treatment that can be used on patients of any age, although there is little experience with children under 6 years old, and is less limited by the patient's status (except pregnancy) than surgery.

Risks

The adverse effects of benzimidazoles include neutropaenia, proteinuria, mild hepatotoxicity (transient increase of aminotransferases), gastrointestinal disturbances and transient alopecia (Annex 2.2.). The potential risks of benzimidazoles include embryotoxicity and teratogenicity which, however, have only been observed in some laboratory animals during the early stages of pregnancy. For special precautions see Annex 2.2.

Medical requirements

Hospitalisation is usually not necessary, but regular follow-up examinations are required. Costs of anthelmintics and repeated medical examinations may be considerable.

Monitoring of patients

Medical and laboratory examinations for adverse reactions are necessary initially every 2 weeks then monthly (129). Leukocyte counts should be checked at 2-week intervals during the first 3 months because in rare instances severe and not always reversible leukopaenia has been observed in early phases of chemotherapy. Serum drug concentrations (ABZ-sulfoxide or MBZ parent compound) should be monitored after 2 and 4 weeks of chemotherapy, respectively, in order to identify levels too high (possibly toxic) or too low (ineffective). For MBZ, it has been recommended to determine serum or plasma levels 4 h after the morning dose. Oral drug doses can be adapted to individual patients in order to achieve adequate serum levels (Annex 2.2.), but such attempts are not always effective. Unfortunately, only few laboratories have the capability to measure ABZ-sulfoxide or MBZ serum drug levels (see also section on AE). Follow-up examinations, including imaging if needed, should be carried out at intervals of about 3 to 6 months for 1 to 3 years after termination of chemotherapy because of the relatively high rate of relapses.

2.3. Alveolar echinococcosis

For detailed information, the reader is referred to several recent monographs or reviews (2, 3, 21, 32, 45, 114, 115, 123, 129).

2.3.1. Causative agent and course of infection

Causative agent

Alveolar echinococcosis is an infection caused by the metacestode stage of *E. multilocularis*, which is characterised by a tumour-like, infiltrative and destructive growth with the potential to induce serious disease with a high fatality rate.

Course of infection

After oral infection with eggs of *E. multilocularis,* metacestodes develop primarily almost exclusively in the liver. This can be concluded from findings in patients with single organ involvement (Table 2.12.). Parasitic lesions in the liver can vary from small foci of a few millimetres in size to large areas of infiltration (15 cm-20 cm in diameter). Primary extrahepatic localisations of the *E. multilocularis* metacestodes are extremely rare. From the liver, the metacestode tends to spread to both the adjacent and distant organs by infiltration or metastasis formation (Table 2.12.). Metastasis formation is due to spreading of germinal cells via lymph or blood vessels (32, 69).

Cases of AE are characterised by an initial asymptomatic incubation period of 5 to 15 years duration and a subsequent chronic course. The fatality rate in untreated or inadequately treated persons is high. In a series of 66 individuals with AE from Germany (period 1960-1972), 70% died within 5 years and 94% within 10 years after diagnosis of the disease (2, 3). According to data from Alaska, in 21 untreated persons, the average survival time after diagnosis was 5.3 years, and all patients died within 14 years (131). However, data obtained from more recent series (diagnosis after 1983), show an improvement of the survival rate which may depend on early diagnosis and other factors.

Until recently, it was believed that the metacestode of *E. multilocularis* usually retains an unlimited proliferative capacity until the death of the patient. However, under the influence of the host's defence mechanisms, the metacestode can degenerate, calcify, and finally die. Therefore, spontaneous cure of AE is possible, but the frequency of such an event is unknown (92).

Table 2.12.
Organ sites of *Echinococcus multilocularis* metacestodes in patients (3, 99)

Single organ involvement* N = 199		Single and multiple organ involvement N = 152	
Organ	**Percentage of cases**	**Organ**	**Percentage of cases**

Liver only	99.0	Liver only	88.7
Skin or muscle only	0.5	Liver and lungs	8.5
Bones only	0.5	Liver and spleen	1.4
		Liver and brain	0.7
		Liver, lungs, brain	0.7

* single organ involvement is indicative for metacestode development after primary infection

2.3.2. Clinical presentation

Age and sex of patients

The age at the time of diagnosis of AE is significantly higher than for CE. In Europe, the peak age group is 50-70 years, range 10-89 years; in Japan, 40-60 years and 7-81 years respectively. The sex distribution of AE is about equal.

Organ sites of metacestodes

The primary site of metacestode development is almost exclusively in the liver (Table 2.12.). The right lobe is predominantly infected, but the liver hilus together with one or two lobes may also be involved. Extrahepatic primarily locations are rare. During the infection, secondary echinococcosis (= metastasis formation) may occur in variety of adjacent or distant organs (Table 2.12.).

Symptoms

Symptoms of AE are primarily cholestatic jaundice (about a third of the cases) and/or epigastric pain (about a third of the cases). In the remaining third of patients, AE is detected incidentally during medical examination for symptoms such as fatigue, weight loss, hepatomegaly, or abnormal routine laboratory findings (3, 123).

Classification and staging of alveolar echinococcosis cases

The European Network for Concerted Surveillance of Alveolar Echinococcosis has recently proposed a classification system for human cases of AE which should:

a) aid the clinician in planning of treatment
b) give some indications for prognosis
c) assist in evaluating the results of treatment
d) facilitate the exchange of information between treatment centres
e) contribute to continued investigation of AE.

The system, denominated as PNM, can be used for describing the anatomical extent of AE and is based on the assessment and ranking of three components at the time of diagnosis (Table 2.13.).

The PNM system is used for staging of AE cases as shown in Table 2.14.

2.3.3. Diagnosis

The diagnosis of AE is based on similar findings and criteria as in CE (2, 3, 129).

Diagnosis of AE in individual patients:

- case history, including epidemiological hints
- clinical findings
- morphological lesions detected by imaging techniques
- immunodiagnostic tests.

2.3.3.1. Imaging

General aspects

This subject has been discussed in various publications (2, 3, 38, 60, 71, 78, 79, 114, 115). In the majority of patients with AE, the liver is involved as the primary focus of metacestode development. Lesions caused by the parasite in the liver can be best visualised by the US and CT techniques. Some cases may benefit from the use of other imaging techniques, such as magnetic resonance imaging (MRI), angiography (AG) cholangiography (CAG), endoscopic retrograde cholangiography (ERC), percutaneous transhepatic cholangiography (PTC) or MRI-cholangiography (MRIC).

Table 2.13.
PNM system for classification of human alveolar echinococcosis

Classification of findings
P: Hepatic localisation of the parasite
PX: Primary lesion cannot be assessed
P0: No detectable lesion in the liver
P1: Peripheral lesions without proximal vascular and/or biliar involvement
P2: Central lesions with proximal vascular and/or biliar involvement of one lobe[a]
P3: Central lesions with hilar vascular and biliar involvement of both lobes and/or with involvement of two hepatic veins
P4: Any liver lesion with extension along the vessels[b] and the biliary tree
N: Extrahepatic involvement of neighbouring organs
Diaphragm, lung, pleura, pericardium, heart, gastric and duodenal wall, adrenal glands, peritoneum, retroperitoneum, parietal wall (muscles, skin, bone), pancreas, regional lymph nodes, liver ligaments, kidney
NX: Not evaluable
N0: No regional involvement (see above)
N1: Regional involvement of contiguous organs or tissues
M: Absence or presence of distant metastases
Lung, distant lymph nodes, spleen, CNS, orbital, bone, skin, muscle, distant peritoneum and retroperitoneum]
MX: Not completely evaluated
M0: No metastasis[c]
M1: Metastasis

a) for classification, the plane projecting between the bed of the gallbladder and the inferior vena cava divides the liver in two lobes
b) vessels means inferior vena cava, portal vein and arteries
c) chest X-ray and cerebral CT negative

Source: European Network for Concerted Surveillance of Alveolar Echinococcosis: PNM system for the classification of human cases of alveolar echinococcosis
Data kindly provided by Professor P. Kern, Ulm

Hepatic lesions

Ultrasonography and computed tomography

In AE, the liver is usually enlarged. In the US and CT, lesions are characterised by heterogenous hypodense masses, often associated with necrotic cavities. The lesion contours are irregular and there is lack of a well-defined wall (Fig. 2.11.). Calcifications are often found and exhibit a typical pattern in regard to shape and distribution: clusters of microcalcifications or irregular plaque-like calcified foci are located in the central or peripheral parts of the lesions.

There may be discrepancies between US and CT patterns, since the two methods yield identical results in only 42% of the cases (78). Hyperechoic haemangioma-like nodules could represent early forms of AE lesions. Quite frequently an extension of the lesions beyond the liver is found toward diaphragm, lungs, pericardium, retroperitoneum, hepatoduodenal ligament and pancreas.

Table 2.14.
Staging of alveolar echinococcosis cases based on PNM classification

Stage of alveolar echinococcosis	PNM classification		
Stage I	P1	N0	M0
Stage II	P2	N0	M0
Stage IIIa	P3	N0	M0
Stage IIIb	P1-3	N1	M0
	P4	N0	M0
Stage IV	P4	N1	M0
	Any P	Any N	M1

Source: European Network for Concerted Surveillance of Alveolar Echinococcosis: PNM system for the classification of human cases of alveolar echinococcosis

Magnetic resonance imaging

Compression or obstruction of inferior vena cava, the hepatic veins or the portal branches (with splenomegaly) may be observed. Pathological changes of the intrahepatic and extrahepatic venous system and of adjacent organs are best visualised by MR imaging. However, calcified lesions are not easily detected. Pathognomonic aspects are represented by multicystic honeycomb-like images. In recent years, angiography has been much less frequently performed, because non-invasive methods, such as CT and MR imaging have become available.

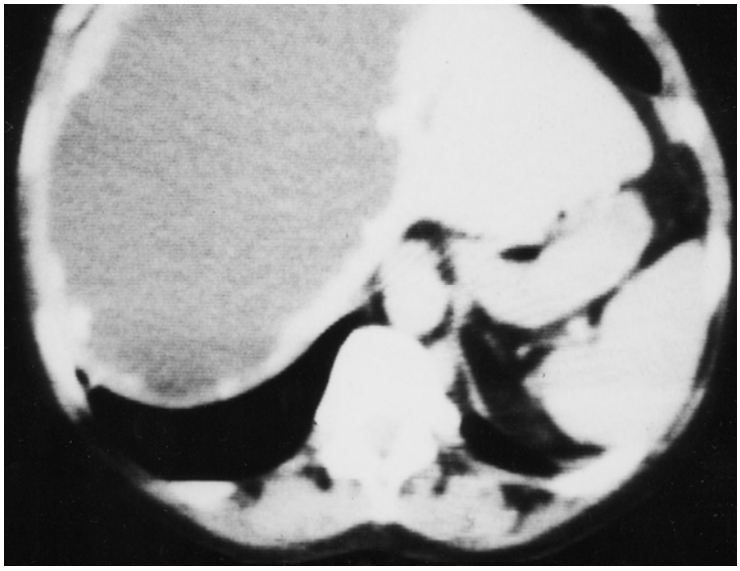


Fig. 2.11.
Computed tomography of liver with lesion caused by *Echinococcus multilocularis*
Photograph: courtesy of Professor R. Ammann, University Hospital, University of Zurich

Cholangiography and endoscopic retrograde or percutaneous cholangiography

Dilated intrahepatic bile ducts are typical findings in cases with involvement of the liver hilus. Displacement of intrahepatic bile ducts, obstructions and other changes can be observed by CAG, ERC or PTC. The non-invasive MRIC will probably become the method of choice in diagnostic cholangiography. An analysis of 18 cholangiograms performed in patients with advanced AE revealed occlusion and/or obstruction of bile ducts in 61%, stretching in 44% and mural irregularities in 18%. In 9 of the 18 patients only intrahepatic bile ducts were involved (78, 118).

2.3.3.2. Diagnostic puncture

Ultrasound-guided fine-needle puncture of liver lesions has recently been used for diagnosing AE, using the biopsy sample for RNA detection by PCR (59). However, the sensitivity of this technique may not be high, since the chances of obtaining sufficient amounts of material are low. More importantly puncture may include the risk of disseminating metacestode cells with subsequent formation of metastases as demonstrated in experimental animals (J. Eckert *et al.*, unpublished findings).

2.3.3.3. Laboratory findings

Haematology and blood chemistry

The routine laboratory tests do not yield specific findings. The blood sedimentation rate is elevated in most of the cases. The numbers of leucocytes and platelets may be depressed in patients with splenomegaly. Lymphopaenia is frequent in advanced cases, and eosinophilia is usually absent. Cholestasis with or without jaundice is observed in patients with intrahepatic bile duct compression or obstruction. Cholangitis and/or liver abscesses, which usually result from bile duct obstruction, are associated with typical alterations of the laboratory parameters. Hypergammaglobulinaemia is present in most of the patients and reflects the specific and polyclonal antibody response. In about one-half of the patients, the presence of specific anti-*E. multilocularis* – IgE can be demonstrated.

2.3.3.4. Immunodiagnosis

Immunodiagnosis of AE is based on similar principles as those for CE (Table 2.15.) (Chapter 2.2.3.8.). However, serological tests for antibody detection are generally more reliable in the specific diagnosis of AE than of CE (Annex 2.1.).

Table 2.15.
Approaches for immunodiagnosis of alveolar echinococcosis in humans

First step: Primary antibody test Usually, for primary testing assays are preferred which exhibit high sensitivity, but may be less specific, whereas in secondary testing assays are employed which have high specificity but may be less sensitive **Subsequent steps**		
↓	↓	↓
Seronegative samples **People without imaging structures or other signs suggestive for AE**	**Seronegative samples** **People with imaging structures suggestive for AE**	**Seropositive samples** **People with or without imaging structures suggestive for AE**
No further serological follow-up In persons with suspected infection risk: Repeated serological examinations after 3 and 6 months, and US imaging if indicated	**Asymptomatic cases** Extended and/or advanced imaging and repeated serological examinations Fine needle biopsy for PCR or immunohistology may be considered in rare cases If lesions are fully calcified, serological and imaging follow-up after 6 months to confirm parasite abortion **Symptomatic cases** Consideration of surgical intervention and/or chemotherapy without further serological examinations	**Asymptomatic and symptomatic cases** Secondary antibody test: for assessment of primary test and exclusion of cross-reactions (Table 2.16.) Em2Plus-ELISA Em alkaline phosphatase-antigen-ELISA Immunoblot for specific bands or similar test (Table 2.16.) Serological differential diagnosis for CE (see text)

2.3.3.4.1. Immunodiagnosis in individual patients

Primary tests for antibody detection

ELISAs with crude *E. multilocularis* antigens achieve high levels of sensitivity, which may exceed that of tests with purified or recombinant antigens, but specificity is mostly lower (Table 2.16.). Due to cross-reactivity, antibodies against *E. multilocularis* antigens can also be detected with assays using *E. granulosus* antigens, such as ELISA or IHAT (hydatid fluid antigen) or IFAT (protoscolex antigen) (7, 64) (Table 2.16.).

Probably the best overall choice for detecting serum antibodies (IgG) in AE cases is the use of an ELISA based on purified antigens of *E. multilocularis,* such as Em2-antigen (48), the Em18-antigen (57), the Em-alkaline phosphatase-antigen (98, the C-antigen (100) or the recombinant antigens II/3-10 (48) and Em10 (52). Tests using these antigens exhibit diagnostic sensitivities approximating 90%-100% (Table 2.16.).

The following approach can be used for immunodiagnosis of human AE.

Primary antibody test: two types of tests are commonly used: **type A tests** which are highly sensitive and specific assays using purified *E. multilocularis* antigens (Table 2.14.); or **type B tests** which are assays with crude *E. granulosus* or *E. multilocularis* antigens. In practice, Type A tests should be preferred as primary tests.

The specificity of the tests in healthy persons is generally very high (data not shown), and also in cases of parasitoses other than CE the specificities are high. However, in some of these assays cross-reactivity occurs in cases of CE (Table 2.16.). The ELISA using purified *E. multilocularis* phosphatase as antigen has apparently outstanding characteristics with a very high sensitivity combined with high specificity, also in cases of CE (Table 2.16.). So far, only one of the assays, which are highly specific for AE, has been made commercially available. This test is based on a mixture of the Em2 and the II/3-10 antigens (Em2PlusELISA™, Bordier

Affinity Products, Crissier, Switzerland). The use of this assay allows to discriminate between AE and CE with a reliability of approximately 95% (48).

Secondary tests for antibody detection

Like in immunodiagnosis of CE, secondary tests may be used for assessment of the results of primary tests, especially when *E. granulosus* antigens or crude *E. multilocularis* antigens have been used for primary antibody screening. Secondary tests may also be needed for excluding cross-reactivity in positive sera. Several test systems have been used in this indication, such as Western blot analysis (56, 57, 77, 127), an enzyme immune test with *E. multilocularis* protoscolex-antigen (6), and the IgG4 determination in ELISA (29, 49, 124, 128) (Table 2.16.). A Western blot test has recently been made available commercially (Echinococcus WB IgG, LDBIO Diagnostics, Lyons, France) which enables discrimination between AE and CE with a reliability of approximately 76% (90).

Antibody response and post-treatment follow-up

For assessing the efficacy of surgical and chemotherapeutical treatment, and of metacestode viability, serological tests are of limited value. However, it has been shown that in part of the treated patients, particularly those with a cured or regressive form of AE, antibody levels detected by the Em2-ELISA, Em2Plus-ELISA, Western blotting, Ig-isotype-ELISA or alkaline phosphatase-antigen-ELISA (29, 47, 66, 98) tend to decline by the time, but only after long periods of one to several years after the therapeutic intervention.

Cellular immune tests show that the *in vitro* lymphoproliferative response to *E. multilocularis* antigen stimulation is high in cured patients who had radical surgery or in patients with dead metacestodes, and is significantly lower in patients that has partial surgical resection or no resection (43, 76). Such assays can be used in scientific studies.

Detection of parasite antigens or DNA in biopsy specimen

Metacestode tissue samples obtained by surgery or fine needle biopsy of organ lesions can be species-specifically identified by the use of PCR (26, 44) or direct immunofluorescence or immunohistochemistry (26).

Table 2.16.
Sensitivities and specificities of assays for antibody detection in human alveolar echinococcosis (AE) (examples)

Antigen	Assay	Percentage sensitivity in cases of AE (cases tested)	Percentage specificity in cases of cystic echinococcosis (cases tested)	Percentage specificity in cases of other parasitoses (cases tested)	Ref.
E. granulosus					
Hydatid fluid	ELISA	97 (140)	–	51 (144)[a]	48
Hydatid fluid	IgG4-ELISA	52 (54)	62 (56)	100 (80)[b]	49
E. multilocularis (Em)					
CH-10: crude	ELISA	96 (140)	39 (124)	97 (144)[a]	48
Em2: partially purified	ELISA	89 (140)	94 (124)	100 (144)[a]	48
Em 10: recombinant[c]	ELISA	86 (140)	93 (124)	98 (144)[a]	48
		93 (74)	89 (64)	100 (30)[d]	52

Em2Plus: mixture of Em2 & Em II/3-10[c]	ELISA	97 (140)	74 (124)	98 (144)[a]	48
Em alkaline phosphatase: purified	ELISA	100 (37)	100 (44)	100 (34)[e]	98
Em C: 30-35 kDa fraction of crude antigen	WB	95 (60)	100 (10)	100 (24)[f]	100
Em 18/16: partially purified	ELISA	91(79)	67 (48)	100 (35)[g]	57

ELISA : enzyme-linked immunosorbent assay
WB : Western blot

a) cases of fasciolosis (20), schistosomosis (17), cysticercosis (20), taeniosis (17), intestinal and tissue nematode infections (70)
b) cases of infections with protozoa (24), trematodes (16), nematodes (32) and of cysticercosis (8)
c) the recombinant antigens Em10 and II/3-10 are functionally identical
d) cases of amoebosis (2), fasciolosis (2), schistosomosis (4), paragonimosis (3), neurocysticercosis (17), and filariosis (3)
e) cases of liver amoebosis (5), malaria (3), schistosomosis (11), trichinellosis (8), toxocarosis (7)
f) cases of schistosomosis (1), paragonimosis (2), diphyllobothriosis (19), toxocarosis (1), and filariosis (1)
g) cases of cysticercosis (28), sparganosis (2), paragonimosis (5)

2.3.3.4.2. Immunodiagnosis in human populations

Early diagnosis of patients with AE is considered to be a prerequisite for efficient management and treatment of the disease (101). Consequently, serological screenings may be offered to populations and communities at risk. Test operating characteristics allow to perform reliable seroepidemiological studies, and thus, to detect asymptomatic cases of AE as well as cases, in which the metacestode lesion has died out at an apparently early stage of the infection. However, it is still difficult to detect liver lesions below 10 mm in diameter either by US examination or by immunodiagnosis. In a Japanese study, 64% of liver lesions detected by US were small, ranging from 8 mm to 50 mm in diameter (109). Cases with lesions below 10 mm in diameter were seronegative (K. Suzuki and N. Sato, personal communication, 1998). Mass screening programmes have used specific immunodiagnostic assays for primary screening followed by ultrasound and other imaging examinations of suspected cases or US examination has been employed as primary screening alternatively complemented by antibody detection. Additional details are described in Chapter 6.2.

2.3.3.5. Pathological and histological examination

In macroscopic sections of the human liver, the metacestode of *E. multilocularis* typically exhibits an alveolar structure composed of numerous irregular cysts with diameters between less than 1 mm and 30 mm (Fig. 2.12.). Due to necrosis of the lesion, cavities filled with liquid and necrotic material may be formed in the central parts of the parasite (32). Microscopically, the cysts consist of a relatively thin PAS-positive laminated layer and a delicate germinal layer often with only a few nuclei; quite frequently the germinal layer is not discernible (Fig. 2.13.). Brood capsules and protoscoleces are rarely formed in the human host (32). The cysts are surrounded by an inner zone of necrotic tissue and outer layers of histiocytes and lymphocytes. In later phases, tissue reactions of chronic inflammation, often with giant cell foreign body reaction, fibrous tissue and calcifications are seen around cysts. Often fibrous tissue proliferation is so intense that cysts are embedded in a very dense and hard stroma, however, the metacestode as a whole is not demarcated at its outer limits by a fibrous capsule like cysts of *E. granulosus*, except in abortive lesions (32). These are characterised by a fibrous wall, which may be partially calcified, and a cavity filled with amorphous necrotic material, in some cases also with folded parasite layers (32, 92).

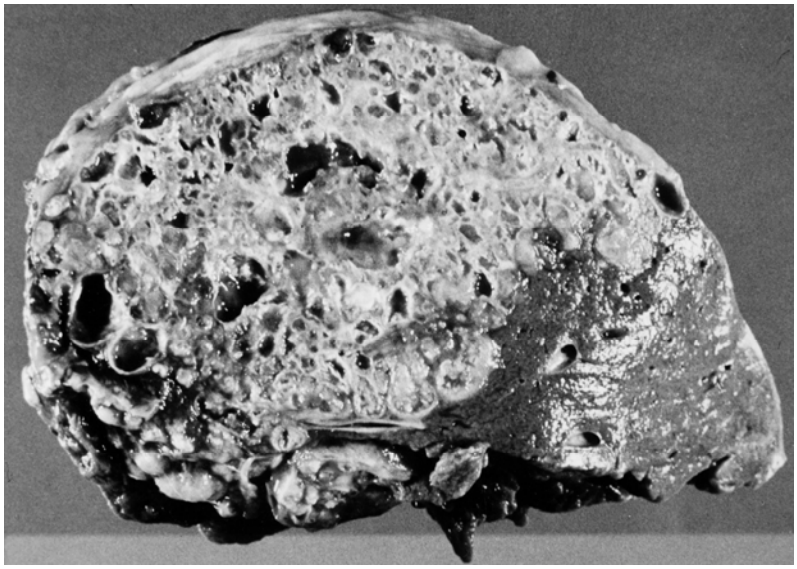


Fig. 2.12.
Macroscopic appearance of human liver with alveolar echinococcosis: multiple small and larger cysts (maximum diameter of a single cyst: 3 cm)
Photograph: J. Eckert, courtesy of the Institute of Parasitology, Zurich

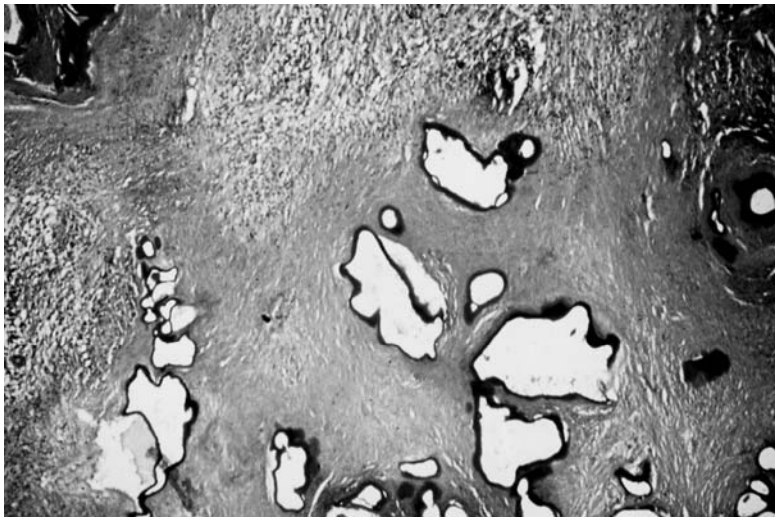


Fig. 2.13.
Histological section of *Echinococcus multilocularis* metacestode in human liver: cysts without brood capsules and protoscoleces
Photograph: J. Eckert, courtesy of the Institute of Parasitology, Zurich

2.3.4. Treatment

General considerations

Treatment of AE involves a variety of options, including surgery and chemotherapy, and requires a specific clinical experience. Therefore, patients should be referred to the recognised national/regional AE treatment centres. As the parasite lesion is comparable to a malignant tumour, early diagnosis of AE is of special importance for successful treatment. Population screening programms for AE in endemic areas of Japan and Europe have clearly shown that early diagnosis reduces morbidity and mortality, as well as costs of the disease (11, 109) (Chapter 6.2.).

The following principles for the treatment of AE are now commonly accepted (129):

- the first choice treatment in all operable cases is radical surgical resection of the entire parasitic lesion from the liver and other affected organs
- in AE patients after radical surgery chemotherapy is indicated for a limited period of time
- long-term chemotherapy is mandatory after incomplete resection of the lesions, in inoperable patients (including cases after interventional procedures) and in AE patients after liver transplantation (further details see below).

2.3.4.1. Surgery

Excision of the parasitic lesion has to be carried out using the procedures of radical tumour surgery (114, 115). Radical or non-radical surgery and liver transplantation require concomitant chemotherapy (see below).

Indications

Resectability of the parasitic lesion in the liver is a prerequisite for radical surgery and must be assessed by imaging techniques before the operation.

Contraindications

Inoperable lesions, extensive lesions, lesions not confined to the liver and diaphragm, but extending to other organs must be managed by alternative therapies after an interdisciplinary consultation.

Benefits

Radical surgery may eliminate the parasites and cure the patient. An early diagnosis of AE can improve prospects for complete cure. Nonradical surgery for reducing the parasite mass and for increasing chances of effective chemotherapy is debatable.

Risks

Lesions cannot always be clearly defined by imaging techniques; incomplete resection leaves invisible remnants of parasitic tissue with a potential for regrowth and dissemination into other organs, even after some years. General risks may be associated with surgical intervention (anaesthesia, stress, etc.), infections (including those transmitted by blood transfusion) or other factors.

Medical requirements

Hospitalisation in a surgical ward is mandatory. The surgical team should be experienced in major liver surgery and in treating AE.

2.3.4.2. Chemotherapy

Extensive studies in animals showed significant parasitostatic efficacy of benzimidazoles against the metacestode stage of *E. multilocularis* and based on this, chemotherapy of AE in human patients has been practiced since 1975 (31). Carefullly controlled clinical studies have revealed that the 10-year survival rate in inoperable or non-radically operated AE patients (including severe forms) on long-term chemotherapy increased to 80%-83%, compared to 6%-25% in untreated historical control patients (3, 4, 55, 131). In addition to chemotherapy, early diagnosis, improved surgery and medical care of patients may contribute to the success of treatment (12, 123).

Indications

There are several indications for chemotherapy, as follows:

- chemotherapy is indicated for a limited period of time after radical surgery. Since residual parasite tissue may remain undetected at radical surgery, post-operative chemotherapy for at least 2 years should be carried

out and patients should be monitored for a minimum of 10 years for possible recurrence

- long-term chemotherapy for several years is mandatory in inoperable AE patients, in cases following incomplete surgical resection of the parasite lesions and after liver transplantation
- pre-surgical chemotherapy is not indicated in cases of AE. However, in rare cases for whom surgery was contraindicated at the time of diagnosis of AE, surgery can be carried out after a prolonged course of chemotherapy.

Contraindications

In view of the severity of AE and the relative low toxicity of the drugs currently used (mebendazole or albendazole), there are only a few contraindications for chemotherapy. In some cases (pregnant women, etc.) certain precautions and limitations or modifications of drug administration are necessary (Annex 2.2.).

Choice of drugs

Two benzimidazoles (mebendazole and albendazole) are preferentially used for chemotherapy of AE (Annex 2.2.).

Mebendazole (MBZ) (Vermox® 500 mg, Janssen, Belgium) is given as 500-mg tablets in daily doses of 40 mg/kg-50 mg/kg bw in three divided doses postprandially. After an initial continuous treatment of 4 weeks, it is advisable to adjust the oral doses in order to obtain plasma drug levels of >250 nmol/l (= 74 ng/ml). The latter level was experimentally determined as effective in rodents (31). These data and results from human trials suggest that mebendazole plasma concentrations in excess of 80 ng/ml-100 ng/ml maintained for long periods may be necessary to achieve high efficacy (132). In special situations, the oral dosage may be higher than the above recommended dose, but a daily dose over 6 g per adult patient should not be given. The duration of treatment is at least 2 years after radical surgery or continuously for many years in inoperable cases, as well as for patients who have undergone incomplete resection or liver transplantation. For some patients, mebendazole has been administered for more than 17 years.

Albendazole (ABZ) (Eskazole®, Zentel®, SmithKline Beecham) is given as 400-mg tablet or as a 4% suspension at daily doses of 10 mg/kg-15 mg/kg bw (in two divided doses). In practice, a daily dose of 800 mg is given to adults, divided into two doses of 400 mg (53). The divided dose is supported by pharmacokinetic data (58). According to the original recommendation of the manufacturer, repeated cycles of 28 days treatment should be followed by a 'wash out' phase without chemotherapy of 14 days. However, recent data from the People's Republic of China (65) and Italy indicate that a continuous ABZ treatment of AE is at least equally or more effective and well tolerated. Sporadically ABZ was given in higher doses of 20 mg/kg/day for up to 4.5 years (65). The duration of necessary chemotherapy has not yet been determined but might well be life-long for most of the patients without complete resection of the AE lesions.

Praziquantel (PZQ) has been used for the treatment of human AE, but experimental data obtained from animal models indicate that its efficacy against the metacestode stage of *E. multilocularis* is far less pronounced than that of the benzimidazoles mentioned above, even when PZQ is given in very high doses (2, 31).

Benefits of benzimidazole treatment

This is a non-invasive treatment with a relatively low toxicity. However, in most patients benzimidazoles are only parasitostatic.

Risks

The main risks are neutropaenia, alopecia and liver dysfunction. Because of the potential embryotoxicity and teratogenicity (only observed in some laboratory animals), it should not be used in women of child-bearing age, unless contraceptive measures are taken, and during pregnancy especially the early stages (Annex 2.2.).

Medical requirements

Hospitalisation is not needed, but regular medical and laboratory checks for adverse reactions and efficacy are necessary. The costs of anthelmintics and repeated medical examinations are high.

Monitoring of patients

In the initial phase, monitoring of AE patients is similar to that in CE patients (Chapter 2.2.4.3.). Subsequently, haemogram and serum transaminases should be checked at intervals of 3 months. At intervals of 6 to 12 months, the patients should be examined in a clinical reference centre, where US and special imaging (for example CT) can be performed to monitor parasitic lesions and their response to chemotherapy. A long-term follow-up of more than 10 years is recommended.

2.3.4.3. Interventional procedures

With AE patients for whom surgery is contraindicated, a number of local complications may occur for which interventional procedures have to be considered (118, 129). Dilation and stent implantation in vessels and/or bile ducts, and endoscopic sclerosing of oesophageal varices are the main interventional procedures performed in AE. Drainage of necrotic liver lesions may be indicated if bacterial infection has occurred. In conjunction with chemotherapy, these procedures can be beneficial for patients.

Indications

Interventional procedures are indicated, when surgery is contraindicated because of disturbances of essential organ functions, i.e. hyperbilirubinemia due to cholestasis, vena cava or portal vein thrombosis, colliquative liver necrosis with risk of rupture into the abdomen, and/or severe bacterial infection or bleeding of oesophageal varices secondary to portal hypertension.

Contraindications

Interventional procedures have the potential risk to spread parasite material and – except the emergent and/or palliative ones – are not indicated when post-interventional chemotherapy is not possible.

Benefits

Interventional procedures together with chemotherapy as options for treatment can improve the life expectancy and quality of life of patients with AE.

2.3.4.4. Liver transplantation

In Europe, liver transplantation (LT) has been carried out in approximately 40 patients with inoperable AE and chronic liver failure (10). In a French series, 21 patients had received liver grafts between 1986 and 1991 for incurable AE (10). Among 15 patients who survived more than one year, ten were alive 6.5 to 11.5 years after transplantation (10). This study has shown that the risk of recurrence of parasite proliferation and metastasis formation after LT is relatively high (10).

Indications

Liver transplantation should only be considered in patients with very severe hilar extension, leading to uncontrolled biliary infections, symptomatic secondary biliary cirrhosis with ascites or severe variceal bleeding owing to portal hypertension (10). Such patients become more rare due to earlier diagnosis of the disease (10) so that the indication for liver transplantation is rather limited. It requires long-term and continuous post-operative chemotherapy (see above).

Contraindications

Liver transplantation is not indicated in extensive AE that is not confined to the liver or for patients with contraindications for prolonged immunosuppressive treatment, and concomitant benzimidazole treatment.

Benefits

Liver transplantations can be a life-prolonging procedure for patients with severe liver dysfunction (10).

Risks

These include general surgical risks, specific risks of long-term immunosuppressive treatment, and induction of proliferation of metacestode remnants and metastases formation (particularly in the brain) under immunosuppression.

Medical requirements

Liver transplantation requires a highly specialised team and equipment with the competence to deal with the current post-transplantation problems as well as with the clinical problems of AE. Supportive medical care includes post-transplantation clinical observation, adaptation of immunosuppressive drugs, and diagnosis and management of complications of immunosuppressive treatment under continuous chemotherapy with benzimidazoles.

2.4. Other forms of echinococcosis

General aspects

Forms of human polycystic echinococcosis (PE) are caused by *E. vogeli* and *E. oligarthrus*, which are confined in their distribution to Latin American countries. Aspects of their biology are described in Chapter 1 (8, 20, 32). Up to 1999, at least 96 cases of human PE have been recorded in 11 countries of Central and South America (Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Venezuela, Surinam, Brazil, Uruguay, Argentina and Chile). Of the 96 cases, 37 were due to *E. vogeli*, three to *E. oligarthrus*, and in the other cases the *Echinococcus* species could not be determined. It appears that this number of cases is only 'the tip of the iceberg' (see also Chapter 4.3.) (8, 20).

2.4.1. Polycystic echinococcosis due to *Echinococcus vogeli*

The metacestode stage of *E. vogeli* is characterised by a polycystic structure and development in visceral organs. In 59 patients with PE, the liver was the most frequently affected organ. In 78% of the patients, the liver was infected alone or together with other organs (spleen, pancreas, stomach, omentum, mesenteries, lung, diaphragma, pericardium, intercostal muscle, etc.) (20). The second most frequently infected organ was the lung 14%, either singly or together with liver or other organs. Single site infections were observed in the liver and lung, but also in other organs (i.e. mesenteries and stomach) (20). Clinical and radiological presentation is very similar to infection with multiple cysts of *E. granulosus*, and differential diagnosis depends on isolation of protoscoleces and morphological hook characteristics (20). Immunodiagnosis using a purified antigen of *E. vogeli* allowed discrimination between cases of PE and CE, but differentiation between PE and AE was not always possible (46). Albendazole has been used for chemotherapy in six cases with success of treatment in four and improvement in two (20).

2.4.2. Polycystic echinococcosis due to *Echinococcus oligarthrus*

The causative agent is the metacestode of *E. oligarthrus*, which is polycystic in structure, and in naturally infected animals, it has been most commonly found in the musculature and the skin, but also in viscera. Only three human cases have been reported to date, two orbital in Venezuela and Surinam and one cardiac in Brazil with 2 cysts (1.5 cm diameter) (20). The diagnosis was based on morphology of protoscolex hooks.

2.5. Ethical aspects

In human echinococcosis ethical aspects have to be considered carefully in activities related to:

a) pure research (e.g. drug testing)

b) optimal and/or novel diagnostic or therapeutic approaches (e.g. diagnostic cyst puncture, therapeutic cyst puncture)

c) population-based studies (mass ultrasonographic or serological screening for CE or AE).

In all the situations, basic human rights have to be respected, according to the Helsinki declaration II (23), CIOMS documents (15, 117) and ethical review committees rules (24). The aim of all these documents is to minimise the risk that medical intervention may bring to the patients.

Risks of medical intervention in echinococcosis may result from the following situations (117):

a) not respecting indications and contraindications in high risk groups of patients (e.g. with young or advanced age, with coagulation defects before liver biopsy)

b) using inadequate instruments (e.g. blunted or nonsterile biopsy needles, imaging equipment of poor quality)

c) carrying the intervention in ways that do not minimise the direct risks (e.g. haemorrhage or anaphylactic shock on liver puncture) or complications (infections, if non-sterile equipment is used, or secondary echinococcosis, if anthelmintic cover is neglected)

d) performing interventions by inexperienced or careless operators (e.g. by not referring patients to a competent centre)

e) making a false interpretation (e.g. results of the serological tests, imaging technologies or biopsy specimens).

General rules related to research

All research activities in echinococcosis aspects necessarily must follow some general rules: any planned research in echinococcosis should avoid a repetition of a previously well done study, have serious justification, with well defined objectives and appropriate study design. The study has to be performed in properly selected and representative population with a careful justification of the necessity of using special vulnerable groups of participants (small children, pregnant or nursing women). The selection of study methods should in optimal way address the objectives, consider necessary sample size and statistical power estimates as well as assure quality control. The personnel involved in the study should be well trained in the use of instruments and procedures, and there should be clearly described emergency procedures. The data have to be properly collected, avoiding bias, respecting confidence rules and defining the way and extent of notifying patients about the study findings. Final data analysis should concentrate on original discoveries, follow basic rules of statistical methodology and respect limitations of the study. Data management, including storing and protecting of the data and their final disposition, has to be decided before the study is undertaken. Other important conditions are: analysis of risks for patients involved and methods to minimise those risks; defining of the study benefits for patients and introduction of an informed consent procedure. This should ensure patient's rights not to participate in the study, or to withdraw his/her agreement to participate any time during the study as well as to regulate any financial aspects of participation or compensation in case of any harm related to the study (15, 23).

Some ethical problems related to the particular situations in echinococcosis research, individual clinical care and population interventions are presented, as follows:

- **Drug testing study**

Drug testing studies are to be best designed by the comparison of an investigational drug versus already available drugs in two randomly selected comparable groups. The consent form is required for each participating patient. The study has to be stopped as soon as the investigational drug is found to be of lower efficacy or too harmful; in that case the group assigned to an investigational drug should be offered a full conventional treatment without any delay (22). In the multicentre study, a uniform protocol has to be

prepared and followed unless it is in conflict with the individual patient's interest according to the best judgement of the researcher.

- **Modification or extension of the standard medical care**

Much progress in the diagnosis and treatment of human echinococcosis originates from the observations of the results of *ad hoc* modified or extended care of the clinical patients, e.g. pharmacokinetic studies, evaluation of the efficacy of chemotherapeutic treatment by imaging techniques, dosing of anthelmintics. In some of these studies, a consent form from the patient may be needed, but in all such studies it is essential that the patient's interest and the benefits for the future patients due to the improved knowledge and experience would outweigh any risk or inconvenience of the modified procedure to the patient.

- **Novel diagnostic or therapy procedures**

It is very important that the initial sporadic clinical observations or experiences that may suggest a novel or improved procedures are described in detail and with maximal objectivity. However, the study aiming at introduction of the novel diagnostic procedures, such as diagnostic biopsy of the liver cysts or the novel therapeutic interventions such as PAIR or liver transplantation should be reserved for selected reference centres before enough experience is gained about their efficacy and safety elsewhere (35).

- **Selection of the optimal diagnostic procedures and treatment methods**

The variety of possible diagnostic procedures and treatment methods available frequently poses a question of the best choice. The choice has to respect the patient's interests, the availability of the diagnostic facilities and drugs as well as the cost of interventions. Unnecessary diagnostic procedures such as diagnostic biopsies, additional radiological or imaging examinations should be avoided. On the other hand, patients should be referred to specialised centres whenever practically possible. The information about the optimal treatment of CE and AE patients is widely available and regularly updated (86, 129).

- **Population-based study**

The population-based study should fully respect the human rights and ethical requirements. First of all, the study should not be undertaken in case the results will be of no benefit for individual participants found to be infected and/or any further use for public health services improvement. Before the population-based study is undertaken, it has to be accepted by the local public health authority and the population at large. When it is impracticable to elicit adequately informed consent from every individual involved in the study an acceptable procedure is to delegate the power of consent to local independent representative body. However, a rule has to be accepted that any individual person involved may refuse his/her participation in the study at any time. The study should be carefully designed and a decision made regarding the way in which any individual participant found to be infected will be further diagnosed, treated and cared. The population-based study have to be performed very carefully as small inadequacies in methodology of the study, in examination of the individual patient and interpretation of the results may lead to the false general conclusions. The documentation of such a study should be as complete as possible, in order of gaining the highest credibility. For example, in mass screening for liver CE, the results should mention – in addition to the age, sex, locality and the number of people examined – the number of persons with any liver space occupying lesion, the number of the persons with lesions suspected for liver CE and the number of patients with confirmed CE by other techniques including surgery and the types of *E. granulosus* cysts (Table 2.5.) or AE lesions (Table 2.13.).

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Annex 2.1.

Determination of performance characteristics for immunodiagnostic assays

F. Grimm

Detailed information on principles of validation of diagnostic assays for infectious diseases can be obtained from a recent review (3). Some basic aspects are described here.

Selection of the cut-off point (positive/negative threshold)

To achieve estimates of the diagnostic sensitivity and specificity (see below) of an assay, for example of an ELISA for serum antibody detection, the results first must be allocated to positive (antibodies detected) and negative (no antibodies detected) categories. The threshold or cut-off point between these categories may be selected by visual inspection of the frequency distributions of test results of groups of infected and uninfected reference individuals (3). However, visual inspection is not precise. Therefore, the cut-off is usually determined by calculating the mean of testing results (optical densities in the ELISA) + 2 or + 3 standard deviations (SD) for groups of individuals that are not infected with a specific agent, for example *Echinococcus*. All testing results above the cut-off point are regarded as positive.

The selection of negative reference groups is crucial. The cut-off point can be based on testing results of individuals that are free of parasites or that are free of a specific agent, i.e. *Echinococcus*, but may harbour other parasites which do not interact with the assay (for example with protozoan parasites in case of an *Echinococcus* assay). Since geographic and ethnic variation is known to occur in antibody response, it might be necessary to determine the cut-off point for each population under evaluation. In cases where information on the parasitological status of the population under study is not available, cluster analysis may provide a powerful statistical tool for the determination of a threshold value (2).

Calculation of diagnostic sensitivity and specificity

- **Definitions**

Diagnostic sensitivity (DS) is defined as the proportion of known infected individuals that test positive in an assay. Infected individuals that test negative are considered as false negatives. Analytical sensitivity defines the smallest amount of the analyte – for example antigen – which is detectable (3). Diagnostic specificity (DSP) is defined as the proportion of uninfected reference individuals that test negative in the assay. Uninfected reference individuals that test positive are regarded as false positives (3). With regard to parasitic infections two types of diagnostic specificities may distinguished:

DSP1: proportion of uninfected reference individuals that test negative in a population of individuals free of parasites;

DSP2: proportion of uninfected reference individuals that test negative in a population of individuals that are not infected with a specific parasite (for example *Echinococcus*), but harbour other parasites or infective agents.

After the cut-off point is established, the testing results of sera can be classified as true positives (TP) and true negatives (TN) if they are in agreement with those of the gold standard (3). The gold standard in human patients with echinococcosis is the diagnosis of the infection by imaging or by other methods of direct parasite identification. Alternatively, they are classified as false positive (FP) or false negative (FN).

Diagnostic sensitivity and DSP are calculated as follows and expressed as percentages:

Diagnostic sensitivity percentage: $\mathrm{DS} = \frac{\mathrm{TP}}{\mathrm{TP} + \mathrm{FN}} \times 100$

Diagnostic specificity percentage: $\mathrm{DSP} = \frac{\mathrm{TN}}{\mathrm{TN} + \mathrm{FP}} \times 100$.

Hypothetical example

Among 100 individuals 15 had confirmed echinococcosis with the following serological testing results:

Test result	Reference individuals	
	With confirmed echinococcosis	Without echinococcosis
Positive	13 = TP	3 = FP
Negative	2 = FN	83 = TN
Diagnostic sensitivity: DS = 13/13 + 2 × 100 = 86.6%		
Diagnostic specificity: DSP = 83/83 + 3 × 100 = 96.5%		

Calculation of predictive values

- **Definitions and general aspects**

The predictive value (PV) can be expressed as positive (PV+) or negative (PV–) value. The PV+ is an indicator of the probability that individuals with positive test results do have the disease, whereas the PV– expresses the probability that individuals with negative testing results do not have the disease. With other words, the PVs are indicators of the probability of the correctness of the diagnosis. The PVs are determined by the prevalence of a disease (P), and both the diagnostic sensitivity (DS) and diagnostic specificity (DSP) of the test. It is important to understand that the PVs are not inherent assay characteristics. Especially positive PVs are strongly dependent on the prevalence of an infection/disease in the population under study, and on the DSP of the assay used.

- **Formulas for calculating predictive values**

Positive predictive value in percentage $= \frac{P \times \mathrm{DS}}{P \times \mathrm{DS} + (100 - P) \times (100 - \mathrm{DSP})} \times 100$

Negative predictive value in percentage $= \frac{\mathrm{DSP} \times (100 - P)}{\mathrm{DSP} \times (100 - P) + (100 - \mathrm{DS}) \times P} \times 100$

P : prevalence of the disease

DS : diagnostic sensitivity

DSP : diagnostic specificity

- **Example for calculating predictive values**

The expected prevalence of echinococcosis in a population is 2%, the available ELISA for a serological survey has a diagnostic sensitivity of 70% and a diagnostic specificity of 90% (1).

Positive predictive value

$$PV+ \frac{2\times 70}{2\times 70+(100-2)\times(100-90)}\times 100=\frac{140}{140+980}\times 100=\mathbf{12.5\%}$$

Negative predictive value

$$PV- \frac{90\times(100-2)}{90\times(100-2)+(100-70)\times 2}\times 100=\frac{90\times 98}{90\times 98+30\times 2}\times 100=\mathbf{99.3\%}$$

The probability for a correct negative result is high, but low for a correct positive result.

- **Examples of predictive values (PV) for different prevalences (P) and diagnostic sensitivities (DS) and specificities (DSP)**

DSP	DS	PV+ P: 10%	PV+ P: 2%	PV+ P: 0.5%	PV– P: 10%	PV– P: 2%	PV– P: 0.5%
90%	70%	43.8	12.5	3.4	96.4	99.3	99.8
90%	97%	51.9	16.5	4.6	99.6	99.9	>99.9
99%	70%	88.6	58.8	26.0	96.7	99.4	99.8
99%	97%	91.5	66.4	32.8	99.7	99.9	>99.9

These examples show that for any prevalence of a disease, the PV+ depends predominantly on DSP, whereas PV– is more dependend on DS. It is to be underlined that in areas with a low prevalence of a disease assays with high DSP's are of crucial importance for reliable seroepidemiological studies.

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Annex 2.2.

Characteristics of benzimidazoles

Source: WHO (1996) (3)

Mebendazole (MBZ) (Vermox 500 mg®, Janssen) is poorly absorbed (<10%) after oral administration. The rate of absorption is increased (up to 8-fold) if the drug is taken during a meal, especially one with a high fat content. After oral administration of standard doses, serum drug levels are highly variable among individuals and are not correlated with the doses given. In blood plasma, >90% of the drug is protein-bound. Based on data from animal experiments, the serum drug concentrations required for effective chemotherapy are estimated to be >250 nmol/l (= 74 ng/l). However, several studies have shown that such serum levels may

not be attained by more than 30% of the patients and that lower (as yet undetermined) levels may be sufficient for of long-term therapy. Mebendazole is rapidly metabolised in the liver and excreted via urine and bile. The elimination half-life times are short (2.5 h-5.0 h) and may be increased in patients with cholestasis and other disturbances of liver function. Serum mebendazole concentrations 4 h after the morning dose have a high degree of predictability for the 24 h average serum concentrations, and the 4-h value has therefore been proposed for monitoring serum drug levels.

Albendazole (ABZ) (Eskazole®, Zentel®, SmithKline Beecham), has similar pharmacokinetic properties to mebendazole with low absorption rates and high interindividual variability of serum drug levels that may lie in the range 200 nmol/l-6,000 nmol/l; average values are 1,000 nmol/l-2,000 nmol/l (albendazole sulfoxide). Serum drug levels are higher in patients with cholestasis and other liver dysfunctions, and intestinal absorption rates are increased by fatty food. The mean half-life elimination time in 14 persons was 8.5 h (SD, 6.0). The effective serum drug levels are not well defined; based on data from animal experiments, they are estimated to be around 650 nmol/l-3,000 nmol/l.

Drug efficacy

Mebendazole and the main metabolite of ABZ – albendazole sulfoxide have anti-parasitic properties.

Animal experiments have shown that long-term treatment with various benzimidazole derivatives (for example: albendazole, fenbendazole and mebendazole) has the following effects against *E. multilocularis* metacestodes: inhibition of metacestode proliferation, resulting in reduction of parasite masses; destruction of protoscoleces and partial destruction of the germinal layer of the metacestode; prevention or suppression of metastasis formation; calcifications; and prolongation of host animal survival.

Long-term animal studies have shown that *E. multilocularis* metacestodes are usually not killed by drug treatment, but that their proliferation is inhibited. The effect of the drugs in animals is therefore not parasitocidal, but parasitostatic. On the other hand, *E. granulosus* cyst may be killed by a long-term benzimidazole treatment.

Adverse reactions

Mebendazole and ABZ are generally well tolerated and adverse reactions are relatively mild. Examples of such reactions from two larger series are presented below.

- Adverse reactions in 70 patients with alveolar echinococcosis under long-term chemotherapy (mean duration: 6.5 years. Number of patients treated: MBZ: 61, ABZ: 4, MBZ/ABZ: 5) were: elevation of transaminases (27%); proteinuria (21%); loss of hair (18%); gastrointestinal disturbances (16%); neurological symptoms (e.g. vertigo) (11%) and leukopaenia (6%) (1).

- Adverse reactions associated with albendazole treatment of 780 patients with CE (the duration of treatment is generally shorter than for alveolar echinococcosis) were elevation of transaminases: (14.7%); abdominal pain: (5.7%); loss of hair: (2.8%); headache: (2.1%); abnormal liver biopsy: (1.7%); vertigo/dizziness: (1.3%); nausea: (1.3%); fever: (1.2%); reversible leucopaenia: (1.2%); abdominal distension: (0.6%); urticaria: (0.5%); jaundice: (0.5%); thrombocytopaenia: (0.3%); allergic shock: (0.3%); bone marrow toxicity: (0.1%); and cyst pain: (0.1%) (R.J. Horton, personal communication, 1997).

In a recent publication, Horton (2) listed 817 adverse events in 3,282 patients with echinococcosis, who had been treated with albendazole. The majority of adverse reactions referred to the liver and the gastrointestinal tract. During 12 years, there was not a single fatal case in patients with echinococcosis related to chemotherapy with ABZ. Two thirds of the patients experienced one or more side effects, but they were mostly of minor importance and reversible. Only in rare instances (3.8%) was a permanent discontinuation of chemotherapy indicated. Allergic reactions may also occur.

Precautions

Patients with CE or AE under chemotherapy should be carefully monitored (Chapters 2.2.4.3. and 2.3.4.2.). Monitoring of serum drug levels is suggested to avoid severe toxic reactions.

Pregnancy and nursing

Under certain conditions, MBZ and ABZ may induce embryotoxic or teratogenic effects in some animals. Although such effects have not been observed in humans, it is recommended that use of these drugs be avoided for pregnant women, or the drugs to be used only in urgent cases in the second or third trimester after a careful benefit/risk analysis. For women of child-bearing age, contraceptive measures are indicated during treatment. Experience with MBZ or ABZ treatment during breast feeding does not appear to put the infant at risk of side effects.

Liver disturbances

For patients with cholestasis or hepatocellular disturbances, the drug doses may have to be reduced. Such patients require frequent monitoring of liver function parameters and of serum drug levels, especially those with chronic cholestasis.

Diabetes

Mebendazole may reduce the insulin requirement; therefore, the serum glucose blood levels of diabetics must be carefully monitored.

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Chapter 3

Echinococcosis in animals: clinical aspects, diagnosis and treatment

J. Eckert, P. Deplazes, P.S. Craig, M.A. Gemmell, B. Gottstein, D. Heath, D.J. Jenkins, M. Kamiya and M. Lightowlers

Summary

Two forms of echinococcosis are known to occur in animals:

a) the intestinal infection with adult or immature stages of Echinococcus *spp., and*

b) the infection of internal organs of intermediate or aberrant host animals with the metacestode stage.

Concurrent intestinal and metacestode infections caused by Echinococcus multilocularis *have been observed in dogs.*

Intestinal echinococcosis

The four species of the genus Echinococcus *infect various species of carnivores as definitive hosts causing the intestinal form of echinococcosis which does not induce any major ill effects to the host. The method used until now for surveys of the* Echinococcus granulosus *infection in dog populations is arecoline purging (average sensitivity of 65%-78%). Coproantigen detection by ELISA (CA-ELISA), which is easier to perform, may replace this method in the near future. On average, this test is at least as sensitive as arecoline purging, but sensitivity approaches 92%-100% when* E. granulosus *burdens are greater than 100 per animal. The specificity of the CA-ELISA is generally high (>95%). Commercial CA-ELISAs are now available, which should be evaluated in further studies in various epidemiological situations. A reliable method for the diagnosis of* E. multilocularis *in foxes and other definitive hosts is the intestinal smear technique (IST) performed at necropsy (sensitivity approximately 80%). The sedimentation and counting technique (SCT) is about 10% more sensitive but requires higher expenditure. A CA-ELISA has been developed for the diagnosis of the* E. multilocularis *in foxes, which has an average sensitivity of approximately 85% and a high specificity of over 95%. This test has also been used for diagnosing the* E. multilocularis *infection in living dogs and cats and for detection of coproantigen in fox faecal samples collected in the field. Furthermore, PCR techniques are now available in specialised laboratories for the specific detection of* E. multilocularis *eggs or DNA in faecal samples of carnivores. These tests are highly specific and detect at least 97% of the infections with gravid worms, but are less sensitive if immature worms or low worm burdens are present. Handling of definitive hosts infected with* Echinococcus *spp. and of all materials potentially contaminated with* Echinococcus *eggs requires special safety precautions. For specific and highly effective chemotherapy of the intestinal* Echinococcus *infection two drugs are now available, namely praziquantel and epsiprantel.*

Metacestode infection in intermediate and aberrant hosts

Infections with the metacestode stage of Echinococcus *spp. occur in a broad spectrum of natural intermediate host species, but also in hosts, which do not play a role in the transmission cycle (= aberrant or accidental hosts). They include humans and other mammals. The* E. granulosus *infection in intermediate hosts (sheep, cattle, pigs, etc.) is typically asymptomatic but symptoms have been described in severe cases, for example in horses. The diagnosis of such infections has to be based on necropsy findings and in special cases on clinical examinations. Immunological assays for the diagnosis of* E. granulosus *metacestodes in intermediate or aberrant hosts are less sensitive and specific than for humans and at present cannot replace necropsy. Chemotherapy is not feasible. Recent results of immunising sheep with a recombinant* E. granulosus *antigen are promising. The infection with metacestodes of* E. multilocularis *of intermediate and aberrant host my cause severe and lethal disease. Such clinical cases have recently been observed in monkeys and dogs. In these animals, various diagnostic methods can be employed, such as ultrasound examination and antibody detection. Albendazole has been used for chemotherapy of dogs with* E. multilocularis *metacestode infection of the liver.* Echinococcus multilocularis *metacestode*

infections of livestock (horses and pigs) do not play a role in epidemiology, as the parasite normally does not produce protoscoleces. However, such infections are indicators for environmental contamination with E. multilocularis *eggs and the potential infection risk for humans.*

Details of several techniques are described in the chapter, and ethical aspects related to echinococcosis in animals are discussed.

3.1. Forms of echinococcosis in animals

The four species of the genus *Echinococcus* infect various species of carnivores as definitive hosts causing the intestinal form of echinococcosis. The metacestode stages of *Echinococcus* species develop in internal organs, predominantly viscera, of natural intermediate host animals and occasionally also of animal species, which normally do not play a role in the life-cycle of the parasite (= aberrant or accidental hosts). Aberrant hosts are humans (Chapter 2) and other mammals. For example, dogs may serve as definitive hosts for *E. multilocularis*, but rarely they are infected with the metacestode stage of this parasite (Chapter 3.3.). The various forms of echinococcosis in animals are presented in Table 3.1.

Table 3.1.
Forms of echinococcosis in animals

Stage of ***Echinococcus*** species	Form of echinococcosis	Animal hosts involved
Adult and immature stages of *Echinococcus* spp.	Intestinal echinococcosis	Exclusively definitive hosts
Metacestode stage		
E. granulosus	Cystic echinococcosis (CE)	Intermediate and aberrant hosts, rarely definitive hosts
E. multilocularis	Alveolar echinococcosis (AE)	Intermediate and aberrant hosts, rarely definitive hosts
E. vogeli	Polycystic echinococcosis (PE)	Intermediate hosts
E. oligarthrus	Polycystic echinococcosis (PE)	Intermediate hosts

3.2. Echinococcosis in definitive hosts

3.2.1. Biological aspects

The definitive host ranges of the four *Echinococcus* species are indicated in Table 3.2. *Echinococcus granulosus* characteristically uses Canidae as definitive hosts, predominantly the domestic dog, but in certain regions wild canids of several genera may by involved in the life-cycle (69) (Chapter 1). The main definitive hosts of *E. multilocularis* are foxes of the genera *Vulpes* and *Alopex*, and less frequently domestic dogs and cats. In North America, the coyote seems to have a significant role in the cycle. Regionally, the wolf may be involved (Table 3.2.). *Echinococcus oligarthrus* typically uses wild Felidae as definitive hosts, whereas *E. vogeli* uses the bush dog and the domestic dog (104, 105, 106) (Table 3.2.) (Chapter 1).

3.2.2. Clinical aspects

In the small intestine, the *Echinococcus* parasites penetrate deeply between the villi into the crypts of Lieberkühn attaching with the suckers and rostellar hooks to the epithelium (129). This intimate parasite-host relationship normally does not cause significant pathology. Minor changes may occur, such as local flattening of epithelial cells slight cellular infiltration of the mucosa and increased mucus production. Excretory/secretory products are released from the scolex region of the parasite and may induce the production of circulating antibodies (Chapter 3.2.3.1.1.).

Table 3.2.
Selected definitive hosts of *Echinococcus* species (105, 138)

***Echinococcus* species**	**Definitive hosts**
Echinococcus granulosus	Canidae: domestic dog (*Canis lupus* f. familiaris), wolf (*Canis lupus*), coyote (*Canis latrans*), dingo (*Canis lupus* f. dingo), silver-backed jackal (*Canis mesomelas*), golden jackal (*Canis aureus*), hunting dog (*Lycaon pictus*), cape silver fox (*Vulpes chama*), red fox (*Vulpes vulpes*), culpeo fox, magellan fox (*Dusicyon culpeus*), raccoon-dog (*Nyctereutes procyonoides*) Hyaenidae: spotted hyaena (*Crocuta crocuta*) Felidae: lion (*Panthera leo*)
Echinococcus multilocularis	Canidae: red fox (*Vulpes vulpes*), arctic fox (*Alopex lagopus*), domestic dog (*Canis lupus* f. familiaris), coyote (*Canis latrans*), wolf (*Canis lupus*), dog fox, corsac fox (*Vulpes corsac*), raccoon-dog (*Nyctereutes procyonoides*) Felidae: domestic cat (*Felis silvestris* f. catus), wildcat (*Felis silvestris*), lynx (*Lynx lynx*)
Echinococcus oligarthrus	Felidae: jaguar (*Panthera onca*), cougar (*Felis concolor*), jaguarundi (*Felis yaguaroundi*), Geoffroy's cat (*Felis geoffroyi*), ocelot (*Felis pardalis*), pampas cat (*Felis pajeros*)
Echinococcus vogeli	Canidae: bush dog (*Speothos venaticus*), domestic dog (*Canis lupus* f. familiaris)

The distribution of *E. multilocularis* in the small intestine of foxes and dogs differs quite markedly from that of *E. granulosus*. Mature *E. granulosus* is predominantly found in the anterior quarter of the small intestine, whereas the site of predilection for mature *E. multilocularis* is the posterior region (131). However, in heavy infections the parasites may be distributed throughout all sections of the small intestine.

Reports on clinical aspects of *E. granulosus* and *E. multilocularis* infection in canids are rare and consist mainly of observations from experimental infections concerned with aspects of the biology of *Echinococcus*. The presence of *E. granulosus* or *E. multilocularis* does not appear to cause any major ill effects to the definitive host even in individuals with heavy infection.

3.2.3. Diagnosis

Diagnosis of the infection with *Echinococcus* spp. in definitive hosts is difficult, because the eggs of all *Echinococcus* and *Taenia* species are morphologically indistinguishable from one another, and the characteristic small segments of *Echinococcus* spp. may be absent from the faeces or can be easily overlooked. Two major diagnostic methods have been extensively used in dogs. These are purgation with arecoline hydrobromide or arecoline acetarsol and necropsy of the small intestine. Both have been reviewed in previously published guidelines of WHO and OIE (95, 138) and are summarised here. Several new techniques are now available for the diagnosis of *E. granulosus* in dogs and *E. multilocularis* in foxes, dogs and other final hosts (see below). For practical reasons the two parasites are discussed separately.

3.2.3.1. Diagnosis of *Echinococcus granulosus* in dogs

3.2.3.1.1. Diagnosis in living animals

Safety precautions

Handling of material containing viable eggs of *E. granulosus* represents an infection risk for humans. Therefore, special safety precautions have to be observed (Chapter 7).

- **Detection of eggs and proglottids**

The *E. granulosus* infection in canids cannot be diagnosed by microscopic egg detection in faecal samples, because these eggs are morphologically indistinguishable from those of *E. multilocularis* and the *Taenia* species. Furthermore, egg excretion is often irregular. Eggs can be detected in faecal samples using routine flotation techniques or on the perianal skin using clear adhesive tape, which is pressed to the skin, transferred to a microscopic slide and examined (25). Proglottids of *E. granulosus* spontaneously discharged by dogs and detected mostly on the surface of faecal samples may allow a correct morphological diagnosis, if they are in good condition.

- **Arecoline purging**

The standard method currently used for surveys of *E. granulosus* infection in dog populations is arecoline purging. It includes the application of arecoline to dogs and the examination of faecal material discharged after purging. The technique has been described in detail in the previous guidelines (95, 138).

Arecoline is the chief alkaloid of the areca nut, the seed of *Areca catechu*. Arecoline hydrobromide is a parasympathomimetic drug with a major action on the smooth muscle of the small intestine, as well as paralysing the worm itself. The subsequent purgation carries the worms out with the faeces. For this activity the drug must be given by the oral route or occasionally per rectum. Dose rates have varied between 1.75 mg/kg and 3.5 mg/kg bw and were suitable for most dogs. Doubling or halving the dose rate does not increase efficacy, but the former may cause excessive vomiting. The drug may be given in tablet or liquid form.

Testing dogs with arecoline hydrobromide has the advantage that if a purge is induced, there is probability that some of the worm burden will be expelled and data for epidemiological studies and for education can be obtained. However, the test has serious disadvantages as shown in a Tunisian study, in which only 68% of 118 dogs purged after a first arecoline dose, and 12% of the dogs failed to purge even after a second dose (117). In the same study, only 65% of 46 infected dogs were detected positive after a single dose and 78% after a second dose (117). The strength of the arecoline test is its absolute specificity which produces 100% positive predictive values throughout the range of possible prevalence, whereas the negative predictive values are much lower at 68% after one dose and 85% after a second dose (117). Moreover, the arecoline test should not be used on pregnant bitches, aged dogs or young puppies. Occasional deaths have been reported following penetration of the intestine with sharp splinters of bone (138). Safety precautions must be taken when collecting and examining purges (Chapter 7).

Since 1958, with the initiation of the hydatid control programme in New Zealand, arecoline surveillance has been adopted for determining prevalence of *E. granulosus* in dogs in several successful control programmes, where literally millions of tests have been carried out. However, with the introduction of effective anthelmintics such as praziquantel, its role in surveillance of control strategies has almost been eliminated, but it still has a role to play in baseline surveys (53, 75, 77, 96, 118). Indeed, arecoline testing has been most useful for epidemiological studies on the comparative rates of infection among Taeniidae.

- **Immunodiagnosis**

Immunodiagnosis of *Echinococcus* spp. in definitive hosts has progressed significantly over recent years (20, 21, 23, 24, 26, 79, 97). Two main approaches have been developed and assessed:

a) detection of parasite antigens in faeces (coproantigen) and

b) serum antibody detection.

Coproantigen detection by enzyme-linked immunosorbent assay

Several groups have described ELISAs for the detection of coproantigens released by cestodes, including *Taenia* species of dogs and humans (2, 3, 28, 29), *E. granulosus* of dogs (1, 3, 20, 21, 22, 30, 31, 71, 83, 114) and *E. multilocularis* of foxes, dogs and cats (24, 26, 30, 34, 39, 76, 90, 91, 116). Coproantigen(s) highly specific for the genus *Echinococcus* can be detected by antibody capture ELISA in dogs experimentally infected with

E. granulosus or *E. multilocularis* by 5-10 days post infection and therefore does not depend on presence of eggs. Faecal antigen conversion to negative status occurred with five days of praziquantel treatment (30). Detection of specific antigen(s) in faecal samples from definitive hosts has the advantage over serum antibody detection in the high probability of correlation with current infection.

- **Capture antibodies**

Rabbit polyclonal antibodies (IgG purified fraction) raised against crude somatic worm extracts or excretory/secretory (E/S) preparation of immature intestinal stages of *E. granulosus* were similar in their ability to capture antigen from faecal supernatants treated with Tween 20 dispensed into microtitre wells for ELISA (2, 30). Somatic extracts are easier to produce, as they do not require *in vitro* maintenance of living preadult or adult tapeworms, but they may be less specific than E/S antigens. Other authors have used rabbit polyclonal antibodies raised against E/S antigens of adult *E. granulosus* and a murine monoclonal antibody against somatic antigen of adult *E. multilocularis* (114).

- **Specificity**

In several studies, detection of *E. granulosus* coproantigens was highly specific, and significant cross-reactions have not been observed in experimental *Taenia* spp. infections. Using faecal samples from necropsied stray dogs (with *Taenia* spp. or intestinal nematodes or helminth-free involving 183 animals), overall specificity was 97% (31). Specificity in 117 dogs infected with *Taenia* spp. was 96% and thus, not different from the overall specificity (31). Very similar degrees of specificity were reported in two other studies with independently developed tests (2, 83).

- **Sensitivity**

When *E. granulosus* worm burdens are greater than 100, the sensitivity of coproantigen tests approaches 92% to 100%; when post mortem worm counts or arecoline purge counts are below 100 worms then sensitivity is variable (29%-70%) with the current coproantigen ELISAs (22, 31, 83). Despite, an overall sensitivity for coproantigen tests of 63% to 77%, more than 90% of the biomass of adult *E. granulosus* present in a target dog population will be detectable (22, 31). The sensitivity of coproantigen ELISA for detection of *E. granulosus* infection in canids is significantly better than that based on antibody serology (22) (see below).

- **Advantages**

For coproantigen tests faecal samples are directly taken from the rectum or the ground and mixed with buffer solution (2, 9). Such samples can be stored for some days in the refrigerator or they may be deep-frozen (–20°C) until use. The coproantigen test can be used for identifying infected dogs during control programmes, including pregnant bitches, old dogs and young puppies. Improvement in sensitivity of the coproantigen test and development of species specificity (i.e. to differentiate *E. granulosus* from *E. multilocularis* infection) should occur following immunochemical characterisation of the faecal antigen(s) (Chapter 3.2.3.2.3.). Preliminary studies indicate these antigen(s) to be rich in carbohydrates (A. Fraser, J.C. Allan and P.S. Craig, unpublished findings). The development of a simultaneous test for *Echinococcus* and *Taenia* species appears to be feasible, but more research is necessary.

- **Availability of the coproantigen enzyme-linked immunosorbent assay**

The *E. granulosus* coproantigen-ELISA is currently produced and used in various laboratories (J.C. Craig, P. Deplazes, Kamiya and others, see: 'Authors and Contributors'). At least two commercial ELISA kits are now available (*Echinococcus*-ELISA from Genzyme-Virotech GmbH, Rüsselsheim, Germany; Chekit® Echinotest from Dr Bommeli AG, Liebefeld-Berne, Switzerland).

- **Coproantigen test and arecoline purging**

It has been recommended that coproantigen tests should now be assessed in further studies with a view to potentially replacing arecoline purgation as the main method of diagnosis of canine echinococcosis during routine surveillance for control programmes (97).

Serum antibody detection

Serum antibodies (IgG, IgA and IgE) can be detected in experimental canine echinococcosis using an *E. granulosus* protoscolex antigen preparation in ELISA (45, 46, 48, 49). Anti-*Echinococcus* antibodies could be detected by 2-3 weeks post infection. However, while sensitivity was reported to be high (73%) for natural canine *E. granulosus* infection in south-east Australia, there was no correlation with worm burden (45). Further application of the *E. granulosus* protoscolex ELISA in endemic areas of Kenya and Uruguay indicated poor correlation (sensitivity 35-40%), with positive worm identification at necropsy (50, 70), or after arecoline purgation (22). Specificity for this test, however, was high (70%-<95%). Similarly, sensitivity was poor for Em2 antibodies in the serum of foxes, but specificity was high (56) (Chapter 3.2.3.2.). A recombinant *E. granulosus* protoscolex antigen was reported to be 100% specific for *E. granulosus* antibodies in dog sera, but sensitivity was significantly below that for the native protoscolex antigen preparation (47).

The potential usefulness of serum antibody detection for the immunodiagnosis of *Echinococcus* in definitive hosts relates, therefore, more to population-based studies than to individual host identification. The high specificity of the above tests enables useful application in determination of presence or absence of *Echinococcus* spp. in dog or fox populations and in estimating relative exposure rates in such populations (50, 56) and may also assist in later stage surveillance of hydatid control programmes when transmission, and therefore, prevalence have become very low, for instance enabling the identification of negligent farms. Although test sensitivity may be increased by combined evaluation of various IgG classes, several basic problems remain, namely the persistence of antibodies after the elimination of the worm burden, the low sensitivity, the unclear specificity and the lack of correlation with the worm burden (79).

3.2.3.1.2. Diagnosis at necropsy

The following recommendations can be given for the diagnosis of the *E. granulosus* infection of carnivores at necropsy.

Collection of material

The small intestine should be removed as soon after death of the definitive host as possible, tied at both ends and placed in a numbered plastic bag or metal container. The material can be deep-frozen until examination at –20°C or at –70°C to –80°C. At the lower temperatures, the eggs of *E. granulosus* are killed (Chapter 7). For transport over long distances, the material can be placed on ice. The injection of a fixative (4%-10% formalin) into the lumen of the intestine is a further option for material preservation, but it is not recommended, as it makes the subsequent examination more difficult, and the use of toxic formalin requires special safety precautions. Therefore, fresh material should be used whenever possible.

Necropsy procedure

Several techniques are used for the diagnosis of the *E. granulosus* infection at necropsy.

- **Direct examination of the intestine**

The intestine can be divided into several sections, and each is placed on a metal tray, opened with a scissors and immersed in physiological saline solution saline at 37°C. Worms adhering to the intestinal mucosa can then be directly counted with the use of a hand lens or stereoscopic microscope. An initial washing and transfer to another tray may assist, when the intestinal contents interfere with observations of the intestinal wall. This method has disadvantages, because small numbers of worms may be overlooked and, where the parasites consist of only one or two segments, these also may escape detection.

- **Sedimentation and counting technique**

Where accurate worm counts are required, the best method is to divide the fresh and unfixed intestine into three or more sections. Open each section along its length, then immerse each in a large baker in physiological saline solution at 37°C for 30 min. This releases most of the worms into the fluid, particularly if intestines are examined immediately after necropsy. Worms needed for morphological or other studies can now be collected from samples of the sediment.

The intestinal wall is then scraped with a spatula. All the material is boiled and washed by sieving to eliminate most of the particulate and coloured material. The washed intestinal contents and scrapings are placed on a black tray and the worms counted with the aid of a hand lens or stereoscopic microscope. Subsampling may be required if large numbers of worms are present.

If intestines are examined immediately after necropsy and the parasites are still viable, it is advisable to remove the intestine after 30 min from the saline solution and count the worms in samples of the sediment. Since most of the worms have detached in warm saline after 30 min from the intestinal wall, scraping of the intestinal mucosa may not be necessary.

- **Parasite identification and differential diagnosis**

Echinococcus granulosus is about 2 mm-6 mm long, it has typically 3 proglottids (up to 6), the genital pore is near and usually posterior to the middle of the proglottid, and the uterus has lateral sacculations (Chapter 1 and Fig. 3.1.).

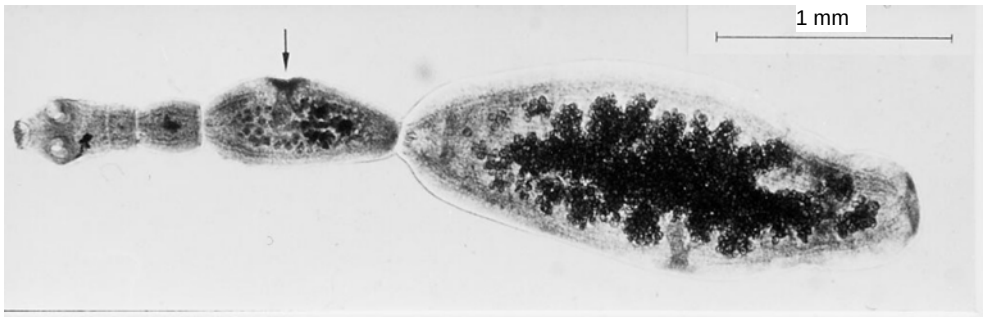

Fig. 3.1.
***Echinococcus granulosus*, gravid worm**
Arrow indicates the position of genital pore
Photograph: courtesy of the Institute of Parasitology, University of Zurich

3.2.3.2. Diagnosis of *Echinococcus multilocularis* in foxes and other final hosts

The technique currently used for the diagnosis of *E. multilocularis* infection in foxes and other final hosts is the parasitological examination of the small intestine at necropsy. Recently, techniques for detecting serum antibodies, coproantigens and copro-DNA have been described as alternatives (26, 34, 35, 39, 41, 79, 88, 90, 91, 92).

3.2.3.2.1. Parasitological diagnosis at necropsy

The following information mainly refers to foxes, but it can also be applied to other final hosts, such as dogs and cats (26, 39).

Collection of material

Whole carcasses of final hosts or the isolated small intestines (ligatured at both ends) should be tightly wrapped up in plastic bags and sent as soon as possible to a specialised laboratory, if necessary placed on ice. Carcasses and intestines can be deep-frozen at –20°C until examined. Fixatives, such as formalin, for preservation of the material are not recommended.

Pre-treatment of necropsy material and safety precautions

In order to reduce or exclude an infection risk for laboratory personnel, the carcasses or intestines should be deep-frozen at –70°C to –80°C for one week before necropsy. This procedure kills the eggs of *E. multilocularis* if the temperature is retained in all parts of the material for at least 4 days at –70°C or for 2 days at –80°C (13, 41, 136). Strict safety precautions should be observed during the whole necropsy procedure. They are indispensable if fresh material is handled, but are also recommended when the material has previously been frozen at –70°C to –80°C. A separate necropsy room with restricted access, protective clothing for the laboratory personnel and the serological examination of all persons involved in the necropsy procedure for specific anti-*E. multilocularis* antibodies once or twice per year are of special importance. Further details of the safety precautions are described in Chapter 7.

Intestinal scraping technique (IST) (26, 39)

The small intestine is placed on a large metal tray and opened in full length with scissors. After removal of coarse material (stones, bones) or large parasites (*Taenia* species, nematodes) deep mucosal scrapings are made using microscopic slides (75 mm × 25 mm × 1 mm) (coverslips are far less suitable as they are thin and fragile). The material adhering to the slide is transferred to a square plastic petri dish (9 cm × 9 cm, Falcon® No. 1012). In the petri dish, the mucosal material is squashed to a thin layer by means of pressure on the slide. In this way, each 3 slides can be placed in the lid and bottom part of each petri dish. By the use of the petri dishes for preparing the squashes, a risk of spillage of mucosal material to the tray, microscope, etc., is excluded.

Each 5 mucosal scrapings should be taken in nearly equal distances from the proximal, middle and posterior third of the small intestine (= total 15 scrapings per intestine). In about two thirds of the foxes, *E. multilocularis* is found in the posterior part of the small intestine, in others the parasites may exclusively be located in the anterior and middle section or distributed in all parts. The mucosal squashes are then examined in transmission light under a stereoscopic microscope at × 120 magnification. With this technique, 44% more infected foxes were detected than by macroscopic examination alone (43). Based on the number of parasites found in the 15 squash preparations the intensity of infection may be assessed in a subjective way as + (low), ++ (medium) and +++ (high).

Sedimentation and counting technique (SCT)

Counting can be done with fresh or previously at –80°C deep-frozen material after washing the intestinal mucosa using a dilution counting technique (85, 107, 138). The following counting technique has recently been used for the quantitative assessment of the *E. multilocularis* burden of foxes (65, 85):

- After deep-freezing at –80°C for 5 days the intestine is incised longitudinally and examined macroscopically for large helminths, and then cut into 20 cm long segments.
- The segments of the intestine are transferred to a glass bottle containing 1 l of physiological saline solution. After vigorous shaking for a few seconds, the mucosa is stripped between two pressed fingers, and the segments of the intestine are removed from the flask.
- The washing fluid with the intestinal material is sedimented several times for each 15 min, and the supernatant decanted until the sediment is sufficiently cleared from coloured particles.
- The sediment is examined in small portions of 5 ml-10 ml in rectangular plastic dishes with a counting grid (9 cm × 9 cm Falcon®, No. 1012) under a stereomicroscope at a magnification of × 120.

Parasite identification and differential diagnosis

Echinococcus multilocularis is characterised by its small size with a total body length up to 4.5 mm, typically 5 proglottids (but variable from 2 to 6), the number and size of rostellar hooks, the position of the genital pore in mature and gravid proglottids (anterior to the middle of the proglottid) and the sac-like uterus (Fig. 3.2.). The latter feature is the most important for routine examinations as the typical sac-like form of the uterus is easily discernible in squash preparations even in autolytic material, in which other parts of the

parasite are hardly visible (Figs 3.2. and 3.3.). *Echinococcus granulosus* is mostly larger (>4 mm body length), has typically 3 proglottids (up to 6), the genital pore is near and usually posterior to the middle of the proglottid, and the uterus has lateral sacculations (Fig. 3.1.).

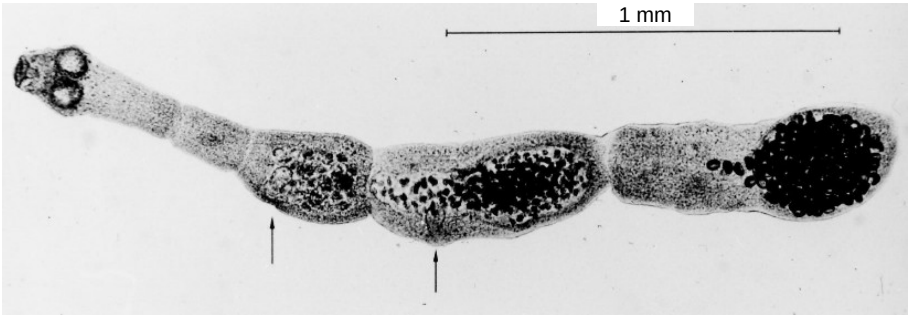

Fig. 3.2.
***Echinococcus multilocularis*, gravid worm**
Arrows indicate position of genital pore
Photograph: courtesy of the Institute of Parasitology, University of Zurich

The adult stages of other cestodes occurring in wild or domestic carnivores belonging to various genera (*Diphyllobothrium*, *Spirometra*, *Mesocestoides*, *Dipylidium* and *Taenia*) can be easily diagnosed using morphological features described in textbooks. The identification of scoleces separated from the strobila or of early immature forms in squash preparations is difficult and may require more detailed examinations on isolated (stained) specimens. Rostellar hook morphology and measurements often cannot be used for diagnosis, as hooks are frequently detached from the scolex after freezing and thawing the material. However, fragments of *E. multilocularis* can be identified with PCR (see below).

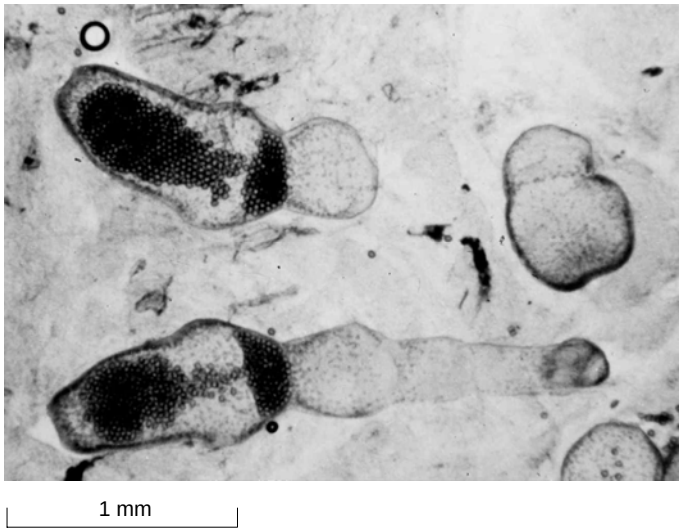

Fig. 3.3.
***Echinococcus multilocularis*, gravid worms in an intestinal squash preparation from a naturally infected dog**
Sac-like uterus clearly visible
Photograph: courtesy of the Institute of Parasitology, University of Zurich

Sensitivity and specificity

The SCT technique detected 91 infected foxes from a total number of 178, and the IST 71 (65). In comparison with the SCT, the sensitivity of the IST was 78%. A very similar sensitivity of 76% was found when the IST was compared with copro-DNA detection by PCR in 165 foxes (35). The specificities of the IST and SCT are very high (around 99%), as the morphological features of *E. multilocularis* allow an unequivocal diagnosis in most cases.

Sample size

In epidemiological studies, several testing parameters and other factors have to be considered, including the sample size. The number of foxes (or other final hosts) that have to be examined in order to detect with high probability at least one infected animal within a population of a certain size can be calculated according to Cannon and Roe (16). An example is presented in Table 3.3., which indicates that in a given fox population, a large proportion of the animals have to be examined, if the parasite prevalence is low (Chapter 5.3.).

Value of the necropsy techniques

The IST has been widely and successfully used for studies on the prevalence of *E. multilocularis* in foxes. It is relatively simple and reliable for field studies, but it underestimates the true prevalence of the parasite by about 20%. The SCT has a higher sensitivity, but it requires more expenditure. Both techniques are time-, labour- and cost-intensive and cannot be applied to living animals. Therefore, alternative techniques are required. Detection of coproantigen and copro-DNA are the most promising options (see below).

Table 3.3.
Necessary sample sizes for detecting at least one animal infected with *Echinococcus multilocularis* at a probability of 99% in a given fox population (16)

Estimated fox population size	Sample size necessary			
	20% prevalence*	10% prevalence	1% prevalence	0.1% prevalence
500	21	42	300	500
1,000	21	43	368	990
5,000	21	44	438	3,009

* Estimated prevalence of infection

3.2.3.2.2. Detection of circulating antibodies

General aspects

Various *Echinococcus* antigens (derived from adult worms, juvenile intestinal stages and oncospheres) may interact with the immune system of the host and lead to the production of specific antibodies (24, 79). Experiences with diagnostic detection of serum antibodies directed against *Echinococcus* antigens are available for dogs infected with *E. granulosus* (45, 46, 47, 48, 49, 50) and for foxes infected with *E. multilocularis* (26, 43, 56).

Antibodies against *Echinococcus multilocularis* in foxes

In an initial study (56), it was shown that specific circulating antibodies against the *E. multilocularis* antigen Em2 could be detected by ELISA in 12% to 60% of about 400 foxes originating from populations infected with *E. multilocularis*. On the other hand, 98 farmed foxes from Norway and dogs with natural or experimental non-*Echinococcus* helminthic infections were free of anti-Em2 antibodies. In the same study, it was observed that not only foxes naturally infected with *E. multilocularis* had circulating antibodies (up to 62%), but also animals from the same endemic area without detectable intestinal infection (up to 57%) (56). The latter fact may be due to antibody persistence after previous *E. multilocularis* infections which were spontaneously eliminated after a few weeks or months. It was concluded that a reliable diagnosis of the intestinal *E. multilocularis* infection in individual foxes is not feasible. In other studies (26, 39, 43), it was shown that a reliable correlation between seroprevalence of antibodies and the prevalence of the intestinal *E. multilocularis* infection in a given fox population does not exist.

Value of antibody detection

The antibody ELISA may be useful as a pre-screening test for areas in which the status of the fox population regarding the *E. multilocularis* infection is unknown. There might be a potential of improving the sensitivity of serology by the use of other antigens.

3.2.3.2.3. Coproantigen detection

Detection of *Echinococcus multilocularis* coproantigens in foxes, dogs and cats

Coproantigens of *E. multilocularis* have been detected by ELISA in experimentally infected foxes, dogs and cats, as well as in the same hosts with natural infections (24, 30, 34, 88, 90, 91, 92). In five dogs experimentally infected with over 10,000 specimens of *E. multilocularis,* coproantigen could be detected from day 5 post infection (p.i.) until the end of the experiment on day 25 p.i. (30). In four foxes experimentally infected with 150,000 protoscoleces of *E. multilocularis* and with worm burdens (four foxes) between 3,720 and 9,240 per animal, coproantigen was first detected at 4 to 6 days until 125 days post infection (p.i.), but with a distinct decline of the levels around 3 and 4 weeks p.i. (90) (Chapter 3.2.3.1.1.).

Specificity

An ELISA using polyclonal rabbit and chicken egg antibodies against *E. multilocularis* antigens (affinity purified coproantigens and somatic adult worm antigens) had very high specificities of at least 99% in large groups of dogs and cats, even if the animals were infected with intestinal nematodes. However, cross-reactivity occurred in 16% of 32 dogs infected with *E. granulosus* (34). Specificity was also high (95%) in wild red foxes (26).

Sensitivity

The overall diagnostic sensitivity of a coproantigen test was 84% in 55 foxes infected with *E. multilocularis*; it reached 95% in 37 foxes harbouring more than 100 worms but dropped to 61% in 18 animals with worm burdens less than 100 (34) (Table 3.4.). The test has also been used for the examination of individual dogs and cats or populations of these animals (34). Coproantigen has also been detected in fox faecal samples collected in the field (92, 103) (Chapter 3.2.3.3.). A comparison of the sensitivities of a coproantigen-ELISA and PCR is presented in Table 3.4.

Table 3.4.
Sensitivities of coproantigen enzyme-linked immunosorbent assay (CA-ELISA) and polymerase chain reaction (PCR) for detecting *Echinococcus multilocularis* in foxes

Coproantigen ELISA[a] (34)			**DNA detection by PCR** (85)		
Numbers of *E. multilocularis* per fox[b]	**Number of foxes examined**	**CA-ELISA positive and percentage sensitivity**	**Numbers of *E. multilocularis* per fox[b]**	**Number of foxes examined**	**PCR positive and percentage sensitivity**
4-20	10	4	4-20	8	7
21-90	8	7	55-100	5	5
Sub-total	18	61%	Sub-total	13	92%
120-350	11	9	120-500	8	8
500-60,000	26	26	>500	14	13
Sub-total	37	95%	Sub-total	22	95%
Total	55	46	Total	35	33
Overall sensitivity		**84%**			**94%**

a) coproantigen-ELISA for detection of *E. multilocularis*
b) sedimentation and counting technique (see page 79)

Predictive values

Examples of predictive values of a coproantigen ELISA for detecting *E. multilocularis* with different prevalences in fox and dog populations are presented in Table 3.5. For epidemiological investigations, especially in animal populations with low parasite prevalences, coproantigen detection by ELISA (with a very high negative predictive value) may be the method of choice. As the positive predictive value of the ELISA is relatively low in such epidemiological situations, positive ELISA results can be further confirmed with the more laborious PCR (Chapter 3.2.3.2.4.) (calculation of predictive values see Chapter 2, Annex 2.1.).

Value of coproantigen detection

The coproantigen ELISA is highly specific, it detects immature and mature stages of *E. multilocularis*, it is correlated with the worm burden and the duration of the infection, and the sensitivity is high enough (95%) to detected more than 100 *E. multilocularis* parasites per animal with high probability. The average sensitivity in foxes with low (<100) and high (>100) worm burdens per animal is approximately 85%, and thus, at least as high than that of the IST (Chapter 3.2.3.2.1.). It has to be considered, however, that sensitivity and specificity depend on the quality of the assay, which is not standardised. Samples from the content of the large intestine obtained at necropsy or fresh faecal material spontaneously excreted by final hosts can be used for the test. The coproantigen ELISA has the potential to replace or to facilitate the labour-intensive procedure of parasite detection at necropsy.

Availability of the coproantigen enzyme-linked immunosorbent assay

A commercial ELISA kit (Genzyme-Virotech GmbH, Rüsselsheim, Germany), which was mainly designed for the detection of *E. granulosus* in dogs, cross-reacts with *E. multilocularis*, but the sensitivity for this parasite is low (53%) (manufacturer's information). Another commercial ELISA (Checkit®-Echinotest, Dr Bommeli AG, Liebefeld-Berne, Switzerland) has a higher sensitivity of approximately 90% for both *E. multilocularis* and *E. granulosus* (manufacturer's information). In addition, several ELISA systems are available in various laboratories. There is a potential for refinement of the coproantigen ELISA. An interesting approach was described by Nonaka *et al.* (90), who developed a sandwich ELISA based on a polyclonal capture antibody against excretory/secretory antigens of intestinal stages of *E. multilocularis* and a monoclonal detecting antibody (76) directed to a homologous antigen. This assay can detect coproantigen in material from heat-treated (70°C for 12 h) or formalin-fixed (1%) faecal samples. However, variable results have been obtained in various laboratories with formalin-fixed material.

Table 3.5.
Examples of predictive values of a coproantigen enzyme-linked immunosorbent assay for detecting *Echinococcus multilocularis* in fox and dog populations
Test parameters: specificity 95% for foxes and 99.5% for dogs; sensitivity 80% for foxes and dogs

Predictive values	Anticipated prevalence of *Echinococcus multilocularis*						
	Fox population				Dog population		
	50%	10%	1%	0.1%	10%	1%	0.1%
Negative predictive value (percentage)	82.6	97.7	99.8	99.98	97.8	99.8	99.98
Positive predictive value (percentage)	94.1	64.0	13.9	1.6	94.7	61.8	13.8

Data from (26)

3.2.3.2.4. Detection of copro-DNA

General aspects

A PCR was described by Bretagne *et al.* (15) for the detection of DNA in faecal samples of foxes. Subsequently, this technique was modified and improved (15, 35, 85, 87, 140).

Sensitivity and specificity

In the modification after Mathis *et al.* (85), taeniid eggs were isolated from faecal samples by a combination of sequential sieving with an in-between step of flotation in zinc chloride solution. Hence, taeniid eggs from large sample volumes could be concentrated in a few microlitres of fluid and be detected by means of an inverted microscope. The DNA isolation from these eggs and PCR were basically performed according to Bretagne *et al.* (15). As determined by necropsy of small intestines of 55 foxes, the specificity of the PCR was 100% (no false-positive result with 20 foxes without *E. multilocularis*), and the overall sensitivity was 94% (Table 3.4.). Two false-negative results were with faeces from foxes harbouring immature worms (4 and 550 worms, respectively). No inhibition of PCR was observed in any sample as was demonstrated by the amplification of a size-modified target in parallel reactions. The tests were done with fresh faeces stored in 70% ethanol, but preliminary results showed that PCR detection was also possible after inactivation of eggs by deep-freezing the faeces or by incubation at +70°C for 2 h.

Predictive values

The predictive values of a PCR described by Mathis *et al.* (85) are presented in Table 3.6. The data indicate that positive PCR reactions are correct with a very high probability. Negative reactions are also reliable, especially at low prevalences (<1%) of *E. multilocularis*.

Table 3.6.
Predictive values of a polymerase chain reaction for detecting *Echinococcus multilocularis* in foxes (85)
Test parameters: specificity 100% (85); sensitivity 94% (Table 3.4.)

Predictive values	Anticipated prevalence of *Echinococcus* in the population			
	10%	1%	0.5%	0.1%
Negative predictive value (percentage)	99.3%	>99.9%	>99.9%	>99.9%
Positive predicative value (percentage)	100%	100%	100%	100%

Other PCR modifications were described, which use faecal material from foxes, but do not require concentration of *E. multilocularis* eggs (35, 66, 135). The sensitivity of the PCR described by Dinkel *et al.* (35) was 89% in average and ranged from 100% (>1,000 gravid worms) to 70% (<10 non-gravid worms).

Availability and value of copro-DNA detection

Commercial test kits are currently not available. Copro-DNA detection has been recommended by Dinkel *et al.* (35) as an alternative to the routine IST. However, the wide use of PCR for field studies will largely depend on the facilities and the costs. On the other hand, copro-DNA detection is already used as a confirmation test in selected cases, especially in dogs and cats (Chapter 3.2.3.3.).

3.2.3.3. Intravital diagnosis of *Echinococcus multilocularis* in dogs and cats

General aspects

Up to now the chances of detecting the *E. multilocularis* infection in living dogs or cats with a certain degree of probability were very low, as spontaneously excreted proglottids are very small and are only occasionally detected on the surface of faecal samples by the animal owner or at laboratory examination. By flotation techniques taeniid eggs may be detected in faecal samples, but morphological differentiation of the eggs of *E. multilocularis*, *E. granulosus* and the *Taenia* species inhabiting the intestine of domestic dogs and cats is not possible.

Detection of coproantigen and copro-DNA

The coproantigen ELISA has been applied for diagnosing or excluding the *E. multilocularis* infection in dogs and cats, using fresh or previously deep-frozen (–20°C) faecal samples (26, 34).

The negative predictive value of this test is very high (99.9%) in dog populations with low prevalence of the parasite (0.1%) (Table 3.5.). This means that a negative result of the coproantigen ELISA is correct with a high degree of probability. The positive predictive values are lower, especially if the parasite prevalence is low. Therefore, in coproantigen-positive cases the diagnosis should be verified by PCR. As this PCR is currently carried out in only a few specialised laboratories, the other option is to treat the coproantigen-positive animals under strict safety precautions with praziquantel (Chapter 3.2.4.).

3.2.4. Chemotherapy

General aspects

A number of drugs has been evaluated for efficacy against *E. granulosus* and *E. multilocularis* infection in definitive hosts (10, 52, 93, 110, 138). Until the late 1970s, treatment of the canine definitive host depended on purging with arecoline hydrobromide. The value of this drug is its expulsion effect on worms for diagnosis. Up to 9 treatments or more might be needed to eliminate all worms in 99.9% of dogs (138).

Choice of anthelmintics and dosage

The current first choice of drug is praziquantel, a isoquinoline-pyrazine derivative (2-cyclohexylcarbonyl-1,2,3,6,7,11b-hexahydro-2-H-pryrazino[2,1-a]isoquinoline-4-one (6, 126) (Droncit®, and other trade names). This has a single dose ED_{90} (= 90% of the worms eliminated) of 2.3 (1.5-3.7) mg/kg bw for *E. granulosus* (138) and of 4.6 (2.1-10.1) mg/kg bw for *E. multilocularis*. The recommended dose of praziquantel for dogs and cats is 5.0 mg/kg/bw for oral treatment and 5.7 mg/kg bw for intramuscular administration. In these dosages, the drug is highly effective against immature and mature intestinal stages of *E. granulosus*, *E. multilocularis*, *Taenia* species and some other Cestode genera (10, 93, 138). However, the drug is not ovicidal (124).

In most of the studies, a single oral administration of praziquantel (5.0 mg/kg/bw) was 100% effective against *E. granulosus* and *E. multilocularis* in all of the treated dogs, and only in some trials low residual worm burdens were reported (10, 93). For example, in one study a single treatment (5.0 mg/kg/bw per os) of five dogs had an average efficacy of 99.9% against *E. multilocularis* (115). Although the efficacy of praziquantel is highly reliable in almost all cases, the possibility of low residual worm burdens in some of the treated animals cannot be excluded, notably if mistakes of drug administration occur. According to a recent observation, 60 viable *E. granulosus* specimens were found in one of four dogs which had been experimentally infected with protoscoleces of the parasite and treated with correct doses of a combination of praziquantel, pyrantel embonate and febantel after 30 days. In addition to the living *E. granulosus* specimens, a single 75-cm long *Spirometra erinacei* tapeworm was recovered from this dog (68). The reason for this apparent lack of full efficacy is unknown, but the case indicates that treatment failure may occasionally occur in practice.

Praziquantel is available as tablets to be administered orally, and an injectable solution for intramuscular administration (subcutaneous injection is less effective against *Echinococcus*) (138). A spot-on formulation for use in cats was 100% effective against *Taenia taeniaeformis*, *Dipylidium caninum* and *E. multilocularis* (44, 72) Medicated baits were used in the People's Republic of China for the treatment of dogs (65 mg praziquantel per bait) (17), and for treating of wild foxes in Germany (50 mg praziquantel per bait) (119).

Praziquantel is safe (safety index in dogs >36) to use in pregnant animals, and dogs tolerate high doses for extended periods without organ damage or disturbance to the reproductive processes (6, 126).

Epsiprantel, a more recently developed drug, is an isoquinoline-pyrazine derivative structurally similar to praziquantel: (2-(cyclohexylcaronyl)-4-oxo-,1,2,3,4,7,8,12b-octahydropyrazino[2,1a][2]benzazepine).
Epsiprantel (Cestex®) is available as coated tablet for oral administration to dogs at 5.5 mg/kg bw and to cats at 2.75 mg/kg bw.

The drug is highly efficacious against *Taenia* species and *Dipylidium caninum* in dogs and cats (18, 84, 93), and also against *Echinococcus* species. In two studies (8, 134), a single oral dose of 5.0 mg/kg bw eliminated an average of 99.9% of the 28-day-old or 41-day-old stages of *E. granulosus* from dogs. However, in these trials only 2 out of 10 dogs were free of *E. granulosus* after treatment. A higher dose of 7.5 mg/kg bw did not

increase the average efficacy, but 6 of 10 treated dogs were parasite-free. A single oral treatment of 8 dogs with 5.1-5.4 mg/kg bw of the drug eliminated 99.9% of 20-day-old stages of *E. multilocularis* from heavily infected dogs; two of the dogs had low residual worm burdens (42). In each of 5 cats, epsiprantel treatment with 2.75 or 5.5 mg/kg bw eliminated 100% of the 20-day-old *E. multilocularis* from all cats (42).

Epsiprantel is well tolerated by dogs and cats (safety indices 90 in dogs, 36 in cats). In contrast to praziquantel, epsiprantel is poorly absorbed by the host, and therefore, a direct action against the cestodes is assumed (84).

Various other drugs, such as nitroscanate and various benzimidazole compounds, are partially effective against *E. granulosus*, but they do not reach the efficacy level of pranziquantel or epsiprantel.

Treatment intervals in control programmes

For control programmes, a single treatment is usually recommended at intervals of six weeks for *E. granulosus* and of 4 weeks for *E. multilocularis*, since the prepatent periods of these species normally exceed 42 and 28 days, respectively. There is, however, evidence that these intervals can be exceeded in control programmes as re-infection intervals may be longer than the prepatent periods. For example, in Uruguay re-infection of dogs with *E. granulosus* occurred between 2 and 4 months after specific chemotherapy (19) (Chapter 6).

Treatment of individual dogs and cats

Dogs infected with *E. granulosus* or *E. multilocularis* and cats harbouring *E. multilocularis* may represent a special infection risk if they live in close association with humans. In order to eliminate the infection risk, euthanasia of such animals may be considered. Another option is chemotherapy of the animals, but this should only be performed under strict safety precautions (Chapter 7). If praziquantel is used for chemotherapy, a 100% efficacy will normally be achieved by a single treatment. Since a low residual worm burden may persist after a single treatment – occasionally after praziquantel treatment, more frequently after epsiprantel therapy – repeated chemotherapy is recommended. The second dose of the drug should be applied within 1-7 days after the first treatment, but for practical reasons treatment may be carried out on two subsequent days. The result of the treatment should be assessed by the coproantigen ELISA and if possible by PCR.

3.2.5. Immunity and immunisation

Immune reactions of canid definitive hosts against infections with *Echinococcus* have been comprehensively reviewed (24, 59, 60). Those reactions of potential diagnostic value, extensively studied in recent years (20, 21, 24, 32, 79), are discussed elsewhere (Chapter 3.2.3.).

So far, studies on stimulation of immunity to infection through previous infection with *E. granulosus* or other taeniid cestodes or vaccination have been only partially successful or not at all. Gemmell *et al.* (53) repeatedly infected dogs eight or nine times with *E. granulosus* purging with arecoline hydrobromide between infections. A proportion of the dogs was unaffected by these infections, but 50% of the remainder demonstrated some degree of resistance by the sixth infection. However, some circumstances of the experiment do not allow definitive conclusions.

A number of experiments to induce immunity in dogs through vaccination have been carried out using non-living vaccines. Such antigens as hydatid cyst fluid, extracts of cyst membranes, or adult worms, worm secretions and protoscoleces have been used as possible sources of a suitable immunogen, but with limited success (59, 60). The effects of oncospheres fed to dogs orally as eggs or hatched and activated or injected have also been studied (59), but the results were generally disappointing. However, a short acting immune response was obtained following parenteral injection of activated oncospheres of *E. granulosus* or activated oncospheres of a number of heterologous species of taeniid cestodes. This immune response affected either the number of *E. granulosus* worms, establishing after a challenge infection, or their growth or oogenesis or all three parameters (59). These partially successful results may encourage further studies into identifying the antigen or antigens capable of conferring resistance.

There are several hints for some degree of acquired immunity to *E. multilocularis* in foxes (Chapter 5.3.), but detailed knowledge is lacking.

3.3. Echinococcosis in intermediate and aberrant hosts

Infections with the metacestode stage of *Echinococcus* spp. occur in a broad spectrum of natural intermediate host species, but also in animals which differ biologically from intermediate hosts in that they do not play a role in the transmission cycle (= aberrant or accidental hosts). The following information on metacestode infections caused by *E. granulosus* and *E. multilocularis* refers to both types of hosts.

3.3.1. Cystic echinococcosis (*Echinococcus granulosus* infection)

3.3.1.1. Biological aspects

Cysts of *E. granulosus* have been found in numerous animal species (intermediate and aberrant hosts), belonging to various groups, including Bovidae, Cervidae, Suidae, Equidae, Camelidae, Giraffidae, Elephantidae, Hippopotamidae, Leporidae, primates and marsupials (104, 105, 130, 138). Rarely, dogs and cats have been identified as hosts for the metacestode stage of *E. granulosus*. The basic structure of *E. granulosus* cysts has been described in Chapter 1.

3.3.1.2. Clinical aspects

Course of infection and organ sites of cysts

Hydatid cysts grow slowly and usually take several years to develop to a size where they may cause disease and symptoms in animals. Fertile cysts may occur within about 6 months in mice, 10-12 months in pigs, but about 2-4 years in sheep (but only 50% of *E. granulosus* cysts are fertile by 6.65 years). Cysts are rarely fertile in cattle in most countries, except where the cattle strain is present (128). The life span of cysts of *E. granulosus* can be very long, for example 16 years in horses and 53 years in man (128).

Hydatid cysts in intermediate host species occur most frequently in the liver and lungs, but they can also develop in other internal organs including the central nervous system, the skeletal muscles and in the marrow cavity of bones. The cysts of *E. granulosus* vary greatly in size and shape (typically unilocular, but sometimes multilobed or multilocular), and may be present in large numbers in one or several organs. The location of cysts and cyst morphology is controlled not only by host factors, but also by parasite factors such as the strain of *E. granulosus* involved. Usually the host and the metacestode of *Echinococcus* coexist well. Initially following infection, there is a cellular response from the host. This resolves and around the parasite a fibrous capsule (adventitial layer) develops, which enlarges to accommodate the cyst as it grows. Under certain circumstances the cellular response from the intermediate host is protracted resulting in the death of the parasite (130).

Clinical effects

The clinical effects of hydatid disease in intermediate host species have been reviewed in detail by Schwabe (121). It is well known that the infection of animals with cysts of *E. granulosus* may be asymptomatic during the whole life-span of the host. On the other hand, it has been postulated that symptoms experienced by humans infected with hydatid cysts may also occur to some degree in infected animals. However, such symptoms may be overlooked, especially in the flock or herd situation. Based on the knowledge of CE in humans (4, 5), it can be assumed for animals that the development of pathological changes is related to various factors, such as the organ(s) involved, the intra-organ site and size of the cyst (s), the cyst number, and their interaction with adjacent structures, particularly with bile ducts, the vascular system, and the bronchial tree. Indeed, some of the published cases show that the metacestode stage of *E. granulosus* may cause severe forms of CE not only in humans but also in animals, for example in horses (see below).

Anaphylaxis has been induced experimentally in sheep with hydatid cyst fluid, but sudden death in sheep or other animals ascribed to *Echinococcus* infection has never been recorded. The biochemical consequences of

infection with *E. granulosus* in intermediate hosts have been reviewed (7, 127). Most of the available data indicate disruption of normal liver function.

Cystic echinococcosis in sheep, goats and cattle

Sheep are typically infected with multiple, pleomorphic *E. granulosus* cysts mainly localised in the liver and lungs, but the spleen, heart, kidneys, the omentum and other organs can also be affected (96). Also in goats, the liver and lung are the main sites of predilection (105). In cattle, cysts are often multiple and unilocular, and the liver and lung are the organs most commonly affected. If cattle are infected with the cattle strain, cysts are predominantly located in the lungs. Less frequently, cysts have been recorded in the spleen, heart, brain and the marrow cavity of bones (105). Multicystic structures, composed of several smaller vesicles, are not uncommon in cattle and have repeatedly been misidentified as the metacestode stage of *E. multilocularis* (105).

Cystic echinococcosis in buffaloes

About 90% of all the hydatid cysts recovered from buffaloes are sterile. Cysts have been recovered most commonly from the lungs, but they have also been reported in the liver, spleen, kidneys, heart, brain, diaphragm and uterus. Several cases of massive secondary CE have also been described in buffaloes, with the involvement of thousands of secondary cysts developing either in the pleural or peritoneal cavities. These cases resulted from the rupture of primary lung and liver cysts respectively (127).

Cystic echinococcosis in horses

In horses, cysts may grow slowly so that fertile cysts do not exceed four centimetres in diameter in horses 11-16 years old and do not induce symptoms (112). However, large cysts in horses may also remain asymptomatic (132). The liver is the organ most commonly affected, but cysts have also been recovered from the lungs, brain, heart, pericardium, pleura, spleen, kidneys and uterus. It is uncommon for the lung to be the only organ affected.

Cases with distinct clinical manifestations may well occur in horses (11, 64). In one case reported from Switzerland (64), a nine years old Irish horse was heavily infected with hundreds of hepatic and pulmonary cysts (1 cm-8 cm in diameter) and showed massive enlargement of the liver (about 6.5 times), increased serum concentrations of liver enzymes, liver function disturbances, hyperbetaglobulinaemia, symptoms of chronic-obstructive lung disease, intermittent colic, anorexia and emaciation.

Cystic echinococcosis in pigs

In pigs, the liver is most commonly affected, but cysts can also be found in the lungs, kidneys, spleen, heart, skeletal muscles and occasionally the testes.

Cystic echinococcosis in wildlife

In naturally infected intermediate wildlife hosts, the site of predilection for larval *E. granulosus* may render the host more susceptible to predation. In moose in Canada, hydatid cysts occur commonly in the lungs. It has been shown that moose heavily infected with hydatid cysts in their lungs are caught more frequently by timber wolves and are usually the first to be shot by hunters (86, 102). Cystic echinococcosis has been recorded in a large number of wild animals, including aberrant hosts (105, 130, 138).

3.3.1.3. Diagnosis of cystic echinococcosis in intermediate hosts

General aspects

The diagnosis of CE in intermediate hosts of *E. granulosus* is mainly based on necropsy findings. Clinical symptoms, notably mild manifestations, may frequently be overlooked. Ultrasound examination for cystic structures may be used for the diagnosis in smaller animals, such as sheep and goats, but it has been also used in the horse (64). In Kenya, ultrasound examination of the lung and liver was used for detecting hydatid cysts

in sheep and goats (n = 260). Sensitivity and specificity of this technique were at 54% and 97% with positive and negative predictive values of 81% and 92%, respectively (113).

Necropsy techniques

For baseline surveys and for surveillance of control programmes necropsy of intermediate hosts for cysts of *E. granulosus* is essential. The principal sites of predilection are the lungs and liver. Here the most important information for determining the epidemiological status of *E. granulosus* is the age-dependent prevalence. Great care is needed to ensure that organisms other than *E. granulosus* are excluded from the analysis.

Where age-dependent prevalence is being studied in young animals, it is essential to thinly slice both the liver and lungs at about 2 mm thickness and submit all lesions for staining and microscopy. Material fixed in formalin can be processed by conventional staining methods for histological examination. The presence of a PAS-positive acellular laminated layer with or without an internal cellular nucleated germinal layer can be regarded as a specific characteristic of metacestodes of *E. granulosus*. Methods such as IFAT or PAP using monoclonal antibodies (27) or PCR (55) may be employed in the differential diagnosis of larval cestodes.

Immunodiagnosis

Immunological tests for the diagnosis of *E. granulosus* metacestodes in animal intermediate hosts are less sensitive and specific than for humans and at present cannot replace necropsy (20, 21).

Serum antibody detection

Currently, there is no suitably sensitive and specific serological test available for ovine hydatidosis or for any other livestock species (79). However, identification of exposure to *E. granulosus* at the flock or herd level by use of mean values for serum antibody activity is possible using hydatid cyst fluid antigens in ELISA and may be useful in hydatid screening and surveillance programmes. The ability to serologically identify *E. granulosus* exposure in lambs, where even autopsy diagnosis is difficult, would be especially useful in surveillance of hydatid control programmes. Hydatid cyst fluid reactive antibodies can be detected in the serum of experimentally infected sheep by 4 weeks post infection, particularly when large oral egg doses were used. In natural infections, sensitivity of serological tests is highly variable, but reports of >90% sensitivity have been recorded by groups using antigen B enriched hydatid fluid extracts in ELISA. Specificity is difficult to assess unless serum from monospecific non-*Echinococcus* helminth infections are tested. When this is done cross-reactivity has been reported to occur with *T. hydatigena*, *T. ovis* and *F. hepatica* infections (20, 79). Unfortunately, few studies have been undertaken to characterise problems with sensitivity and specificity for immunodiagnosis of CE in animals (20). Recombinant antigens may improve specificity, but sensitivity problems are likely to remain.

Detection of circulating antigen

This method does not appear to be useful for immunodiagnosis of ovine hydatidosis (79).

DNA technology

Several DNA techniques are now available which allow the identification of *Echinococcus* species and of *E. granulosus* strains using metacestode material from intermediate hosts. Details are described in Chapter 1.

3.3.2. Alveolar echinococcosis (*Echinococcus multilocularis* infection)

3.3.2.1. Biological aspects

Metacestodes of *E. multilocularis* have been reported from a large number of species of rodents and other small mammals representing at least eight families, namely Soricidae, Talpidae, Sciuridae, Cricetidae, Arvicolidae, Muridae, Dipodidae and Ochotonidae (104, 105). The epidemiological significance of the various families and species as intermediate hosts for *E. multilocularis* differs, and this is discussed in Chapter 5.3.

E. multilocularis may also infect aberrant hosts, including humans (Chapter 2) and various animal species. In recent years, there were several reports from endemic areas on such cases (Table 3.7.).

Table 3.7.
Examples of aberrant host animal species infected with the metacestode stage of *Echinococcus multilocularis* in Germany, Japan and Switzerland

Species	Country and prevalence	References
Domestic dog (*Canis lupus* f. familiaris)	Germany: sporadic cases	51
	Switzerland: several cases	33, 38, 58
	Belgium: single case	82
Horse (*Equus caballus*)	Japan: 1993-1994: 0.82% of 1,100 horses infected	94
Domestic pig (*Sus scrofa domesticus*)	Japan: 1993-1994: 0.14% of 1.1 million slaughtered pigs infected	94
	Switzerland: liver lesions in 10% of 90 pigs kept outdoors, 2.9% of 522 sows sero-positive*	33, 122
Wild boar (*Sus scrofa*)	Germany: 9% of 23 livers with lesions	99
Nutria (*Myocastor coypus*)	Germany: single case	139
Monkeys (various genera: *Gorilla*, *Macaca, Lemur*, etc.)	Germany: single case in zoo	109
	Japan: several cases in zoos	94, 123
	Switzerland: several cases in zoos	12, 33

* highly specific test: EmG11-ELISA

3.3.2.2. Clinical aspects

The effect of *E. multilocularis* on its natural and experimental intermediate hosts or on accidental hosts is much more profound than those experienced by intermediate hosts infected with *E. granulosus* due to the tumour-like proliferation of the metacestode stage which may cause severe and lethal alveolar echinococcosis.

Alveolar echinococcosis in intermediate hosts

Possibly as an adaptation to its comparatively short lived intermediate hosts, metacestodes of *E. multilocularis* develop rapidly in natural intermediate host animals, but proliferation may be slow or inhibited in certain host species. Death of the intermediate host can occur, usually around five months after infection. Development of the parasite occurs initially in the liver, but it can metastasise to other parts of the body. Typically development is rapid with protoscoleces being produced two to three months after infection. In naturally infected intermediate hosts, proliferation and increase in size of the parasite usually ceases following protoscolex production.

Clinical symptoms and pathological changes in experimentally infected rodents include, during the advanced stage of the disease, enlargement of the abdomen, increased total body weight (due to metacestode proliferation), but loss of total body mass, weakness, apathy, anorexia, ascites, intensive cellular infiltration of the liver, peritoneal cavity, other abdominal organs, and sometimes of the lungs with metacestode tissue, and finally death.

Alveolar echinococcosis in aberrant hosts

Domestic and wild pigs, horses, dogs, monkeys, and some other animal species have been described as aberrant hosts of the metacestode stage of *E. multilocularis* (Table 3.7.). In several cases, cysts of *E. granulosus* with an atypical polycystic structure have been confused with metacestodes of *E. multilocularis*. Therefore, the diagnosis has to be based on several reliable criteria (Chapter 3.3.2.3.).

Horses and swine in Japan had nodular, small (1 mm-20 mm) liver lesions, most of them showing signs of suppressed development of the metacestode (94). Similar observations were made in European wild and domestic pigs (38, 99, 122). Experimental infections of domestic pigs in Europe by oral administration of eggs (98) or intraperitoneal implantation of metacestode tissue (101) had shown that the parasite can persist for some time, but finally dies out.

In contrast, pathological changes can be very pronounced leading to clinical manifestation of disease and death in monkeys and dogs. For example, an orang-utan in a Japanese zoo showed clinical signs of emaciation, poor appetite, and severe jaundice (123). The liver was markedly enlarged with metacestode lesions 10 cm-20 cm in diameter. Protoscoleces were not observed in this case, but they were seen in other species of monkeys (109; J. Eckert, unpublished findings). Dogs with metacestode infection of the liver and/or the peritoneum had shown abdominal enlargement, ascites, hyper-γ-globulinaemia and other symptoms (33, 58). Recently, concurrent infections of the liver (with the metacestode stage of *E. multilocularis*) and the intestine (adults stages of the parasite) were observed in dogs from Switzerland for the first time (33).

3.3.2.3. Diagnosis

The diagnosis of metacestodes of *E. multilocularis* in intermediate and aberrant hosts should always be based on several criteria, including the results of macroscopic and histological examinations, and – if possible – morphology and size of protoscolex hooks. For the identification of very small and atypical lesions, the use of additional diagnostic procedures may be necessary, such as immunohistology with monoclonal antibodies, DNA-hybridisation techniques or PCR (57, 65, 79, 120). In living animals, such as monkeys and dogs, US examination of the liver and other abdominal organs in conjunction with antibody detection using specific tests (Em^2-ELISA, etc.) are the main diagnostic methods (58).

3.3.3. Chemotherapy

General aspects

Chemotherapy of animals against infection with larval *Echinococcus* has been comprehensively reviewed (4, 5, 36, 89, 138). Several groups of drugs including cytostatics, antibiotics, sulphonamides, antiprotozoal compounds and several anthelmintic drugs have been tested for their efficacy against the metacestode stage of *Echinococcus*. The efficacy trials for these drugs have been mostly carried out in rodents, but some have also been tested in domestic livestock species. The most promising results were obtained with anthelmintics of the benzimidazole group.

Based on data from animal experiments, benzimidazoles have been routinely used in recent years for chemotherapy of CE and AE in humans (Chapter 2). At present, there is no routine treatment of domestic animals against CE or AE since the application of benzimidazoles in effective dosages would be too expensive. To date, there is only one report that a benzimidazole compound (albendazole) has been used for the treatment of dogs with alveolar echinococcosis in the liver (33, 58).

Benzimidazoles

The first report of the anthelmintic effects of this group of drugs against the metacestode stage of taeniid cestodes was made by Thienpont *et al.* (125), when they described the effects of mebendazole against larval *Taenia taeniaeformis* in mice. There followed a number of studies using other benzimidazole derivatives, such as albendazole, fenbendazole and flubendazole. They were tested in rodents with secondary hydatidosis induced experimentally by intraperitoneal injection of protoscoleces of *E. granulosus*. It was found with dose rates equivalent to 30 mg/kg-50 mg/kg bw, daily, for 60-80 days, that severe damage or killing of *E. granulosus* cysts occurred (4, 36).

Extensive trials with benzimidazoles have also been carried out in rodents infected with metacestodes of *E. multilocularis* (36). In trials using long-term treatments with albendazole (60 days), fenbendazole (60-200 days), flubendazole (60-200 days) and mebendazole (60-300 days) at 30 mg/kg-50 mg/kg bw, there was up to 99% reduction in metacestode weight, as compared with untreated controls. However, despite this substantial reduction in weight, the metacestodes remained viable after treatment in most trials, contrasting with the results obtained with *E. granulosus* using the same dose rates for a shorter time.

Trials with benzimidazoles in domestic livestock species have only been carried out using mebendazole in sheep and pigs infected with metacestodes of *E. granulosus*. To achieve killing of protoscoleces in hydatid cysts in sheep mebendazole had to be administered daily at 50 mg/kg bw for up to three months (53).

Praziquantel

Praziquantel has also been tested for its effects on hydatid cysts in animals, but the results were disappointing. The drug was found to inhibit the development of secondary cysts of *E. granulosus* in mice by up to 97% if administered a few days before infection with protoscoleces. However, if administered a few days after infection, the inhibitory effects were less marked, peaking at around 78%. Praziquantel does not have a significant inhibitory effect on the proliferation of the metacestodes of *E. multilocularis* in rodents, when administered at 30 mg/kg-50 mg/kg bw, daily for up to 60 days. Sheep infected with *E. granulosus* have been treated with praziquantel administered subcutaneously (50 mg/kg bw) or per os (100 mg/kg bw), but no visible effects on their cysts were observed (108).

In one study (108), the effects of praziquantel at a dose rate of 500 mg/kg daily were observed on equine strain secondary CE in mice. The ultrastructure of the germinal layer of the cysts was monitored. A number of changes were noted in the cysts from the treated groups compared with untreated groups. These changes were observed after 21 days of treatment in cysts judged to be alive by other criteria. Praziquantel is effective against protoscoleces of *E. multilocularis* in rodents after prolonged periods of treatment (36), and also protoscoleces of *E. granulosus* are susceptible to the drug *in vitro* and *in vivo* (89, 133).

3.3.4. Immunisation

It is now well known that the density-dependent constraint in the transmission cycle of *Taenia* species and *E. granulosus* is acquired immunity of the intermediate hosts (53). Several years ago, an important advance in the prophylaxis against infection of intermediate host species with larval taeniid cestodes has been made in Australia and New Zealand. A recombinant vaccine against *Taenia ovis* infection in sheep has been successfully developed using antigens derived from oncospheres (73, 78, 80). Vaccination trials found that 50 µg of protein of the vaccination products per sheep, using saponin as adjuvant induced 94% protection against challenge infection. A similar technology is currently being applied to develop a vaccine against *E. granulosus* and to determine how it can be applied. Recent trials with a recombinant *E. granulosus* (Eg95) vaccine have shown that in sheep protection levels of 97% to 98% against challenge infection with *E. granulosus* eggs can be achieved (61, 62, 81). A high level of immunity (about 80%) persists for 6 months, and in pregnant ewes vaccinated before lambing high level of antibody is transferred to the lambs (62). According to the present status of knowledge, several vaccinations are recommended in order to obtain a high levels protection in a flock (62).

3.4. Ethical aspects

Ethical aspects have to be considered in all fields related to echinococcosis in which animals are involved. These are predominantly the following:

a) control of dog populations

b) sampling of wild carnivores

c) sampling of small mammals

d) animal experiments with metacestode stages of *Echinococcus* spp.

e) drug testing and other therapeutic interventions.

Control of dog populations

In campaigns against *E. granulosus*, the control of dog populations may be unavoidable. The usual measures taken to reduce the numbers of dogs are extermination of stray dogs and spaying of bitches (100). In some instances, stray dogs were exterminated by shooting, in others they were captured and euthanised.

These measures have to be carried out by well trained personnel with permission of the responsible authorities under veterinary supervision. Great care has to be taken in all phases of the control programme (shooting, capture, transport, maintenance, euthanasia, etc.) that a high standard of animal protection is practised. This is not only for the benefit of the animals, but it is also important for the acceptance of the campaign by the public. The WHO has published guidelines for control of dog and cat populations (137).

Sampling of wild carnivores

For surveys on the prevalence and geographic distribution of *E. multilocularis*, the examination of foxes at necropsy is the currently used method. Shooting by experienced hunters is the best of the existing possibilities for collecting wild carnivores for necropsy studies. Live traps may be used for the study of the behaviour of wild carnivores or for sampling of material from the living animal.

Sampling of small mammals

For studies on intermediate hosts of *E. multilocularis* sampling of small mammals, predominantly rodents, by trapping is necessary. Various traps are currently used for this purpose (54, 120) (Chapter 5.3., Annex 5.3.2.). Great care has to be taken that only those types of traps are used which normally kill the animals instantaneously or in which the animals stay alive under acceptable circumstances. Frequent inspection of the traps is a precondition to avoid animal suffering as much as possible in those cases in which the traps do not function in the proper way. Trapping should only be performed with permission of the wildlife authorities under consideration of national or international rules on endangered animal species.

Animal experiments with metacestode stages

Metacestodes of *E. granulosus*, and more frequently of *E. multilocularis*, are maintained in laboratory animals, predominantly in mice, jirds (*Meriones unguiculatus*), and cotton rats (*Sigmodon hispidus*) (111). Such experiments have to be carried out under the local rules of animal welfare. If such rules do not exist, international standards should be applied. Information can be provided through the WHO Working Group on Echinococcosis.

The extension of *E. granulosus* cysts and the infiltrative proliferation of *E. multilocularis* metacestodes may lead to large parasite masses in the liver, peritoneal cavity and other sites and cause discomfort, pain and finally death of the animals. Most of the experiments can be performed and reliable data obtained if animals in the asymptomatic phase of the infection are used. Therefore, it is the ethical responsibility of the researcher to plan the experiments in a way that discomfort and suffering of the animals can be kept on the lowest possible level. In addition, professional care of the animals and good maintenance are necessary.

Echinococcus multilocularis metacestodes are normally maintained in the laboratory by serial passages in rodents. If such isolates are not needed for longer periods, they can be maintained alive for many years by cryopreservation (14, 37, 40). In this way, the number of animals normally needed for maintaining the isolates can be reduced. For some research purposes *in vitro* cultures of *E. multilocularis* cysts can be used (63, 67, 74).

Drug testing or other therapeutic trials

In WHO guidelines on echinococcosis (138), detailed recommendations for testing drugs against the metacestode stage of *Echinococcus* species were given. The main aim of these recommendations was to stimulate researchers to plan the experiments in a way that reliable results can be expected. During recent years, inadequate techniques have been used in part of the studies for drug testing (for example: evaluation of drug efficacy only by electron microscopy of metacestode tissue), so that the results were unreliable. The use of experimental animals for inadequate experiments is unethical. The same applies for repetitive work. Animal

experiments that are only performed because previously published results were unknown to the researcher have to be regarded as unethical. As access to data banks or international exchange of information is possible today to most of the researches, repetitive work can be avoidable in most instances. The same ethical aspects have to be considered in other therapeutic trials, such as immunotherapy (e.g. cytokine therapy).

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Chapter 4

Geographic distribution and prevalence

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Summary

Due to the lack of representative and well documented data from many countries, this chapter can only provide an incomplete and preliminary picture of the present geographic distribution of echinococcosis and the epidemiological situation. However, the available data indicate that human echinococcosis continues to be a significant public health problem in numerous countries. From several regions there are alarming indications of increasing human health risks caused by echinococcosis. These facts should be reasons for health authorities to establish internationally co-ordinated systems of surveillance and risk assessment and to improve and support measures for control and prevention.

Echinococcus granulosus *has a world-wide geographic distribution and occurs in all continents. High parasite prevalences are found in parts of Eurasia (for example Mediterranean region, Russian Federation and adjacent independent states, the People's Republic of China), Africa (northern and eastern regions), Australia and South America. In some European countries or regions the annual incidence (AI) rates of hospital cases of human cystic echinococcosis (CE) vary between <1.0 and >8.0 per 100,000 population. In Xinjiang, a highly endemic province of the People's Republic of China, the average AI was 8.7 per 100,000 in 1990, but up to 42 per 100,000 in one of the counties. High incidence rates or prevalences have also been recorded from countries in northern and eastern Africa (prevalences up to >3%) and South America (example: Uruguay AI of 6.5 per 100,000 population in 1997). A few islands are now free of* E. granulosus *(Iceland, Greenland) or 'provisionally free' (New Zealand, Tasmania, southern Cyprus). The occurrence of* E. granulosus *is sporadic or has not been reported from other regions, including countries in northern and central Europe, in the Pacific Region, and in the Caribbean. The synanthropic cycle with domestic dogs as final hosts and sheep or other livestock animals as intermediate hosts predominates as an infection source for humans world-wide.*

Echinococcus multilocularis *is distributed in the northern hemisphere, including endemic regions in central Europe, most of northern and central Eurasia, parts of North America, and possibly an isolated focus in northern Africa (Tunisia). In central Europe, endemic areas were known to exist in only four countries by the end of the 1980s, but recent studies revealed a much wider geographic range, including at least twelve countries (Austria, Belgium, the Czech Republic, Denmark, France, Germany, Liechtenstein, Luxembourg, the Netherlands, Poland, the Slovak Republic and Switzerland). A new focus was detected in 1999 on the Norwegian Islands of Svalbard (Barent's Sea). In central European countries and regions the prevalence of* E. multilocularis *in red foxes varies from <1% to >60%. Presently, there are indications of emerging risk factors, such as increasing parasite prevalences in foxes, growing fox populations and progressive spread of foxes to cities. Human cases of alveolar echinococcosis (AE) were recorded in recent years from seven central European countries. Retrospective country-wide or regional AIs of verified human AE were low (0.02-1.4 cases per 100,000 population). More recent serological/imaging surveys of larger and smaller population groups in endemic areas revealed local prevalences between 11 and 40 cases per 100,000 individuals, respectively. Up to May 1999 more than 400 live AE patients from six countries were registered in an ongoing pilot study, most of them from France, Switzerland, Germany and Austria. In the Mediterranean region, human cases of AE are recorded from Turkey and Iran.* E. multilocularis *is endemic in large regions of the Russian Federation and adjacent countries (Belarus, the Ukraine, Moldova, Georgia, Armenia, Azerbaijan, Uzbekistan, Kazakhstan, Turkmenistan, Tajikistan, Kyrgyzstan). In the People's Republic of China,* E. multilocularis *is mainly distributed in the western and central parts, including regions of the provinces Xinjiang, Qinghai, Ningxia, Gansu, Inner Mongolia, Sichuan and Tibet. In Gansu a survey of 3,331 persons revealed AE in 135 cases, corresponding to a local prevalence of approximately 200 per 100,000 population. In Japan,* E. multilocularis *is endemic in Hokkaido where the parasite spread from approximately 8% to 90% of the area between 1981 and 1991 and is*

responsible for a considerable number of human AE cases. The endemic area of North America includes the northern tundra zone of Alaska (USA) and Canada, and further south a northern central region, including parts of three Canadian provinces (Alberta, Saskatchewan, Manitoba) and thirteen States of the United States of America (Montana, Wyoming, North and South Dakota, Nebraska, Minnesota, Iowa, Wisconsin, Michigan, Missouri, Illinois, Indiana and Ohio). There are indications of widening of the range of the parasite. Most human cases have been reported from the northern zone and only two from the northern central region.

Echinococcus vogeli *and* Echinococcus oligarthrus, *the causative agents of human polycystic echinococcosis (PE), are endemic in Central and South American countries. To date, at least 96 human cases of PE have been diagnosed in an area stretching from Nicaragua in the north to Chile, Argentina and Uruguay in the south, but it is assumed that the real extent of the disease is not yet fully assessed.*

The purpose of this chapter is to provide an overview of the approximate geographic distribution and prevalence of echinococcosis, and the most important transmission cycles in various parts of the world. However, it is difficult to draw a clear picture of the current situation because published data are quite often incomplete (period and area of study not mentioned, number of examined individuals not given, methods of data sampling and assessment not described, only percent values presented, wrong use of the terms prevalence and incidence, criteria for the description of high or low prevalence not clearly defined, etc.), some data are only listed in reports or in abstracts which are not generally accessible via databases, and for some countries or regions information is lacking. Therefore, an attempt was made to characterise the epidemiological situations by presenting selected examples of prevalence or incidence rates of echinococcosis which may not be representative for a given country/region but can provide at least some basic information on the magnitude of the problem. For an improved world-wide epidemiological assessment, a new approach for uniform data collection is needed urgently.

Technical note

The reader is referred to the specific definitions of the terms 'prevalence' and 'incidence' (see 'Glossary of Terms and Abbreviations'). For prevalence data obtained from relatively small groups of humans, the term 'group prevalence' is used, in order to indicate that the information may not be representative for larger groups or populations. n: stands for number of individuals examined; (120/250) means that 120 of 250 examined individuals (animals or humans) were infected. G: is the abbreviation for genotype.

4.1. *Echinococcus granulosus*

Transmission of *Echinococcus granulosus* occurs predominantly in a synanthropic cycle with domestic dogs as definitive hosts and livestock animals as intermediate hosts. The spectrum of intermediate host species in this cycle depends on the strain of *E. granulosus* (Chapter 1), regional or local differences in the availability of various intermediate host species and other factors. Highest prevalences of cystic echinococcosis (CE) in humans are found in populations involved in sheep raising, thus emphasising the overwhelming public health significance of the dog-sheep cycle and the sheep strain of *E. granulosus*. In some regions or countries, sylvatic cycles of *E. granulosus* exist and may play a role as an infection source for both domestic animals and humans.

4.1.1. Global distribution of *Echinococcus granulosus*

Echinococcus granulosus has a world-wide geographic range and occurs in all continents including circumpolar, temperate, subtropical and tropical zones (38, 162). The highest prevalence of the parasite is found in parts of Eurasia, Africa, Australia and South America (Fig. 4.1.). Within the endemic zones, the prevalence of the parasite varies from sporadic to high, but only a few countries can be regarded as being free of *E. granulosus* (see below).

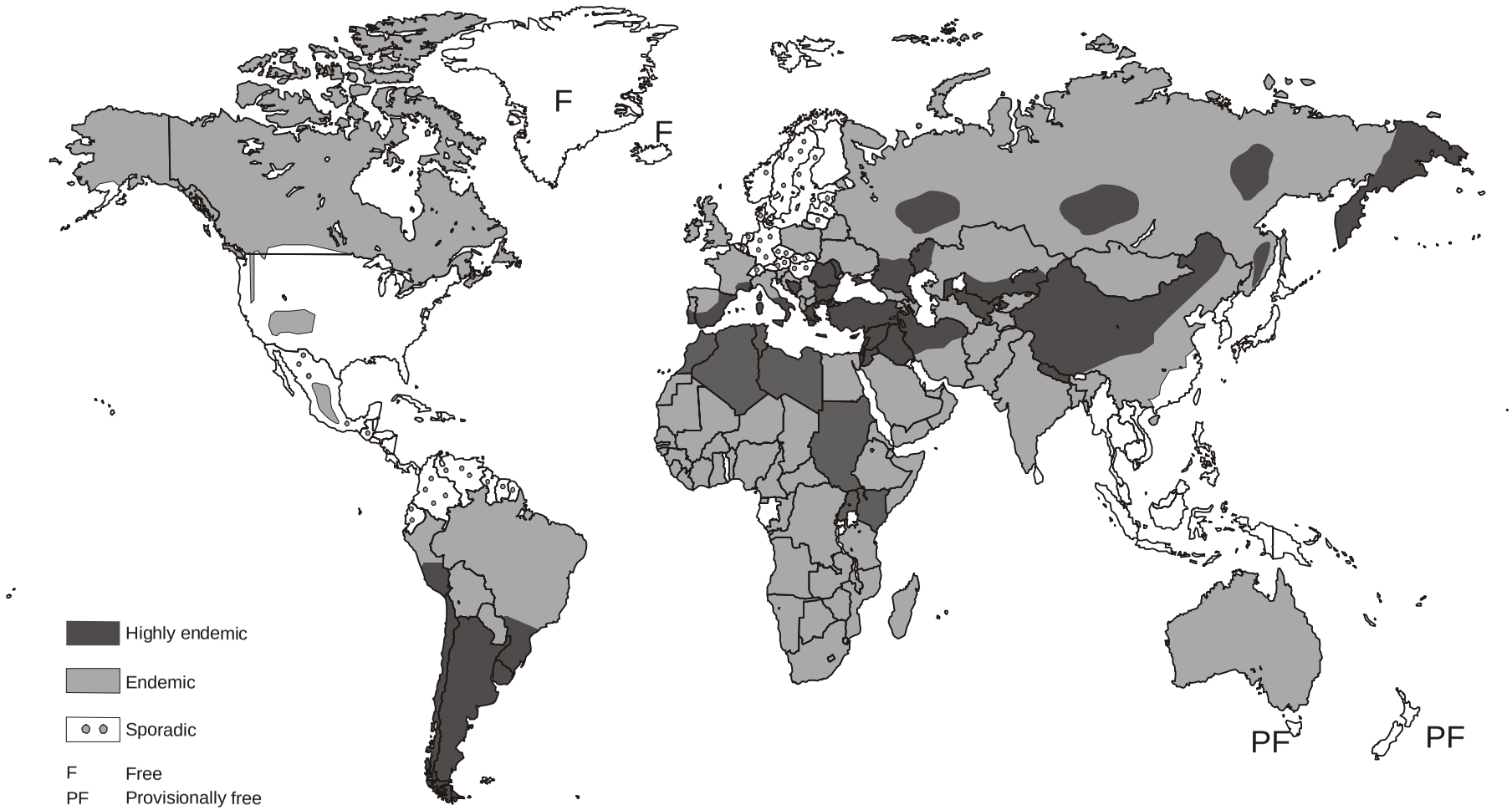


Fig. 4.1.
Approximate geographic distribution of *Echinococcus granulosus* (1999)
Source: F.L. Andersen *et al.* (7, 8), J. Ci-Peng (33), P.S. Craig *et al.* (38), A.S. Bessonov (18) and P.M. Schantz *et al.* (162); for further references, see text

Note: exact identification of endemic and highly endemic areas in all regions is not possible because of incomplete or lacking data

4.1.2. *Echinococcus granulosus* in Europe

Geographic range

In Europe, *E. granulosus* has an uneven geographic distribution with very low prevalence rates in some of the northern and central countries, but with medium or high prevalences in regions of southern, south-eastern and eastern regions. Iceland and Greenland are free of the parasite (55, 162).

Animal hosts, transmission cycles and strains of *Echinococcus granulosus*

Transmission of *E. granulosus* occurs predominantly in synanthropic cycles, involving domestic dogs as definitive hosts and sheep, goats, cattle and pigs as intermediate hosts. Wolves (*Canis lupus*), red foxes (*Vulpes vulpes*), wild ruminants (red deer: *Cervus elaphus*; roe deer: *Capreolus capreolus*, etc.) and wild pigs (*Sus scrofa*) have been found occasionally to be infected with *E. granulosus* in some countries, but normally do not play a significant role in disease transmission (162). In southern England, *E. granulosus* was found in 0.1% of 843 red foxes (152). Several strains of *E. granulosus* have been identified in Europe, including the sheep, cattle, horse and pig strains (Chapter 1).

4.1.2.1. Northern Europe (Iceland, Greenland, Norway, Sweden, Denmark, Finland and the Baltic States)

Echinococcus granulosus has been eradicated in Iceland, and the last human case of CE was diagnosed in 1960 (162, 179). In Greenland, which belongs to Denmark, the parasite has never been recorded (137). Endemic foci of *E. granulosus* were known to occur in northern Norway and Sweden, with reindeer as intermediate hosts and dogs as definitive hosts (174). Infection rates of slaughtered reindeer had already decreased in previous years to low levels, namely, 0.17% in Norway (1981-1982) and 0.27% in Sweden (1960-1972) (174). More recent information is apparently not available. One rare case of hydatid cyst infection in a reindeer was diagnosed in 1992 in north-eastern Finland, but faecal examination of 95 local dogs did not detect eggs consistent with those of *E. granulosus* (138). According to the Office International des Epizooties (137), *E. granulosus* cysts have not been recorded in 1997 from slaughtered animals in Norway, Sweden, Denmark and Finland, but in the latter country in wild animals (137). The Baltic States, Lithuania and Latvia have reported cysts in cattle and pigs, respectively, but there are no records from Estonia (137). In Lithuania, 5 cases of human echinococcosis were officially registered in 1996, corresponding to an annual incidence of 0.14 per 100,000 population (153).

4.1.2.2. Western and south-western Europe (United Kingdom, Republic of Ireland, France, Spain and Portugal)

In the United Kingdom (UK), *E. granulosus* is endemic, with transmission occurring in England, Wales, Scotland and Northern Ireland (38). Human cases of CE occur predominantly in two foci with hill sheep farming, i.e. mid/south Wales (principally Powys county) and north-west Scotland (principally Hebrides Islands) (38). A recent report indicates that disease transmission continues in the County of Powys, where 4.3% and 6.0% of 96 sentinel sheep acquired the infection in two areas with control measures as compared to 10.4% of 48 sheep from an area without control interventions (115, 143). In Powys, the annual incidence of human CE has declined from 3.9 per 100,000 population in 1974-1983 to 2.3 per 100,000 in 1984-1990 (115). In Ireland, only the horse strain appears to be present (162, 174; P.R. Torgerson, personal communication, 1999), but *E. granulosus* is not recorded officially from livestock animals (137). Little data are available from France where a nation-wide slaughterhouse survey in 1989 revealed the following average infection rates with hydatid cysts: 0.42% in sheep and goats, 0.13% in cattle, and 0.009% in pigs (170). The highest infection rates were recorded from 8 departments in the south of the country (170). In a 1994 survey, 0.31% of 43,148 slaughtered cattle were found to be infected in the Midi-Pyrenees (21). In 1966-1970, the average annual incidence rates of human CE were 10 and 4.5 per 100,000 population in Corsica and some eastern regions, respectively (62, 174), but recent reports are not available. In Spain, *E. granulosus* is highly endemic in various regions, as documented by the prevalence rates of hydatid cysts in sheep of over 5 years of age in 1993 (percentages in parenthesis): Navarra (19.8%), La Rioja (47.8%), La Mancha (no data), Guadalajara (data for 1989: 80.3%), Manserja (11.5%), Madrid (35.0%), Aragon (79.8%), and Extramadura (10.5%) (74) (Table 4.1.). In La Rioja, a control programme has reduced the infection rate from 1993 to 1998 in sheep

from 47.8% to 27.4%, and in dogs from 1.4% to 0.9%, respectively (92). In the same area, the annual incidence of human CE declined from 9.4 in 1993 to 5.6 per 100,000 population in 1998 (92). Between 1993 and 1996, an average of 396 (range: 354-449) hospital cases of human CE was recorded in the entire country, corresponding to annual incidence rates of 1.1 per 100,000 in 1993 and of 0.9 in 1996 (74). Information on Portugal is based on older data (Table 4.1.). The nation-wide incidence of human CE is estimated to be 2.2 per 100,000 population (47).

4.1.2.3. Central Europe

Exceptional or sporadic occurrence of *E. granulosus* cysts in slaughtered livestock animals is recorded in official reports for 1997 from Belgium (cattle), Luxembourg (cattle), Switzerland (cattle), Germany (cattle) and Austria (cattle) (137). There are no reports from the Netherlands; in the Slovak Republic and Hungary the last cases were diagnosed in slaughter animals in 1995 and 1993, respectively (137). Although the accuracy of these data cannot be assessed, it is evident that the occurrence of *E. granulosus* in domestic animals is currently sporadic in countries of this region in central Europe. This is supported by recent data. For example, the infection rate of sheep in the Slovak Republic varied between 0.04% to 4.6% in 1988-1994 (172). In the Czech Republic, the prevalence rates in 1994-1995 were also very low in large numbers of animals: up to 0.73% in sheep, 0.003% in cattle and 0.005% in pigs (107). In Switzerland, the infection rate in cattle declined from an average of 1.48% in 1969 to sporadic occurrence in 1999 (J. Eckert, personal communication, 1999). Representative data from recent years on the infection rate of dogs in central European countries appear to be lacking.

Data on human CE from this region are limited. In Switzerland, a total of 228 new cases were diagnosed between 1984-1992 with an annual average of 25 cases. On average, 25% of these cases were diagnosed in Swiss nationals and 75% in foreigners (majority from Iberian Peninsula, Italy, the Balkan States and Near East) (61). The annual incidence of CE in the total population of Switzerland was 0.38, in Swiss nationals 0.09, and in foreigners 1.49 per 100,000 population (61). In three federal states of Austria, the annual incidence rates varied between 0.21 and 0.67 in 1983-1992 (14).

In Poland, hydatid cysts were found in 1997 in 18.7% of sheep and goats (2,439/13,005), 0.007% of cattle (119/1.550 mio.), and 4.5% of pigs (770,364/16.907 mio.) (51). Domestic dogs are regarded as definitive hosts (no data on prevalence). Human CE is regularly diagnosed in Poland: 14 cases were officially recorded in 1998 and 18 in 6 months of 1999, but these data appear to be incomplete (Z.S. Pawłowski, personal communication, 1999).

4.1.2.4. Eastern Europe (see Chapter 4.1.4.)

4.1.2.5. Southern and south-eastern Europe (Italy and Balkan countries)

Echinococcus granulosus is endemic or highly endemic in most of the countries of the region. In Italy, the country-wide infection rates in sheep varied between 11% and 87% in 6 regions in 1980s (72). The highest prevalences in dogs and livestock animals have been reported from the island of Sardinia, where the annual incidence of human CE was 8.0 per 100,000 in 1990 (Table 4.1.). The average incidence rate of human CE for the period 1980-1984 was 1.92 per 100,000 population in the entire country and 0.46 to 10.1 in various regions (72). High endemicity was also recorded in recent years from some of the Balkan countries, including Yugoslavia, Romania, Bulgaria and Greece (Table 4.1.); there are no or incomplete reports from other countries of the region. Due to the deterioration of the economic situation and the cessation of control programmes in Bulgaria, echinococcosis in animals and humans has re-emerged in recent years. Between 1971-1982 and 1983-1995, the infection rates in dogs and sheep have increased from 4% to 7% and from 19% to 32%, respectively (181). During the same periods the average nation-wide annual number of surgical cases (new and readmitted) of human CE has increased from 176 to 291, and the corresponding annual incidence rates rose from 2.0 to 3.3 per 100,000 (182). In 1995, the average incidence rate by district showed variations from 1.9 to 15.8 per 100,000, with high endemicity particularly in southern parts of the country (182).

Table 4.1.
***Echinococcus granulosus* in Europe: examples of prevalence or incidence**

Country/region	Period[a]	Prevalence in animals (percentage)				Incidence in humans	Ref.
		Dogs	Sheep	Cattle	Pigs	Annual incidence (100,000 population)[c]	
Spain							
La Rioja (+ C)[d]	1995-1998	0.5-0.9[b]	27.4-38.7	–	–	6.2 (5.6-7.1)	92
2-7 regions	1993	0-1.4 (2 regions)	10.5-79.8 (7 regions)	–	–	1.0 (0.9-1.1) (1993-1996)	74
Portugal							
Entire country	1944-1968	10.4 (1972)	2.2[b]	4.3[b]	4.6[b]	2.2 (210 surgical cases per year)	47
Italy							
6-9 regions	1984-1992	–	11.4-86.9	–	–	1.9 (0.46-10.1)	72
Sardinia	1990s	16-20	86.9[b]	23.7[b]	17.6[b]	8.0 (1990)	35, 62
Yugoslavia							
Montenegro	(1997)	65.4[b]	7.8[b]	–	–	–	101, 102
Romania							
Entire country	1987-1994[e]	21.6 (0.5-87.5)[f]	39.9	32.8	–	Frequent	139, 140
Banat county	1990-1997	16.6-66.6[g]	11.0-19.6	9.9-19.5	0.5-18.9	505 cases = 63 per year	36
Bulgaria							
Entire country	1983-1995	7	32	19	1.5	3.3	181, 182
Greece							
Macedonia	(1994)	–	100[b]	56.6[b]	9.3[b]	3.4 (entire country: see ref. 62)	62, 82
Turkey							
Various regions[h]	1987-1997	0.9/40.5	57.1	9.4	–	4.4 (average: 1987-1994)	5
Cyprus							
Greek Cypriot Community[i]	1998	0	0.006[b]	0.022[b	–	1990-1993: no new cases under the age of 20	62
Turkish Cypriot Community[j]	1997	–	78.7 (n: 286)	–	–	5.7	62

a) period of survey or year of publication in brackets
b) for these data, the numbers of animals examined are given in the publications, but had to be omitted from the Table for technical reasons
c) hospital cases
d) + C: data from an area with control measures
e) data for dogs from 1987-1994, for sheep and cattle period of examination not given
f) range, depending on group of dogs
g) stray dogs
h) dogs: regions of Ankara (0.9 %) and Kars (40.5 %), sheep and cattle: region of Konya
i) southern part of the island
j) northern part of the island
– no data given

4.1.3. *Echinococcus granulosus* in the Eastern Mediterranean

4.1.3.1. Turkey

Turkey has a high prevalence of *E. granulosus* in dogs and livestock animals, and a high incidence of CE in humans (5, 62) (Table 4.1.). Between 1987 and 1994, the Ministry of Health has recorded 21,303 hospital cases of human CE, with an annual average of 2,663 (range: 2,295-2,958) (5). Related to a total population of approximately 61 million, the average annual incidence rate is 4.4 per 100,000 inhabitants.

4.1.3.2. Cyprus

In Cyprus, *E. granulosus* was widespread before the 1970s, and the average annual incidence of human CE was 12.9 cases per 100,000 population (62). Strict control measures in the Greek Cypriot Community have reduced the infection rates in animals to very low levels, and human cases did not occur in persons under the age of 20 between 1990-1993 (62) (Fig. 4.1.). However, in the Turkish Cypriot Community, where control measures have only been implemented recently, echinococcosis remains a public health problem. Between 1990 and 1996, a total of 80 surgical cases was recorded in this part of the island, with a population of 198,000, resulting in an annual incidence rate of 5.7 per 100,000 (62). If patients who had been operated abroad are added to the local surgical cases, the incidence rate for 1995 was estimated to be at least 25 per 100,000 (62).

4.1.3.3. Gulf Littoral States

The Gulf Littoral States include the Sultanate Oman, the United Arab Emirates (UAE), the States Qatar, Bahrain and Kuwait, the Kingdom of Saudi Arabia, the Republic of Iraq and the Islamic Republic of Iran (42). The epidemiological situation regarding the *E. granulosus* infection in animals and humans has been reviewed comprehensively in 1997 (42, 43).

Geographic range

The countries of the Gulf region have always had endemic foci or regions of *E. granulosus* in animals hosts associated with CE cases in humans (42, 43). This is confirmed by recent reports from various countries.

Animal hosts and transmission cycles

The principal definitive hosts of *E. granulosus* in the Gulf region are domestic dogs which are mostly kept as guard or shepherd dogs or live as stray dogs, often in large populations (42). Like in most other endemic regions, a wide range of infection rates of dogs have been found in previous surveys: 15% in stray dogs of eastern Saudi Arabia, 23% in Kuwait, 38% in Iraq and 34% in Iran (42). These data are complemented by more recent reports on prevalence rates of *E. granulosus* in dogs. Iran: western parts (Zanjan area) 33.0% of 115 stray dogs (in 1995) (135), western regions 82% of 55 stray dogs (year not given) (199), 27.2% of 390 sheepdogs from 13 provinces with highest prevalence of 63% in Isfahan province (69); northern Iraq 49.5% of 97 stray dogs (1991-1998) (157). In Iran, wild carnivores have been reported as hosts of *E. granulosus* with infection rates of 22% in jackals (*Canis aureus*), 33%-100% in wolves (*Canis lupus*) and 12% in red foxes (*Vulpes vulpes*) (42).

Important intermediate hosts of *E. granulosus* in the Gulf region are sheep, goats, cattle and camels (42). The seasonal importation of millions of livestock animals, including hydatid cyst carriers, for religious sacrifice from endemic countries (Sudan, Ethiopia, Somalia, Kenya, Uganda and Australia) (42) represents another potential source of infection for local dogs. In Saudi Arabia (El-Gassim), average infection rates of sheep and goats (n: 88, 771) with hydatid cysts were 2.5% and 5.0%, respectively (169). In the UAE, hydatid cysts are regularly found both in local and imported animals (42). In Iran, high infection rates were found in 1995-1996: sheep 19.1% (1,047/5,477), cattle 22.9% (441/1,923) (135), and also in camels (11%-64%) and buffaloes (42). From northern Iraq, the following prevalence rates were reported for 1991-1998: sheep 15.0% (191/1,270), goats: 6.2% (34/550), and cattle: 10.9% (35/320) (157). Wild ruminants were identified as carriers of hydatid cysts in Iran, such as wild sheep (*Ovis ammon orientalis*), a gazelle (*Gazella subgutturosa*), and

wild boars (*Sus scrofa*) (42, 201). In the whole region, synanthropic cycles with dogs and livestock animals predominate, while the epidemiological role of wildlife or mixed cycles appears to be of minor importance.

Strains of *Echinococcus granulosus*

In Iran, two strains of *E. granulosus* were identified using mitochondrial DNA markers: the sheep strain (G1) in sheep, goats and human patients, and the camel strain (G6) in camels (201).

Cystic echinococcosis in humans

Like in many other endemic regions, it is difficult to summarise data on the prevalence or incidence of CE in humans. In Saudi Arabia, CE accounts for approximately 0.3% of all general surgeries, and in Kuwait the prevalence is estimated to be 1.6-3.6 cases per 100,000 population (42, 43). Due to special epidemiological circumstances in Oman, the prevalence of *E. granulosus* in animals and humans is apparently low (86). In the entire Iran more than 5,000 patients with CE were treated surgically in a 14-year period (1980-1993), corresponding to an annual average of 357 cases and an estimated annual incidence of at least 0.5 per 100,000 (about 70 million inhabitants) (11). In northern Iraq (Arbil province), 99 hospital cases were recorded between 1990 and 1998 resulting in an annual average of 12.4 cases and an estimated prevalence of 2 cases per 100,000 (157). Previous assessments had estimated the nation-wide prevalence rates at 1-20 cases per 100,000 (157).

4.1.3.4. Levant countries

The Levant countries include Jordan, the Palestinian Autonomy, Israel, Syria and Lebanon. Detailed epidemiological information on this area was reviewed in 1997 (1).

Geographic range

All of the Levant countries belong to the endemic zone, where *E. granulosus* occurs in animal hosts and causes CE in humans (1).

Animal hosts, transmission cycles and strains

As in the Gulf countries (Chapter 4.1.3.3.), synanthropic cycles with dogs and livestock animals are of principal importance in the Levant region. This is substantiated by findings of *E. granulosus* in dog populations, for example in Jordan with prevalences of 14.5% and 9.7% in groups of 173 and 341 dogs, respectively (1) and 9.4% of 340 stray dogs (66), or in northern Israel with a prevalence of 10.7% in 206 dogs (80). High infection rates in dogs have also been reported in previous years from Syria and Lebanon (1).

The spectrum of intermediate hosts includes domestic ruminants, camels, and donkeys (1). Only two examples of the prevalence rates of hydatid cysts is livestock animals are given here: these rates in Jordan were 12.7% for sheep (n: 4,549), 0.9% for goats (n: 4,200), 12.9% for cattle (n: 275), and 17.2% for donkeys (n: 122); camels (1/9) were also infected (1); in northern Israel 5.9% for sheep (n: 874), and 5.3% for goats (n: 616) (83). Morphological, biological and biochemical features suggest that the sheep, horse and camel strains of *E. granulosus* occur in Jordan (1, 129), but confirmation by molecular techniques has apparently not yet been done.

Cystic echinococcosis in humans

Human cases of CE continue to occur in all Levant countries. Recent data are not available from all countries, but two examples are given to characterise the situation in this area. In Jordan, 676 surgical cases have been recorded between 1985 and 1993, and the annual surgical incidence rates in different regions of the country were estimated to be 0.5 to 8.2 cases per 100,000, with a mean for the entire country of 2.9 per 100,000 (99). In the Palestinian Autonomy and Israel, the estimated annual incidence was high in previous years, reaching 9 per 100,000 population in 1968 in the entire area and 53 per 100,000 in 1980-1989 in Yirka, an Arab/Druze village in northern Israel (1). In southern Israel, during 1995-1999, the mean annual surgical incidence rates in Jews and Bedouins (Muslims) were 0.41 and 1.08 per 100,000 population, respectively (65).

4.1.4. *Echinococcus granulosus* in the Russian Federation and adjacent countries

Previous studies have shown that CE in humans represented a considerable public health problem in the Russian Federation and the adjacent independent states (125, 149, 162). At present, it is difficult to obtain current information from this area as research and surveillance activities in this field have been greatly reduced since 1990. According to local experts there are no indications for a decrease in prevalence of *E. granulosus* in animals and humans and of the resultant public health consequences, but there are recent reports on CE as an emerging problem, for example in Kazakhstan (165).

Geographic range

According to reports published between 1985 and 1990, it can be assumed that *E. granulosus* in animals and humans has a wide geographic distribution in the Russian Federation. Endemic or highly endemic areas have been identified in many parts of the country (Fig. 4.1.), for example in the south-west region between the Black Sea and the Caspian Sea (Machačkala, Stavropol, Krasnodar, Rostov and Volgograd), in the western central region (Samara, Ufa, Kazan and Perm), southern Siberia (Omsk, Novosibirsk, Tomsk and Kranoyarsk), central Siberia (Jakutsk), north-eastern Siberia (Chukotka), and eastern and south-eastern Siberia (Magadan, Kamchatka and Amur region with Kkabarovsk). This description is not comprehensive, but is indicative of the magnitude of the problem which results from the wide distribution of *E. granulosus* in vast areas.

Highly endemic areas are also known to exist in adjacent countries: In the west: Belarus, the Ukraine (southern part) and Moldova; in the south-western Caucasus region: Georgia, Armenia and Azerbaijan; in the south: Kazakhstan, Turkmenistan, Tajikistan, Uzbekistan, Kyrgyzstan; and also Mongolia and the People's Republic of China (for the latter two countries: Chapters 4.1.5. and 4.1.6.).

Animal hosts and transmission cycles

In the large endemic region of the Russian Federation and adjacent countries, *E. granulosus* is transmitted in various cycles involving different definitive/intermediate host assemblages (149, 162).

- **Sylvatic cycles**

In the northern taiga and tundra zone, stretching from Fennoscandia in the west to the Bering Strait in north-eastern Siberia, *E. granulosus* is perpetuated in a cycle with the wolf (*Canis lupus*) and wild cervids (elk and reindeer) as hosts (162). In Yakutia or Sakha (north-eastern Siberia), approximately 40% of wolves (9/23), 68% of elks (*Alces alces*) (34/50) and 1.0% of reindeer (114/11,304) were *E. granulosus* carriers, according to older studies (87). In other regions jackals and wild boar are principal hosts of a sylvatic cycle.

- **Mixed cycles**

Several cycles of this type are known to occur, for example domestic dog and elk or wild reindeer in north-eastern Siberia; jackals and farm animals in Azerbaijan and Uzbekistan, and domestic dogs and wild boars in Belarus. In previous years, high infection rates of *E. granulosus* have been found in domesticated reindeer (25%-70%) and also in domestic dogs (149, 162).

- **Synanthropic cycles**

Cycles involving domestic dogs and farm animals (sheep, goats, cattle, pigs or camels) occur widely distributed in the Russian Federation and adjacent countries (149, 162). According to older reports, prevalences of *E. granulosus* in these animals were high in various regions. This was also observed in some more recent surveys. For example, in the Russian Republic of Bashkortostan (capital: Ufa) 38% of sheep, 7.9% cattle and 3.1% of pigs were infected with hydatid cysts in 1994 (73). From Uzbekistan, the following prevalences were recorded for the period 1984-1996: sheep 47.2% (5,174/10,953), cattle 20.8% (852/4,089); pigs 7.7% (–/2,598), and 10.1% (53/522) or 17.7% (56/316) of two groups of dogs (130). In southern districts of Moldova, 72.6% of sheep, 49.1% of cattle and 18.2% of pigs were infected in 1992-1996 (22).

Recent data suggest that in the south of Kazakhstan the prevalence of *E. granulosus* in adult sheep has increased from 38% in 1986 to 62% in 1999 (165).

Strains of *Echinococcus granulosus*

Predominantly morphological and biological studies (results are usually in agreement with genetic analyses) have provided evidence for the occurrence of several strains of *E. granulosus*, including the cervid strain in the northern tundra zone and Far East; the sheep, cattle and pig strains in many parts of the Russian Federation and adjacent countries, and the camel strain in Kazakhstan and Middle Asia (19, 59, 60, 158, 162, 168, 197).

Cystic echinococcosis in humans

In Russia, during 15 years (1983-1997) a total of 2,863 cases of human echinococcosis (CE and AE) was officially recorded (average of 191 cases per year) (109; A.S. Bessonov, personal communication, 2000). However, these figures indicate only the 'tip of the iceberg'. In 1993, 140 cases of CE were recorded in the Chukotka region (north-eastern Siberia) alone (112). An annual average of 3.8 new cases of human CE was reported from the Russian Republic Bashkortostan (total: 46 cases, 1983-1994) (73). From the southern districts of Moldova, an incidence rate of 15.5 per 100,000 population has been reported for the period 1992-1996 (22). In Kazakhstan, the annual incidence of surgical cases increased from 0.9-1.4 cases per 100,000 population during 1974-1990 to 2.5 and 4.4 cases per 100,000 in 1997 and 1999 respectively, representing an increase in annual case numbers from 221 in 1990 to 659 in 1999 (165, 183). This increase of incidence rates has been most marked in the south of the country in the Zhambyl Oblast from 3.8 in 1990 to 10.3 per 100,000 in 1997, and the South Kazakhstan Oblast from 2.7 in 1990 to 3.6 per 100,000 in 1997 (165). Another focus of high endemicity in the north-west of Kazakhstan is in the Uralsk region (P.R. Torgerson, personal communication, 1999). In Kyrgyzstan, in 1998 the incidence was 14.1/100,000 with 661 cases recorded (range 9.9 to 17.9 cases/100,000) (183). These examples indicate that CE is of considerable public health significance.

4.1.5. *Echinococcus granulosus* in Mongolia

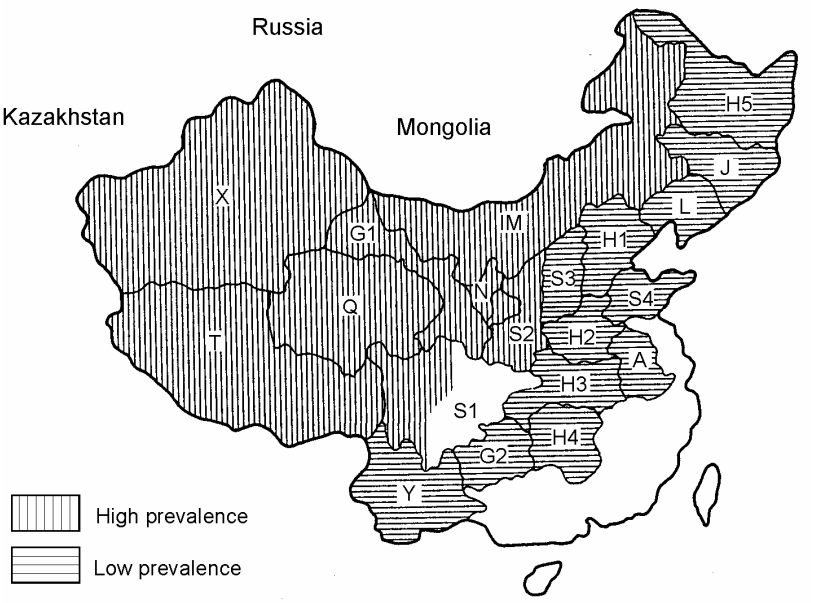
Little published information seems to exist on the epidemiological situation in Mongolia, but a recent study in the north-western part of the country has shown that 5% of 334 semi-nomadic pastoralists were strongly seropositive in the ELISA for antibodies to *E. granulosus* antigen B. This finding suggests that CE is likely to be a public health problem in that area (194).

4.1.6. *Echinococcus granulosus* in the People's Republic of China

Human CE constitutes one of the major public health problems in the People's Republic of China, as documented in a number of informative reviews and other publications which can only be cited selectively herein (7, 30, 33, 38, 162, 163, 167, 195).

Geographic range

Echinococcus granulosus is endemic in at least 21 of the People's Republic of China's 31 provinces, autonomous regions and municipalities, covering approximately 87% of the country's entire territory (195). The highest prevalences in animals and humans occur in the pastoral and semi-pastoral western provinces and regions, including the provinces of Xinjiang, Qinghai, Gansu, Ningxia, Inner Mongolia, Tibet, and parts of Sichuan and Yunnan with a wide range of geographic, climatic and socio-ecological conditions (7, 38, 167). Epidemiological surveys indicate that the prevalences of the parasite decrease from west to east. *Echinococcus granulosus* and *E. multilocularis* may occur sympatrically in some areas (Figs 4.1. and 4.2.).



A : Anhui
G1 : Gansu
G2 : Guizhou
H1 : Hebei
H2 : Henan
H3 : Hubei
H4 : Hunan
H5 : Heilongjiang
IM : Inner Mongolia
J : Jilin
L : Liaoning
N : Ningxia
Q : Qinghai
S1 : Sichuan
S2 & S3 : Shanxi
S4 : Shandong
T : Tibet
X : Xinjiang
Y : Yunann

Fig. 4.2.
Approximate geographic distribution of cystic echinococcosis in the People's Republic of China
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Animal hosts

The principal animal hosts of *E. granulosus* in the People's Republic of China are domestic dogs and sheep, but also other domesticated herbivores are also frequently found to be infected, including goats, cattle, yaks, pigs, horses, donkeys, camels and farmed red deer (*Cervus elaphus*) (162; H. Duolong and D.J. Jenkins, personal communication, 1999). Some examples of prevalences of *E. granulosus* in animal hosts are presented in Tables 4.2. and 4.3. Of wild animals, wolves (*Canis lupus*) and foxes were identified as definitive hosts, and Blue sheep, antelopes and *Ochotona* as intermediate hosts (33).

Table 4.2.
***Echinococcus granulosus* in the People's Republic of China: examples of prevalence in domestic dogs**

Province/region	Period	Number of dogs examined	Percentage infected (and range)	References
Xinjiang				
Northern counties	1983-1990	4,318	17.7 (7.1-70.0)	114
Southern counties	1986-1990	4,795	5.6 (0-51.8)	114
Qinghai	–	303	(19.5-82.3)	167[a]
Gansu				
Counties	–	360	9.2 (6.6-16.7)	167[b]
Sichuan				
Western parts	1997-1998	–	21.2	148

a) Xu, 1995, cited in 167
b) Wang, 1996, cited in 167
– no data given

Transmission cycles

Synanthropic cycles involving domestic dogs and predominantly sheep or other domesticated herbivores appear to be the most common transmission patterns and the main infection sources for humans in the People's Republic of China. The findings of *E. granulosus* in wild carnivores and herbivores are indicators for the existence of sylvatic or mixed cycles, which are apparently of minor importance.

Table 4.3.
***Echinococcus granulosus* in the People's Republic of China: examples of prevalence in intermediate host animals[a]**

Province	Percent prevalence (numbers of animals examined)					
	Sheep	Yaks	Cattle	Goats	Pigs	Camels
Xingjiang	0-99 (431,326)	41 (41)	0-88 (15,673)	4-42 (2,769)	0-38 (10,761)	–
Gansu	8-77	30-76	51	14-32	30-70	–
Qinghai	10-100 (45,263)	5-100	72	–	5-20	–
Ningxia	52	–	81	3	24	19
Tibet	56	–	66	–	–	–
Inner Mongolia	15-48	–	–	–	–	35
Sichuan	82 (4,104)	50 (3,645)	–	41 (125)	–	–

a) the data were published by various authors between 1984 and 1995 and are summarised by Shi (167); see this paper for original references

– no data given

Strains of *Echinococcus granulosus*

Analyses of DNA sequence variation have recently shown that the common sheep strain (genotype G1) and the camel strain (genotype G6) of *E. granulosus* occur in northern Xinjiang (200).

Cystic echinococcosis in humans

From 6 highly endemic provinces or autonomous regions (Xinjiang, Gansu, Qinghai, Ningxia, Tibet and Inner Mongolia), a total of 26,065 surgical cases of CE was recorded during four decades between 1951-1990 (30), corresponding to an annual average of 651 cases. The analysis of 15,289 surgical cases from Xinjiang has indicated a steady increase of case numbers which reached an average of 1,218 cases per year during 1986-1990. This increase was thought to be due in part to an improvement of medical services (127). Statistics on age distribution showed that surgical cases reached a peak at 6-15 years, indicating a high infection pressure for children (Chapter 2). Up to 1993 additional cases were recorded, increasing the total number to 27,716 cases during the period 1951-1993 (167).

Based on the analysis of 15,289 of the cases diagnosed between 1951-1990 in the province of Xinjiang, an average annual incidence of 8.7 cases per 100,000 population was calculated for 1990, but the local rates in the various prefectures ranged between 0.07 (Hetian Prefecture) and 28.4 (Tacheng Prefecture), with even higher incidences in some counties, for example in the Yumin County (Tacheng Prefecture) with 42.2 cases per 100,000 population. In Xinjiang, there were 46 counties with high annual incidence rates of surgical cases (>5 cases/100,000), 15 with medium (1-5 cases/100,000), and 23 counties with low rates (<1 case/100,000) (127). These data, which possibly underestimate the actual number of cases, are indicative of a very serious situation in some of the highly endemic areas. This is also supported by recent prevalence studies. For example, in western Sichuan 3,999 individuals were examined in 1997-1998 for echinococcosis by abdominal sonography and chest X-ray; in 2.1% of them *E. granulosus* cysts were detected (148), representing a group prevalence of

2,100 per 100,000 individuals. In another study in southern Qinghai, 3,702 individuals were examined in 1997-1998 using the same methods, and 7.6% had lesions indicative of CE or AE, but unequivocal differentiation was not achieved (193). Data from this group allow the calculation of a group prevalence of 7,590 per 100,000 individuals for both forms of echinococcosis. As pointed out in other parts of this Chapter, group prevalences, particularly those from smaller population groups at high infection risk, may not be representative for larger populations or regions, but they can provide an indication of the severity of the problem and can serve as statistical parameters for comparisons between different endemic foci.

4.1.7. *Echinococcus granulosus* in southern Asia

Echinococcus granulosus appears to occur in most of the countries of the area, including Afghanistan, Pakistan, Nepal, Bhutan, Bangladesh, India and Sri Lanka (162).

4.1.7.1. Afghanistan and Pakistan

In Afghanistan, *E. granulosus* has been found (according to older surveys) in 73% of 105 stray dogs in Kabul (113) and also in various livestock animals (67). In one recent study in Pakistan (Feisalabad), the following overall infection rates were reported for 1998 (numbers of animals examined): sheep 3.5% (n: 480,000), goats 4.3% (n: 200,000); cattle: 5.3% (n: 31,200); buffaloes: 2.4% (n: 16,800), and camels: 12.5% (n: 500) (9).

4.1.7.2. India, Bangladesh and Sri Lanka

In India, *E. granulosus* has a wide geographic distribution, as indicated by reports on parasite prevalences in livestock animals from various parts of the country, for example from Uttar Pradesh in the north and several states in the south. To give only a few examples, a recent study in southern India (States of Karnataka, Maharshtre, Kerala, Tamil Nadu, Pondichery and Goa) revealed overall prevalences of *E. granulosus* cysts of 7.0% in sheep (106/1,519), 7.1% in cattle (31/439), 9.4% in water buffalo (*Bubalus bubalus*) (46/489), and 11.5% in pigs (10/87) (78). Another study carried out from 1995 to 1997 in Pondichery showed higher infection rates of 37.8% in 325 sheep and of 47.6% in 680 goats (44). In the region of Uttar Pradesh, infection rates in sheep were 2.9% (9/312), in goats 1.4% (39/2,710), and in pigs 0.9% (27/2,980) (45). *Echinococcus granulosus* is also known to occur in livestock animals (cattle, buffaloes, sheep and goats) in Bangladesh, with infection rates between 2.4% to 56%; human cases are reported also, but not well documented (128).

4.1.7.3. Nepal and Bhutan

In Nepal, *E. granulosus* was found at necropsy and in coproantigen surveys in dogs in Kathmandu; the coproantigen prevalence was 5.7% (5/88) and 1.8% (3/171) in two groups of dogs, respectively (17). The infection rates in buffaloes were 5% (n: 3,065), 8% in sheep (n: 150), 3% in goats (n: 1,783) and 7% in pigs (n: 143) (Joshi, cited in 38). *Echinococcus granulosus* cysts have also been found in yaks on 17 (31%) of 55 farms surveyed in various regions (95). Human cases of CE were reported but not quantified (96).

4.1.8. *Echinococcus granulosus* in South-East and East Asia

Sporadic cases of human CE, either imported or locally acquired, have been reported from various countries in this region, including Laos, Thailand, Vietnam, Malaysia, Indonesia, the Philippines, Korea, Japan and Taipei China, but neither in a previous review (162) nor in the recent literature could information be found on the current prevalence of the parasite and the general epidemiological situation.

4.1.9. *Echinococcus granulosus* in Oceania, Australia and New Zealand

There are little published epidemiological data for the islands of the Pacific region. *Echinococcus granulosus* had not been recorded in Papua New Guinea until 1989, but the subsequent finding of sterile *E. granulosus* cysts in cattle was suggestive for the occurrence of the parasite on that island (6). In contrast, detailed information is available on New Zealand and Australia (162).

4.1.9.1. New Zealand

Prior to 1959, approximately 80% of adult sheep and 10% of dogs in New Zealand were infected with *E. granulosus*. Since then, a national eradication campaign has been operating (Chapter 6) which reduced the annual prevalence of hydatid cysts in livestock to <1 per million in 1993 (80). In 1995, 26 million lambs, 7.3 million adult sheep, 1.3 million calves/vealers and 2.5 million adult cattle were slaughtered, and cysts consistent with those of *E. granulosus* were found in only 13 animals from different properties (80), which corresponded to 0.35 cases per million. In 1999, it was anticipated that New Zealand could enter the new millennium 'hydatid-free' (80).

4.1.9.2. Australia: Tasmania

Before the implementation of an echinococcosis control programme in the island state of Tasmania in 1960, the prevalence of *E. granulosus* in animals was high, with approximately 12% in dogs, 52% in 3-year-old sheep and 17% in cattle. Between 1946 and 1958, the average annual incidence of human CE was 9 cases per 100,000 population. The causative parasite has been identified as a distinct strain of *E. granulosus* (Tasmanian sheep strain, G2) (Chapter 1) and was transmitted entirely via a synanthropic cycle, involving domestic dogs, sheep and cattle (162, 180). The control programme has interrupted disease transmission to humans since 1977 or earlier, but occasional cases are still recorded and are considered to represent 'old infections' (180). *Echinococcus granulosus*-infected dogs have not been detected since 1985-1986, infection of cattle was reported in the mid 1980s to the early 1990s only on King Island (Bass Strait), and infection of sheep in Tasmania was reduced to a few cases each year. In 1996, the island state of Tasmania was provisionally declared 'free' of *E. granulosus* infection with respect to dogs and sheep. Since then, approximately 1 million sheep and 170,000 cattle have been inspected at slaughter in Tasmania, and cysts consistent with those of *E. granulosus* were detected in only 3 sheep (0.0003%) and 12 cattle (0.007%) (180).

4.1.9.3. Australia: mainland

The epidemiological situation on the mainland of Australia is more complex than in the state of Tasmania as there is a wildlife reservoir of the *E. granulosus* infection.

Geographic range

Echinococcus granulosus is widely distributed on the mainland of Australia, as indicated by reports published over the years on the occurrence of the parasite in domestic or wild animal hosts in Western Australia (120), Queensland (116), New South Wales (88), the Australian Capital Territory (Canberra) (89), and Victoria (77). Great differences in prevalence exist among various regions. For example, in Western Australia, 78% of 304 cattle found to be infected with *E. granulosus* cysts originated from the Kimberley region, whereas the percentages of infected animals from 6 other locations/areas were much lower (0%-8%) (120).

Animal hosts, transmission cycles and strains of *Echinococcus granulosus*

There are several transmission cycles on mainland Australia:

a) the synanthropic cycle, involving domestic dogs and domestic herbivores (predominantly sheep) as hosts
b) the sylvatic cycle with wild dogs (dingoes, feral dogs or dingo/feral dogs hybrids) and red foxes (*Vulpes vulpes*) as definitive hosts and macropod marsupials (kangaroos) as intermediate hosts, and
c) the mixed synanthropic/sylvatic cycle.

The only strain of *E. granulosus* involved in cycles *a)-c)* is the common sheep strain (G1) (Chapter 1).

There is no recent information on the prevalence of *E. granulosus* in domestic dogs. Previous studies of wild dogs in regions considered to be endemic have shown prevalences ranging from 48%-90% (162). More recently, a study in Victoria has revealed that 14 of 15 (93%) wild dogs (dingoes, feral dogs or dingo/feral dogs hybrids) harboured *E. granulosus* and that they are considered to represent an important source of infection for sheep and macropods (77). It has been proposed that some of the rural cases of human CE may be the result of ingestion of eggs spread by wild dogs and/or foxes (77). Red foxes (*Vulpes vulpes*) have been

found infected with intestinal stages of *E. granulosus* in localities in south-eastern New South Wales (162), the Australian Capital Territory (3 infected of 45) (89) and north-eastern Victoria (150).

Data on the prevalence of *E. granulosus* in sheep, cattle and pigs have been reviewed comprehensively by Schantz *et al.* (162), but there appears to be no subsequent report on the assessment of the current epidemiological situation for livestock intermediate hosts.

As shown in various studies (89, 162), wild animals (such as feral pigs, and several species of marsupials) acquire natural infection and harbour *E. granulosus* cysts. For example, in one study in Victoria, 4 of 17 Eastern Grey kangaroos (*Macropus giganteus*) and 2 of 10 wombats (*Vombatus ursinus*) harboured hydatid cysts (89).

Cystic echinococcosis in humans

Human cases of CE continue to occur on mainland Australia. In the 4-year period between January 1991 and December 1994, the National Notifiable Diseases Surveillance System of Australia recorded 170 human cases of CE (annual average: 42.5 cases). Notifications were received from all States and Territories, with the majority of reports from Queensland, New South Wales and Victoria (116). Assuming an approximate total population of 18.1 million people, an average annual incidence of 0.23 cases per 100,000 population can be calculated. However, a case-finding study from 1987-1992 (90, 126), based on records from 38 hospitals or health services in New South Wales and four hospitals in the Australian Capital Territory, revealed 195 new cases (annual average over 5 years: 39), compared with a total of 40 officially notified cases from these two states in the overlapping period 1990 to 1994 (annual average over 5 years: 8) (126). This latter comparison suggests that human CE is seriously under-reported in Australia.

4.1.10. *Echinococcus granulosus* in Africa

Echinococcus granulosus has been recorded from most of the African countries. Several reviews of the epidemiological situation were published between 1995 and 1997 (8, 38, 162).

4.1.10.1. North African countries

Geographic range

Previous and recent reports describe the endemic occurrence of *E. granulosus* in dogs and livestock and of human cases of CE in all north African countries bordering the Mediterranean Sea, including Morocco, Algeria, Tunisia, Libya and Egypt (84, 97, 142, 166) (Fig. 4.1.).

Animal hosts, transmission cycles, and strains of *Echinococcus granulosus*

The parasite is transmitted predominantly in a synanthropic cycle involving dogs (large dog populations and many stray dogs) and various livestock animals (sheep, goats, cattle and camels). Older and more recent studies have shown high prevalences of *E. granulosus* both in dog populations and in one or more livestock species in various countries and regions (Table 4.4.). In all countries where the camel has been reported as intermediate host, it is considered to be important for the local maintenance of the life-cycle (162). Wild carnivores can be hosts of *E. granulosus*, for example the Golden jackal (*Canis aureus*) in Algeria (121) and a fox species (*Vulpes rueppelli*) in Egypt (63). Two strains of *E. granulosus* known to occur widely in North Africa are the sheep strain and the camel strain (121).

Cystic echinococcosis in humans

Recent data emphasise that CE in humans continues to be a significant public health problem in Morocco, Algeria, Tunisia and Libya (Table 4.5.), and apparently to a lesser extent in Egypt where the annual incidence of hospital cases in estimated to be less than 1 per 100,000 population (166). Community-based ultrasound studies have revealed alarmingly high prevalences of CE of approximately 1%-2% in Tunisia and Libya (Table 4.5.). The prevalence of 1.7% in a large study in Libya involving 20,220 individuals corresponds to a group prevalence of 1,676 per 100,000 individuals.

Table 4.4.
***Echinococcus granulosus* in northern Africa: examples of prevalences in animals[a]**

Country/region	Period[b]	Animal species	No. of animals examined/ infected[c]	Percentage of infected (range)[d]	References
Morocco					
Khemisset Province	(1997)	Dog	103/34	33.0	142
5 regions	1980-1985	Sheep	4,014/–	9.9 (0.7-25.9)[e]	97
4 regions	1980-1985	Goat	1,660/–	3.2 (1.4-5.2)[e]	97
5 regions	1980-1985	Cattle	4,844/–	42.0 (23.3-56.9)[e]	97
Algeria	1989	Camel	250/2	0.8	32
Tunisia					
9 regions	1982-1996	Dog	568/–	22.5 (4.4-45.7)	104
8 localities	1993-1996	Sheep	410/269	65.6	110
Libya					
5 localities	1985-1988	Dog	92/33	35.9 (0-60.0)	15
6 localities	(1998)	Sheep	367/58	15.8 (0-37.9)	85
6 localities	(1998)	Goat	184/7	3.8 (0-8.2)	85
6 localities	(1998)	Camel	514/248	48.0 (38.7-55.2)	85
Egypt	1992-1996	Camel (imported)[f]	400,159	4.3-8.2/year	79

a) for more detailed information for previous years, see Andersen *et al.* (8)
b) period of examination or year of publication in parenthesis
c) necropsy studies
d) if not otherwise indicated, ranges refer to various localities or regions
e) average calculated from percent values
f) imported from the Sudan
– no data given

4.1.10.2. Sub-Saharan Africa

Geographic range

As shown in Figure 4.1., *E. granulosus* has been recorded from most of the sub-Saharan countries over a vast area stretching from the Sahel zone to southern Africa.

Animal hosts, transmission cycles and strains of *Echinococcus granulosus*

Previous or recent reports indicate wide variations in the prevalences of *E. granulosus* in dog and/or livestock populations in various countries and regions (Table 4.6.). Areas of high endemicity are known to occur in eastern Africa, including at least parts of Sudan, Ethiopia, Kenya and Uganda (38, 121). Large parts of western, central and southern Africa apparently have lower prevalences but an accurate assessment is difficult due to lack of recent and more comprehensive data. Several strains of *E. granulosus* have been identified in various parts of Africa, i.e. the sheep, cattle, horse, lion strains (121) (Chapter 1). Synanthropic cycles involving dogs and livestock animals are most important, but wildlife-cycles exist involving a number of wild carnivores (jackal species, hyena, lion, etc.), wild ruminants and pigs (121).

Cystic echinococcosis in humans

Several ultrasound surveys have confirmed the high prevalence of CE in humans in certain population groups and areas. In 1985-1987, a large study was performed in semi-desert regions of 4 countries (Sudan, Ethiopia, Kenya and Tanzania) involving 18,565 persons of various ethnic groups. The average prevalence of CE was

1.8%, with a range between 0 and 5.6 in various regions and population groups (122). High prevalences were also observed in north-eastern Turkana in Kenya: approximately 7.5% before (1983) and 3.1% 10 years (1992) after the initiation of control measures (data extracted from a graph) (121). In 1996, 3,224 persons were screened in southern Ethiopia, and 16 (0.5%) were positive for CE (123). In Mauritania, the annual incidence of CE was estimated to be 1.2 per 100,000 population for the period 1996-1997, based on the number of cases diagnosed at the National Hospital in Nouakchott (20).

Table 4.5.
***Echinococcus granulosus* in northern Africa: examples of incidences or prevalences of cystic echinococcosis (CE) in humans[a]**

Country/region	Period	Number of cases of CE	Annual incidence per 100,000 population (hospital cases)	References
Morocco				
Entire country	1980-1992	13,973	4.8	64
Entire country	1980	702	3.6	64
Entire country	1992	1,347	5.3	64
Infrane Province	1980-1992	230	15.8	64
Algeria	1970-1979	–	3.4-4.6	166
Tunisia				
Entire country	1982-1985	–	0-56.0	166
Entire country	1977-1982	4,124	–	166

Country/region	Period	Number of people examined/infected	Percent prevalence (ultrasound survey)	References
Tunisia				
3 studies	1986-1991	4,263/98	2.3% (0.4-3.6)	166
Libya				
Northwest	1992	4,103/57	1.4%	166
North	1997	20,220/339	1.7%	166

a) for more detailed information for previous years, see F.L. Andersen *et al.* (8)
– no data given

4.1.11. *Echinococcus granulosus* in North America

Geographic range

Two strains of *E. granulosus* are known to occur in North America, the cervid strain and the sheep strain (161, 162). The former is prevalent in the holarctic zones of the tundra and boreal forests of Alaska and Canada and occurs also under favourable conditions at lower latitudes; it is transmitted in a sylvatic cycle. The sheep strain occurs sporadically in sheep husbandry areas of the western United States of America (USA), including parts of Utah, Arizona, New Mexico, California and other western states, and is perpetuated in a dog-sheep cycle (Fig. 4.1.).

Definitive and intermediate hosts and transmission cycles

Several assemblages of definitive and intermediate hosts have been reported from North America (162):

a) wolf and wild ungulates, mainly moose and caribou, but also mule deer (*Odocoileus hemionus*) (Alaska, Canada, northern Minnesota)

b) coyotes and wild ungulates (northern California)

c) domestic dog and wild ungulates (northern California)

d) dog-sheep (Utah, Arizona, New Mexico, California and other western states)

e) dog and pig (Mississippi valley)

f) dog-horse (162).

The assemblages *a-c* are attributed to the cervid strain of *E. granulosus*, and *d* to the sheep strain (59), while the status of *e* and *d* has not yet been defined. Older studies have revealed high prevalences of *E. granulosus* in wolves (20%-70%) and moose (29%-59%), but lower rates in coyotes (<10%) and in sheep (about 5%-10%) (161, 162).

Cystic echinococcosis in neither humans nor animals is a reportable disease in the USA, so there is no systematic collection of data. However, observation and inquiry suggest that the cycles in dogs and pigs may no longer occur and that the presence of the sheep strain of *E. granulosus* (previously reported in California and Utah) has been reduced to very sporadic occurrence.

Table 4.6.
***Echinococcus granulosus* in sub-Saharan Africa: examples of prevalences in animals[a]**

Country/region	Period[b]	Animal species	Number of animals examined/infected[c]	Percentage infected (range)[d]	References
Mauritania					
Nouakchott	1995	Dog	–	Low rate	20
–	1995	Camel	–	54-60	20
Nigeria					
Plateau State	(1988)	Pig	360/12	3.3	3
Borno State	1990-1993	Camel	24,531/501	2.0	4
Sudan	1992-1996	Camel[e]	400,159	4.3-8.2/year	79
Ethiopia					
3 localities	(1996)	Dog	110/-	14.5	93
3 localities	(1996)	Cattle	2,717/-	24-46	93
2 localities	(1996)	Sheep	630/-	2-26	93
Djibouti					
Djibouti	(1996)	Sheep	–	12.0	31
Djibouti	(1996)	Goat	–	9.6	31
Djibouti	(1996)	Cattle	–	4.4	31
Kenya					
Nairobi	(1994)	Dog	58/42	72.4	192
Turkana, northeast	1992	Dog	–	~38[f]	121
Kajiado District	(1995)	Sheep[g]	612/125	20.4	133
Kajiado District	(1995)	Goat[g]	575/96	16.7	133
Zambia	1984-1985	Cattle[g]	482/69	14.3	144

a) for more detailed information for previous years, see Andersen *et al.* (8)
b) period of examination or year of publication in parenthesis
c) necropsy studies
d) if not otherwise indicated, ranges refer to various localities or regions
e) exported animals examined in Egypt
f) data extracted from a graph
g only livers examined
– no data given

Cystic echinococcosis in humans

Historically, and presently, the majority of patients diagnosed with the sheep strain of *E. granulosus* in the USA have been immigrants who acquired the infection in their countries of origin. The national origins of these persons have changed over time, reflecting changes in the flow of immigration. Until 1970, the majority of patients were of Italian and Greek origin, whereas in recent decades, patients from Middle Eastern and South American countries have been in the majority (53; P.M. Schantz, unpublished findings, 1999). Autochthonous infections with the cervid strain occur sporadically in the north and with the sheep strain and possibly other strains in other parts of the USA, mainly in populations at relatively high risk, such as sheep farmers in known endemic areas.

4.1.12. *Echinococcus granulosus* in Central and South America, the Caribbean and the Falkland Islands

Geographic range

Echinococcus granulosus is known to occur in animal hosts and sporadically in humans in countries of Central America, such as Mexico, Guatemala, El Salvador and Honduras, but has apparently not been identified in the Caribbean islands (161, 162, 179). To date, only few systematic surveys have been made, so that the current epidemiological situation of this region remains sketchy. A more recent abattoir survey (1992) in Mexico (Los Reyes, La Paz) revealed that 0.27% of about 40,000 inspected pigs were infected with *E. granulosus* cysts (187). In South America, *E. granulosus* occurs in most countries, but highest prevalences are observed in parts of Argentina, Bolivia, Brazil, Chile, Peru and Uruguay where the parasite causes significant public health problems (161, 162, 179) (Fig. 4.1.). The regions most affected by *E. granulosus* are: Argentina: Patagonia, Pampas, Coast; Bolivia: south-western parts; Brazil: Rio Grande do Sul; Chile: southern central valley regions, including Chilean Antarctica; Peru: central and southern highlands; Uruguay: entire country (Table 4.7.). In the Falkland Islands, *E. granulosus* is endemic at a low prevalence (1993: 0.47% of sheep infected with cysts) (151).

Definitive and intermediate hosts

In areas where specific control programmes are not performed the prevalence of *E. granulosus* is high, with infection rates of approximately 10% to 70% in dogs and about 25% to over 70% in sheep. Hydatid cysts may also be frequently found in goats and cattle, but less frequently in swine and horses. Some examples of infection rates in dogs, sheep, cattle and swine are presented in Table 4.7. In the southern highlands of Peru 2.1% to 8.3% of camelids (llamas, alpacas and vicunas) were infected with cysts of *E. granulosus* (131) (for other hosts, see below).

Transmission cycles and strains of *Echinococcus granulosus*

The synanthropic cycle with domestic dogs as definitive hosts and sheep as intermediate hosts, is regarded as most important in the endemic areas of South America (179). In this cycle, other intermediate hosts can be included (see above). In some countries of Central America (Mexico, El Salvador, Honduras) and northern South America (Ecuador), *E. granulosus* cysts have been reported mainly in pigs indicating that a dog-pig cycle exists (161). *Echinococcus granulosus* infection has also been reported in foxes (*Dusicyon* sp.) and hares (*Lepus* spp.) in Argentina, but this is believed to represent a 'spillover' from the dog/sheep cycle rather than an independent sylvatic cycle (161).

According to previous records, there is evidence of the occurrence of the pig strain of *E. granulosus* in Central America, of the sheep strain in several South American countries, and of the horse strain in Chile. The sheep strain was made responsible for human cases of CE. Recent studies on genetic variants using molecular techniques revealed a more complex situation and the occurrence of additional strains in South America (98, 156). In these studies the following strains of *E. granulosus* were identified in Argentina, Brazil or Chile: common sheep strain (G1), Tasmanian sheep strain (G2), cattle strain (G5), camel strain (G6), and pig strain (G7). Humans were found to be infected with G1, G2, G5 and G6. The camel strain, which was previously believed to have reduced or no infectivity for humans, was found in goats and humans (156).

Table 4.7.
***Echinococcus granulosus* in South America: examples of prevalences in animals**

Country/region	Period[a]	Percent infected animals[b]				References
		Dogs	Sheep	Cattle	Swine	
Argentina						
Rio Negro Province	1980 (– C)	42	61	–	–	28
	1990 (+ C)	7.9	6.7	–	–	28
	1996 (+ C)	2.8	5.5	–	–	28
	1997 (+ C)	2.3	18	–	–	28
Tierra del Fuego	1975 (– C)	90	75	–	–	198
	1996 (+ C)	2.5	1.2			198
Brazil						
Rio Grande do Sul	1977 (– C)	–	26	28	–	159
	1993 (+ C)	–	6	25	–	159
Chile						
Region XII	1979 (– C)	71.4	60[c]	50[c]	–	27, 84
	1995-1997 (+ C)	0.35	1.3	–	–	
Region XI	1982 (– C)	54	88	–	–	26
	1996-1997 (+ C)	6.5	10.4	–	–	26
Region X	1992 (– C)	58	85			26
	1996 (+ C)	0.7	90	–	–	26
Tierra del Fuego	1977-1978 (– C)	68	69	45	–	27
	1995-1997 (+ C)	1.7	0-1.2	–		27
Peru						
Central highlands	1989-1993 (– C)[d]	12	28	50	2.8	131
Southern highlands	1989-1993 (– C)[d]	31	13-50	16-69	9.1	131
Uruguay (not defined)	1991 (– C)	11	43	64	–	141
	1993 (+ C)	–	16	–	–	141
	1996 (+ C)	0.74	0.17[L]	23	–	141
	1998 (+ C)	–	7.6[L]	–	–	25
			18.0[A]	–	–	25

a) – C or + C: periods without or with control measures
b) as far as recorded in the cited sources, numbers of animals examined were high (dogs at least several hundred, sheep, cattle and swine mostly several thousand) so that percentage values can be regarded as representative
c) estimated by means of mathematical model
d) data for sheep, cattle and swine (1989), and dogs (1993)
– no data given
L lambs
A adults

Cystic echinococcosis in humans

Annual incidence rates of human CE based on hospital or surgical cases are presented in Table 4.8. They indicate that human CE remains a significant public health problem in particular in South American countries and regions, although control measures have been introduced in some of these areas (10) (Table 4.7.). This statement is supported by prevalence studies based on ultrasound examination of larger population groups. For example, in Uruguay 1.4% of 6,027 persons (from 7 towns in 4 states) had confirmed asymptomatic CE (146). More recently (1993-1994), in the same country (Department of Florida), sonographic evidence of asymptomatic CE of the liver was found in 1.6% of 9,515 individuals (29), and in another study (1991-1992) of 1 village (La Paloma, central Uruguay), 3.5% of new CE cases were detected in a group of 1,149 persons

(34). The data of these three studies correspond to group prevalences of 1,400, 1,600 and 3,481 per 100,000 individuals, respectively.

Table 4.8.
***Echinococcus granulosus* in South America: examples of incidence or prevalence of cystic echinococcosis in humans**

Country/region/ province	Period	Number of new cases[a]	Average per year	Annual incidence per 100,000 population[b]	Ref.
Argentina					
Entire country	1988-1992	–	464	1.42	111
Patagonia					111
Tierra del Fuego		–	3	4.3	
Santa Cruz		–	4	3.5	
Rio Negro		–	65	16.9	
Chubut		–	84	32.0	
Neuquen		–	162	67.0	
Pampas					111
Buenos Aires		–	80	0.74	
Coast					111
Corrientes		–	4	0.6	
Other parts		–	62	–	111
Chile					
Entire country	1978-1989	6,956	580	(4.0)	189
Brazil					
Rio Grande do Sul	1973-1984	470	31	0.33	46, 159
	1980-1991	>600	>50	(0.54)	
Peru					
Entire country	1989-1992	975	244	1.1	131
Uruguay					
Entire country	1972	552	552	17.4	141
	1995	293	293	9.2	141
	1997	–	–	6.5	Annex 6.1.1.

a) hospital or surgical cases
b) data calculated by authors of this Chapter are given in parentheses

4.2. *Echinococcus multilocularis*

The geographic distribution of *E. multilocularis* depends on the occurrence of its natural definitive and intermediate hosts, which are wild canids, mainly foxes, and small mammals, predominantly arvicolid and cricetid rodents, respectively. Some other species of wild canids as well as domestic dogs and cats are also susceptible and may act as definitive hosts in some regions (Chapters 3 and 5.3.). The prevalence of the infection and the dynamics of transmission also depend upon innate susceptibility of hosts, the nature of relationships between them, host population densities, seasonal fluctuations, host age, diversity of diets, and other factors. The spatial distribution of *E. multilocularis* is highly variable, and significant differences may exist in the prevalences of the parasite between larger regions and even within small habitats of only a few hectares. This results in a focal or patchy distribution which is usually not reflected in maps showing the general geographic distribution (Fig. 4.3.). It should be considered that prevalence data also depend on the diagnostic techniques used and the number of hosts examined (Chapter 3).

Fig. 4.3.
Approximate geographic distribution of *Echinococcus multilocularis* (1999)
Source: J. Eckert (57), J. Eckert & P. Deplazes (58), P.M. Schantz *et al.* (162); for additional references, see text

Note: exact identification of endemic and highly endemic areas in all regions is not possible because of incomplete or lacking data

4.2.1. Global distribution of *Echinococcus multilocularis*

Echinococcus multilocularis has an extensive geographic range in the northern hemisphere (Fig. 4.3.), including endemic regions in central Europe, most of northern and central Eurasia (extending eastward to Japan) and parts of North America. In Eurasia, parts of Turkey, Iran and possibly northern India (report of 1 human case) seem to represent the southern limits of the range of *E. multilocularis*, but little information is available in this respect (162, 163). In North America, the range of the cestode reaches from Alaska southward to the States of Nebraska, Iowa, Illinois, Indiana and Ohio (103, 162). Two autochthonous human cases of AE were reported from a mountainous region of northern Tunisia, which might be an indication of the occurrence of *E. multilocularis* in northern Africa, but further information is not available (162).

4.2.2. *Echinococcus multilocularis* in Europe (excluding the Russian Federation and adjacent countries (Chapter 4.2.4.)

The first cases of AE in humans were diagnosed in southern Germany in 1852. Since then, cases of this disease have been reported continuously from several countries.

Geographic range

By the end of the 1980s, areas endemic with *E. multilocularis* were known to exist in only four countries of central Europe: Austria, France, Germany and Switzerland (55, 56, 58, 162, 163). Recent studies have shown that the parasite has currently (1999/2000) a much wider geographic range, including at least 12 European countries: Austria, Belgium, the Czech Republic, Denmark, France, Germany, Liechtenstein, Luxembourg, Poland, the Slovak Republic, the Netherlands and Switzerland (58, 68, 100, 108, 154) (Fig. 4.4.). The recent finding of *E. multilocularis* in eastern Poland and the Slovak Republic supports the hypothesis that the endemic areas in central and eastern Europe, formerly regarded as separate, are coherent (Chapter 4.2.4.). According to a communication of the Animal Health Authorities in Troms and Finnmark, Norway, metacestodes of *E. multilocularis* were found in rodents in 1999 on Spitsbergen island, which belongs to the Norwegian Svalbard Island group situated in the Barent's Sea (171). Within the endemic area shown in Figure 4.4., there are marked differences in the spatial distribution of the parasite, as indicated by its prevalence in red foxes. The current situation in the various regions and countries can be briefly summarised as follows (abbreviations: N: north, E: east, S: south, W: west) (see also Table 4.9.):

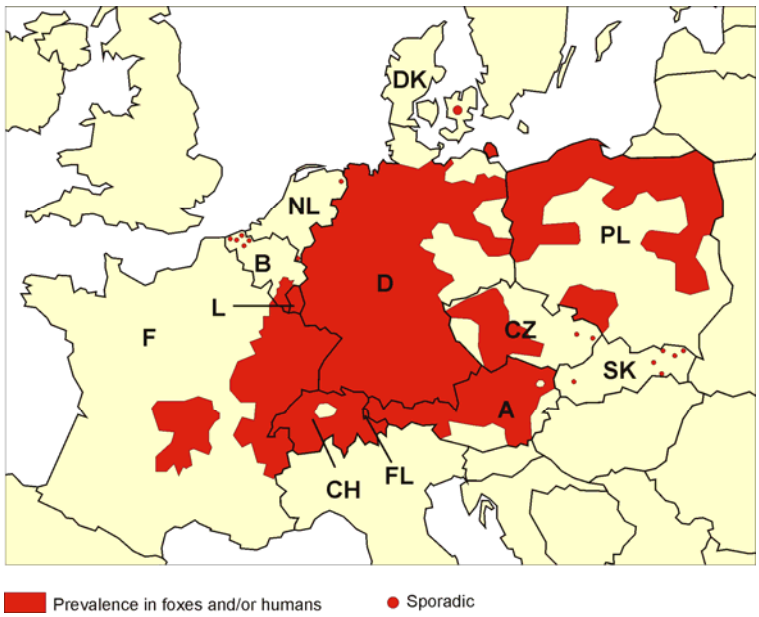


Fig. 4.4.
Approximate geographic distribution of *Echinococcus multilocularis* in central Europe (1999)

Table 4.9.
***Echinococcus multilocularis* in central Europe: examples of prevalence in red foxes**
Diagnosis at necropsy (N) if not otherwise indicated
PCR = diagnosis by DNA detection

Regions and countries	Period	Foxes examined/ infected[a]	Average prevalence and (range) percentage[b]	References
Belgium				
Flanders	1996	50/1	2.0	188
Luxembourg Province	1993-1995	145/74	51.0	118
	1997-1998	65/17	26.1	70
The Netherlands	1996-1997	272/5	1.8	186
Luxembourg	1990-1992	255/13	5.1	2
France				
Lorraine	1983-1987	513/112	21.8	12
Doubs	1996	39/24	61.5	70
Germany				
North and East				
Schleswig-Holstein	1990-1994	699/3	0.4	132
Mecklenburg-Vorpommern	1991-1994	3,576/21	0.6	106
Brandenburg	1992-1994	6,529/267	4.1	177
Sachsen-Anhalt	1992-1996	3,344/21	0.6	147
Saxony	1990-1995	2,155/0	0	68
North-West				
Lower Saxony	1991-1997	5,365/706	13.1	191
North-Rhine Westfalia	1993-1998	414/*117*	28.3	70, 178
Central				
Hesse	1989-1990	162/47	29.0	16
Thuringia	1990-1995	8,923/1,631	18.3	196
South-West and South				
Rhineland-Palatinate	1996-1997	1,145/340	29.7	94
Saarland	1994-1995	251/50	19.9	2
Baden-Württemberg	1995-1998	6,013/*2,225*	37.0	70
Bavaria	1988-1994	3,969/1,128	28.4	134
Switzerland				
(21/26 cantons)[c]	1990-1998	7,457/2,217	29.7 (3-53)[b]	55, 56, 57, 58, 70, 71
Liechtenstein	1990-1992	129/45	34.9	71
Austria				
(5/9 federal states)[c]	1989-1998	3,778/294	7.8 (0-34.2)	70, 173
Czech Republic				
(5/5 regions)[c]	1994-1998	1,528/214	14.0 (2.5-22.9)	108, 145
Slovak Republic				
Eastern and western parts	1998-1999	56/6 (N+PCR)	10.7	54
Poland				
18/43 districts[c]	1993-1998	2,951/76	2.6 (<1-36)[b]	124

a) numbers in italics calculated from average percent values
b) ranges: except areas where *E. multilocularis* was not found
c) number of areas with infected foxes/number of areas investigated

Northern region

- **Scandinavia:** in 1999, *E. multilocularis* was recorded for the first time in red foxes in Denmark (100) and in the same year metacestodes of *E. multilocularis* were detected in rodents on the Norwegian island of Spitsbergen/Svalbard (Barent's Sea) (171).

Western region

- **Belgium:** province of Luxembourg (SE) with high prevalence of *E. multilocularis* in foxes, lower prevalence in Flanders (NW)

- **the Netherlands:** only few infected foxes in the provinces of Groningen (NE) and Limburg (SE) close to the German border (186)

- **Luxembourg:** low prevalence of *E. multilocularis* in foxes

- **France:** a large endemic area in the east, including approximately 15 departments from Ardennes (capital: Charleville-Mézières) (48) in the north along the borders of Belgium, Luxembourg, Germany and Switzerland to Savoie (capital: Chambéry) in the south; a smaller area in the Massif Central (region of Auvergne).

Central and eastern region

- **Germany:** infected foxes reported from most of the 16 federal states (*Bundesländer*), except from the 3 small 'city states' (Hamburg, Bremen, Berlin) and the state of Saxony (E). Highest prevalences of *E. multilocularis* in the southern parts of the country and in some of the central federal states

- **Switzerland:** *E. multilocularis* reported from 21 of the 26 cantons with highest prevalences in the north-western and north-eastern parts north of the Alps. The average prevalence of *E. multilocularis* in foxes is high

- **Liechtenstein:** high parasite prevalence in foxes

- **Austria:** infected foxes reported from 6 of the 9 federal states (*Bundesländer*), with highest prevalence in two western states (Vorarlberg and Tyrol) and decreasing in areas further east

- **the Czech Republic:** *E. multilocularis* in foxes found in 5 regions of the country with rather high average prevalence rates

- **the Slovak Republic:** infected foxes found in eastern and western parts of the country in preliminary studies

- **Poland:** the northern regions have higher prevalences of *E. multilocularis* in foxes (2.2% to 11.8%) than the southern parts of the country (0.4%) with an overall prevalence of 2.6%.

Isolated human cases of AE have been reported in previous years from other European countries, for example, from the UK (most likely imported), the Slovak Republic, Hungary and Greece (108, 162). In Sweden, a case of multicystic echinococcosis has been diagnosed as AE, but was not documented (174). Furthermore, several species of rodents infected with metacestodes of *E. multilocularis* were recorded from various countries, e.g. Slovenia, Bulgaria and Romania (108). However, these reports have not provided enough information to confirm the enzootic status of *E. multilocularis* in those countries.

Since 1989, *E. multilocularis* has been found in foxes in an increasing number of European countries (see above). As reliable information from earlier surveys is not available for these countries it cannot be decided whether these findings indicate the first identification of hitherto unnoticed endemic areas or recent extensions of the geographic range of the parasite (58).

Definitive hosts

In central Europe, the principal definitive host of *E. multilocularis* is the red fox (*Vulpes vulpes*). In recent years, large numbers of foxes have been examined at necropsy for *E. multilocularis* in various European countries (Table 4.9.). The data reflect an alarmingly wide geographic range of the parasite with average prevalences varying from less than 1% to over 60%. It should be considered that in some smaller areas, the local prevalences can be much higher than the averages calculated for larger regions or countries. For example, in one focus in Baden-Württemberg, Germany, the prevalence of *E. multilocularis* in 53 foxes was 75% (155). In some regions of Germany and France, data of long-term studies have suggested an increase of the prevalence of *E. multilocularis* in foxes during recent years (70, 94, 154). However, the spatial and seasonal distribution of the parasite in foxes is extremely complex, and potential effects of various parameters (fox population density, land use patterns, etc.) on the population dynamics of the parasite are insufficiently known (70). Therefore, definitive conclusions cannot yet be drawn.

Domestic dogs (*Canis familiaris*) and cats (*Felis catus*) have been found to be infected in some countries, but mostly less frequently than foxes. In three studies carried out between 1988-1998, 278 cats from the highly endemic area of Baden-Württemberg have been examined at necropsy for *E. multilocularis*; 3 animals (1.1%) were infected (70). In the same area, of 145 dogs, none could be identified as parasite carrier in 1998. A recent study, including 660 dogs and 263 cats originating from randomly selected populations in an endemic area of eastern Switzerland, has shown that an average 0.30% of the dogs and 0.38% of the cats were infected with *E. multilocularis* (49). Higher prevalences may be expected in dogs and cats that have regular access to infected rodents, especially in highly endemic foci. For example, in a small focus in western Switzerland 12% of 41 dogs were identified as carriers of *E. multilocularis* (76).

Intermediate hosts

In central Europe, the common vole (*Microtus arvalis*), the water vole (*Arvicola terrestris*) and the Muskrat (*Ondatra zibethicus*) are regarded as important intermediate hosts, but also some other rodent species have been found to be infected, e.g. the snow vole (*Microtus nivalis*), earth vole (*Pitymys subterraneus*), red-backed vole (*Clethrionomys glareolus*) and house mouse (*Mus musculus*) (57). However, neither the range of potential intermediate hosts nor their significance in disease transmission have been adequately studied. The average prevalence of *E. multilocularis* in rodents is generally low (<1% to 6%) (57), but in certain foci higher prevalences have been determined. For example, in a highly endemic focus in Switzerland, 11 of 28 *A. terrestris* were infected with metacestodes of *E. multilocularis* (164). In France, it has been shown that large populations of *A. terrestris* Scherman increase the risk for the acquisition of AE in humans (190). Surprisingly, high and increasing prevalences of *E. multilocularis* metacestodes were found in muskrats in various counties of Baden-Württemberg, Germany; 15%-39% of muskrats (n: 33-315, total: 702) were infected in 1995-1997 as compared with 0%-4.1% (n: 123-814, total: 2,583) in 1981-1985 (154). It can be assumed that muskrats play a greater role in maintenance of the life-cycle than previously anticipated.

Aberrant host animals

In recent years, infection with the metacestode stage of *E. multilocularis* has been undoubtedly identified in a number of aberrant hosts (which do not play a role in the transmission cycle), including domestic pigs (*Sus scrofa domesticus*), wild boars (*Sus scrofa*), domestic dogs (*Canis familiaris*), nutria (*Myocastor coypus*), and various species of monkeys in captivity (55, 117, 176). Such infections are indicators of the existing infection risk for humans.

Transmission cycles

The cycle of *E. multilocularis* in central Europe is predominantly sylvatic, involving red foxes as definitive hosts and rodents as intermediate hosts. The sylvatic cycle is not restricted to unpopulated rural areas, but also occurs within villages or even in cities. For example in Zurich, Switzerland, the prevalence of *E. multilocularis* in foxes (n: 349) was 47% in the urban area and 67% in the adjacent recreational suburban region. In the urban area, *Arvicola terrestris* was identified as intermediate host with infection rates of 20% (n: 60) in 1997 and 9% (n: 75) in 1998 (50). Growing fox populations and their increasing spread to cities may represent new risk factors.

In addition to the sylvatic cycle, a synanthropic cycle exists with domestic dogs and cats as final hosts and rodents as intermediate hosts. This is concluded from the fact that dogs and cats have been found naturally infected with egg-producing stages of *E. multilocularis*. Dogs are highly susceptible to the infection, while cats are apparently less susceptible. Considering the estimated population sizes of red foxes, dogs and cats and the prevalences of *E. multilocularis* in these hosts, a model calculation carried out for the canton of Zurich has shown that foxes had to be regarded as the main contaminators of the environment with eggs of the parasite (58) (Chapters 5.3 and 6.2.).

Alveolar echinococcosis in humans

Although official reporting and surveillance systems for *E. multilocularis* do not exist in most of the European countries, some reliable data are available, which were derived from retrospective case finding studies or sero-epidemiological surveys, the latter combined with ultrasound imaging examinations. Since 1983, autochthonous and well documented cases of human AE have been reported from the following countries: Austria, France, Germany, Liechtenstein, Poland and Switzerland (58), and according to a recent report also from Belgium (70). The annual country-wide or regional incidence rates calculated from retrospective data of confirmed cases are generally low and vary between 0.02 and 1.4 per 100,000 population (Table 4.10.). Recently, a pilot project for an European Network for Concerted Surveillance of Alveolar Echinococcosis (main investigator Professor A. Vuitton, France) was established within the framework of the 'Community Programme for Prevention of Acquired Immune Deficiency Syndrome (AIDS) and some other Communicable Diseases' of the European Commission (http://www.eurechinoreg.org) (70). In the first year of the network's activities, the following numbers of live AE patients could be registered up to May 1999, but case registration has not yet been completed (in parenthesis are cases which have been recorded but could not yet be evaluated and included in the register for technical reasons): Austria: 33, France: 112 (+ 69), Germany: 82 (+ 20), Belgium: 3; Switzerland: 54 (+ 50), Poland: 7, Greece: 1; total live patients with AE: 292 (+ 139).

Table 4.10.
***Echinococcus multilocularis* in central Europe: examples of incidence of human alveolar echinococcosis[a]**

Country and region	Period	Number of new AE cases[b]	Average per year	Annual incidence per 100,000 population	References
Switzerland					
Entire country	1956-1969	122	8.7	0.15	58, 61
Entire country	1970-1983	145	10.4	0.18	58, 61
Entire country	1984-1992	65	7.2	0.10	58, 61
Canton Jura	1970-1983	6	0.4	0.74	58, 61
Austria					
Entire country	1983-1990	14	1.8	0.02	13
Germany					
Bavaria	1985-1990	50	10	0.09[b]	134
France					
Franche Comté	1971-1989	85	4.5	0.5	23
Doubs	1960-1992	56	1.7	1.4[c]	24

a) predominantly based on retrospective case finding studies
b) only cases confirmed by clinical, pathological and other data
c) calculated from data of screening survey (serology and ultrasound imaging)

In a study conducted in Switzerland, 17,166 blood donors living in endemic areas of northern Switzerland were examined during 1984-1985, and asymptomatic AE was detected in 2 persons, corresponding to a verified group prevalence of 11.6 cases per 100,000 individuals (75). A similar study was carried out between 1987 and 1991 in an agricultural population of 7,884 persons in the department of Doubs, France (24). In this group, 8 cases of active disease and 5 with inactive lesions were identified. The 13 confirmed cases in this

population permits calculation of a verified group prevalence of 165 cases per 100,000 individuals, and a regional prevalence of 11 cases per 100,000 when the total population of the study region (122,986) is considered. A recent study (1996) including 2,560 persons living in a highly endemic rural community in Baden-Württemberg, Germany, revealed 1 seropositive person with active AE, corresponding to a verified group prevalence of approximately 40 per 100,000 (155), and a local prevalence of 24 per 100,000, if the total population of the 3 villages under study (4,131) is used as basis for calculation. It has to be considered that the results of smaller studies from highly endemic foci may not be representative for larger regions.

4.2.3. *Echinococcus multilocularis* in the Eastern Mediterranean and northern Africa

In this region Turkey and Iran are known as endemic areas of *E. multilocularis*, but only limited information is available thus far.

4.2.3.1. Turkey

Between 1934 and 1983, a total of 157 human cases of AE was diagnosed, i.e. an average of 3.1 new cases per year (174, 185). Patients originated from all 7 provinces of the country, but 86% were from eastern and central Anatolia, and only 0.7% to 5.5% from other regions, including the European Marmara province (174, 185). It is not known whether the cycle of *E. multilocularis* is established in the latter region or whether human cases have been imported. More cases were diagnosed after 1983 through 1995, increasing the total number to approximately 207 (5). According to another report (160), during the period of 1979-1993 at least 7-10 new cases of AE were diagnosed in the country per year. Although little information exists on *E. multilocularis* infection in animal hosts in Turkey, this country should be considered as endemic because human cases of AE have been diagnosed regularly. It is assumed that foxes serve as final and 'rats' as intermediate hosts, but there is apparently only one report on the detection of *E. multilocularis* in a fox in north-west Turkey (5).

4.2.3.2. Iran

A recent study in the northern part of Iran (Ardabile province) revealed that of 130 wild carnivores 22.9% of red foxes (*Vulpes vulpes*) and 16% of jackals (*Canis aureus*) were infected with adult stages of *E. multilocularis* (199). Metacestodes of *E. multilocularis* were not found in 1,500 rodents, predominantly belonging to the genera *Microtus* or *Meriones* (191). During a period of 3.5 years, 37 human cases of AE were diagnosed in various hospitals, most of them in the Ardabile province (199).

4.2.3.3. North Africa

Two autochthonous human cases of AE have been reported from a mountainous region of northern Tunisia, which might be an indication of the occurrence of *E. multilocularis* in north Africa, but additional information is not available (162).

4.2.4. *Echinococcus multilocularis* in the Russian Federation and adjacent countries

The situation in this area has been summarised by various authors (18, 108, 162, 163), mainly based on older data because of the lack of recent information (see also Chapter 4.1.4.).

Geographic range

In recent years, there has been no indication for a reduction of the previously known endemic areas (18). In the western region, these include Belarus, the Ukraine and Moldova. In the north of the Russian Federation, the endemic zone extends from the Barent's Sea (Arkhangelsk) region in the north-west to the Chukotka region, Bering Strait, Kamchatka peninsula and northern Kuriles in the far east, and in the south from the region between the Black Sea and the Caspian Sea through the Omsk, Novosibirsk, Irkutsk regions to the Amur region, Chabarovsk and the island of Sakhalin (18) (Fig. 4.3.). Although data from many regions are lacking, it can be assumed that the parasite occurs in wide areas of the Russian Federation. *Echinococcus multilocularis* also occurs in the independent states south of the Russian Federation: Georgia, Armenia and Azerbaijan in the Caucasian region, and further east in Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan and Kyrgyzstan (18).

Definitive hosts

In the area, at least 9 species of carnivores have been identified as definitive hosts of *E. multilocularis*: Arctic fox (*Alopex lagopus*), red fox (*Vulpes vulpes*), corsac fox (*Vulpes corsac*), wolf (*Canis lupus*), jackal (*Canis aureus*), raccoon-dog (*Nyctereutes procyonoides*), the spotted cat (*Felis libyca*), domestic dog (*Canis familiaris*) and domestic cat (*Felis catus*) (18). The prevalence rates of *E. multilocularis* in arctic foxes in the far north of the Russian Federation and in red foxes in Chuktoka and Kamchatka were found to be high, reaching 26% to 76% and 15% to 24%, respectively (18). High prevalences of *E. multilocularis* in red foxes have also been reported from southern Siberia and from parts of Kazakhstan; in the same region 14% to 39% of dogs were also found to be infected (18). Natural *E. multilocularis* infection of dogs has also been reported from other regions of the Russian Federation (e.g. Taimyr and Chukot districts and Yakutia) and from Uzbekistan. In Yakutia, *E. multilocularis* infection was reported in 18% of 307 rural dogs (162). According to a recent communication (P.R. Torgerson, personal communication, 1999), the principal carnivores infected in Kazakhstan are the red fox, the corsac fox and the spotted cat with prevalences of up to 18%-25%.

Intermediate hosts

More than 30 species of small mammals have been identified as intermediate hosts in the Russian Federation and adjacent countries (18). Intermediate hosts which are important in these regions include voles and lemmings of the genera *Microtus, Arvicola, Clethrionomys*, *Lagurus* and *Lemmus*, and the muskrat (*Ondatra zibethicus*), a species introduced to Eurasia from North America. Surveys in Eurasia have shown that infection rates of rodents with metacestodes of *E. multilocularis* were generally low (1%-11%). However, high rates have been observed in some species, including the northern red-backed vole (*Clethrionomys glareolus*) (up to 46%), root vole (*Microtus oeconomus*) (52%), Siberian lemming (*Lemmus sibiricus*) (21%), muskrat (*Ondatra zibethicus*) (5.8%) and bobac marmot (*Marmota bobac*) (4.5%) (18). According to a recent communication (P.R. Torgerson, personal communication, 1999), *Rhombomys opimus* and *Myospalax myospalax* are the most important intermediate hosts in Kazakhstan with *E. multilocularis* prevalences of up to 3%.

Aberrant host animals

Multilocular, sterile bladders were observed in domestic ruminants and regarded as metacestodes of *E. multilocularis* (119). However, experimental infections of larger groups of pigs, lambs and calves with eggs of *E. multilocularis* have shown that partial development of the parasite in the liver of these animals is possible, but the metacestodes perish at an early stage of establishment. It was concluded that domestic ungulates do not play a role in disease transmission (119).

Transmission cycles

In various regions, different definitive/intermediate host assemblages predominate in disease transmission, for example: Far north: arctic fox and lemmings (Siberian, hoofed) or narrow sculled vole, in some parts (Yakutia) dog and voles; south of Siberia: red fox and muskrat; Prybalkhashie (Kazakhstan): red fox or corsac fox and muskrat, or dog and muskrat; Kazakhstan: fox and steppe lemming; forest-steppe districts: fox and voles or wood mice; desert regions: fox and great gerbil (18).

Alveolar echinococcosis in humans

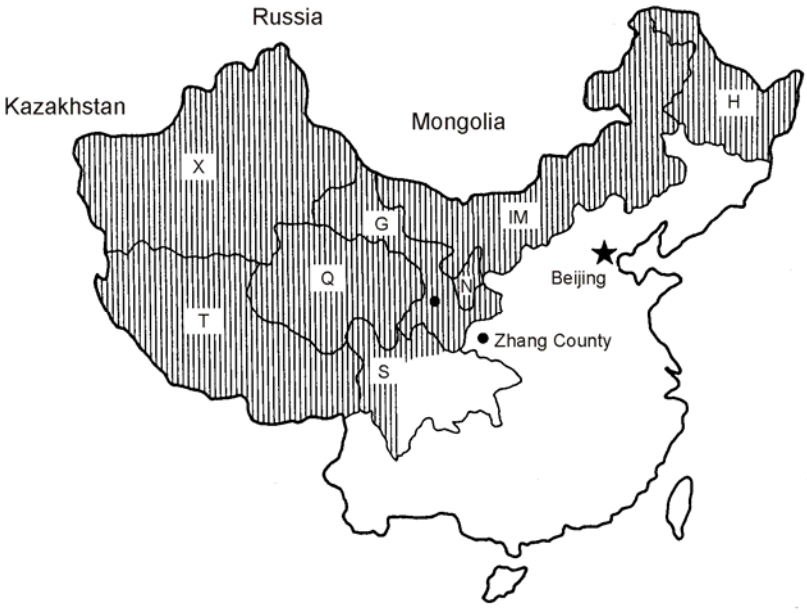
Human AE is known to occur throughout the range of *E. multilocularis* in the Russian Federation (Fig. 4.3.). According to older data collected 20 to 30 years ago (18) very high prevalence of AE (10 or more infected persons per 100,000 population) has been reported from areas in the far east of the Russian Federation (Chukot and Koriak region and Kamchatka), and further west in Yakutia, the Omsk, Tomsk and Altai regions. Reports of high prevalence (1-10 infected persons per 100,000 population) came from areas adjacent to Yakutia and Altai Territory as well as from Tuva Republic (south of Krasnojarsk Territory), Magadan region in the far east, and parts of Kazakhstan. In other areas, lower prevalences were recorded. The current situation remains unclear as recent data are lacking, except for a report from Kyrgizstan where 3 cases of human AE were detected in 1990 (cited in 18).

4.2.5. *Echinococcus multilocularis* in Mongolia and the People's Republic of China

In central Asia, *E. multilocularis* has a more or less contiguous distribution involving parts of Kazakhstan (Chapter 4.2.4.), Mongolia and the People's Republic of China (38). As epidemiological data do not appear to exist for Mongolia (194), the following chapter is focussed on the situation in the People's Republic of China. The first series of human AE cases in the People's Republic of China was reported rather late in 1965. Subsequent observations have disclosed that the infection is widespread and the public health consequences are serious in some rural communities of the country (37, 38, 163).

Geographic range

Echinococcus multilocularis is distributed mainly in the western and central parts of the People's Republic of China, including regions of the provinces Xinjiang, Qinghai, Ningxia, Gansu, Inner Mongolia, Sichuan and Tibet, but sporadic human cases have also been reported from the north-eastern province of Heilongjiang (33, 195) (Fig. 4.5.). Hospital and public health records indicate that there are two major regional foci of human AE in the People's Republic of China. The most serious occurs in the central regions of the People's Republic of China, involving south Gansu, southern Ningxia Hui Autonomous Region (AR), eastern Qinghai and northern Sichuan. The other main endemic region is situated in northern Xinjiang Uyghur AR especially along the central Tian mountains and the Kazakhstan border (38, 39, 163); it is assumed that these two foci are probably contiguous (39). The environmental conditions in these endemic localities range from the very dry (e.g., Ordos desert, Ningxia) to moist mountain valleys (e.g., Gansu, Xinjiang) and high plateaus (Qinghai). *Echinococcus multilocularis* coexists with *E. granulosus* in several provinces of the People's Republic of China.



H : Heilongjiang
G : Gansu
IM : Inner Mongolia
N : Ningxia
Q : Qinghai
S : Sichuan
T : Tibet
X : Xinjiang

Fig. 4.5.
Approximate geographic distribution of alveolar echinococcosis in the People's Republic of China
Reproduced from (33) with permission from the editors

Definitive hosts

Several species of wild canids have been found to be infected with adult stages of *E. multilocularis*, namely the red fox (*Vulpes vulpes*), corsac fox (*Vulpes corsac*), Tibet fox (*Vulpes ferrilata*) and wolf (*Canis lupus*) (33, 91, 163). Information on the prevalence of *E. multilocularis* in these hosts is relatively sparse; some examples are presented in Table 4.11. Although based on small numbers of examined animals, the prevalence data indicate

that infection rates in canids of the genus *Vulpes* are high. The tapeworm also occurs in domestic dogs, including stray dogs, with remarkably high prevalences.

Table 4.11.
***Echinococcus multilocularis* in the People's Republic of China: examples of prevalence in definitive hosts**

Animal species	Period[a]	Province	Number of animals examined/infected	Percentage infected	References
Red fox (*Vulpes vulpes*)	1989	Xinjiang	36/11	30.6	162[b]
	1985	Ningxia	20/3	15.0	162[b]
	1991	Sichuan	32/19	59.4	162[b]
Corsac fox (*Vulpes corsac*)	1989	Inner Mongolia	3/2		162[b]
Tibet fox (*Vulpes ferrilata*)	1999	Sichuan	–	44.4	91
Wolf (*Canis lupus*)	1989	Xinjiang	2/1		162[b]
Dog (C*anis familiaris*)	1992	Gansu	58/6	10.3	37
	1991	Sichuan	28/4	14.3	162[b]
	1999	Sichuan	–	12.1-25.0	91

a) identical with year of publication of original paper
b) for original references see this paper
– no data given

Intermediate hosts

The spectrum of rodents found to be infected with metacestodes of *E. multilocularis* includes the Brandt's vole (*Microtus brandti*), another vole (*Pitymis irene*), jird (*Meriones unguiculatus*), mole rat (*Myosplax fonatnieri*), grounds squirrels (*Citellus daurious* and *C. erythroqenys*) and house mouse (*Mus musculus*). Furthermore, two species of lagomorphs, namely the pika (*Ochotona curzoniae*) and the woolly hare (*Lepus oiostolus*) were also infected (33, 38, 91, 163). Where substantial numbers of small mammals were examined in previous studies, the highest prevalence was found in the vole, *Microtus brandti* (2.4%, 64/2,635) in Inner Mongolia, and the pika, *Ochotona* sp. (4.2%, 9/214) in Sichuan Province (33, 38, 163). In a recent survey in Sichuan, 25% of *Pitymys irene*, 6.7% of *Ochotona curzoniae*, and 7.1% of *Lepus oiostolus* were found to be infected with the metacestode stage of *E. multilocularis* (91). Infection of domestic livestock with metacestodes of *E. multilocularis* has been reported from various provinces (see below).

Aberrant host animals

There are several reports of infection of sheep and yaks with the metacestode stage of *E. multilocularis* (163). For example, such infection was recently recorded in 0.3%-1.9% of yaks in Sichuan (91). However, the diagnosis needs to be confirmed, as confusion with atypical, multicystic forms of larval *E. granulosus* is possible. According to present knowledge (Chapter 4.2.4.), domestic livestock animals are aberrant hosts which do not play a role in disease transmission.

Transmission cycles

Evidence suggests that sylvatic cycles of *E. multilocularis* exist in various parts of the People's Republic of China with foxes as definitive hosts and rodents as intermediate hosts. The spectrum of potential intermediate hosts is apparently broad and may differ in various regions. Furthermore, synanthropic cycles exist, with dogs as definitive hosts, and pikas and other small mammals as intermediate hosts. For an area in south Gansu, it has been suggested that the existence of a cycle involving domestic dogs (10% infected with *E. multilocularis* in Cao Tan Commune, Zhang County) and rodents, together with poor living conditions, might account for the high infection rate in humans (38, 163). A canine distemper epidemic in 1990 has eliminated almost all dogs and reduced the numbers of red foxes in that area.

Alveolar echinococcosis in humans

In the 1980s, when attention began to focus on echinococcosis as a public health problem in the People's Republic of China attempts were made to measure the numbers of cases and distinguish the cystic and alveolar forms of disease. In 1992, approximately 500 human cases of AE were reported from Ningxia (257 cases), Xinjiang (88 cases), Gansu (71 cases), Sichuan (49 cases), Qinghai (37 cases), Heilongjiang (1 case) and Tibet (1 case) (163). However, there is evidence that these data do not reflect the actual epidemiological situation. In recent years, approximately 350 cases of AE have been detected alone in the Gansu region (39). An ultrasound mass screening survey with serological confirmation in south Gansu between 1991 and 1997 revealed a group prevalence of human AE of approximately 4% (135/3,331) (39). Taking into account the population size in this rural area, this is equivalent to a local group prevalence of approximately 200 per 100,000 (39). However, it has to be considered that AE has a focal distribution, thus a group or local prevalences may not be representative for larger regions.

4.2.6. *Echinococcus multilocularis* in Japan

Echinococcus multilocularis is believed to have been introduced into Japan through infected red foxes translocated to Rebun Island, northwest of Hokkaido, from islands in the middle Kuriles from 1924 to 1926, for the purpose of controlling the vole population. On Rebun Island, 131 human cases of AE were diagnosed between 1937 and 1989, but no further cases were detected thereafter (105). Another outbreak occurred about 1960 in eastern Hokkaido, followed by the spread of *E. multilocularis* to central and western parts of the island (105). Between 1981 and 1991, the parasite spread from approximately 8% to 90% of the area of Hokkaido (175). Foxes infected with *E. multilocularis* in urban areas may represent a new risk factor for humans (184).

Geographic range

Currently, Hokkaido is the only region in Japan with documented endemic occurrence of *E. multilocularis* (105). Between 1985 and 1996, red foxes infected with *E. multilocularis* have been found in eastern Hokkaido (districts: Nemuro, Kushiro and Abashiri), in central areas (districts: Sohya, Kamikawa, Tokachi, Rumoi, Sorachi, Hidaka and Ishikari) and western Hokkaido (Shiribeshi, Iburi, Hiyama and Oshima) (105).

Definitive hosts

Red foxes (*Vulpes vulpes*), domestic dogs and cats, and raccoon-dogs (*Nyctereutes procyonoides*) have been identified as definitive hosts in Hokkaido (136). The average prevalence of *E. multilocularis* in red foxes in different districts varies between <10% and >30%; this shows an increasing tendency during recent years in some of the districts of Hokkaido (105).

Intermediate hosts

The spectrum of intermediate hosts identified thus far includes 3 species of voles of the genus *Clethrionomys*, *Apodemus argenteus*, *Mus musculus*, *Rattus norvegicus* and two species of *Sorex* (136). Rates of infection in voles (*Clethrionomys rufocanus* and *C. rutilus*) varied from 4% to 22%. Occasional infections seen in wood mice, house mice, Norwegian rats, swine and horses are not considered to be of significance in disease transmission.

Aberrant host animals

The spectrum of aberrant hosts identified in Hokkaido comprises the domestic pig (*Sus scrofa domesticus*) and horse (*Equus caballus*), and various species of monkeys kept in zoos (136) (Chapter 3).

Transmission cycles

Red foxes and *Clethrionomys rufocanus bedfordiae* are regarded as particularly important for the sylvatic transmission cycle because of their high susceptibility to *E. multilocularis*, their wide geographic distribution, their numerical dominance and other features (105). Dogs are taken into consideration as a potential source of infection in a synanthropic cycle.

Alveolar echinococcosis in humans

A summary of the annual numbers of new human cases of AE diagnosed between 1937 and 1997 is presented in Table 4.12.

Table 4.12.
***Echinococcus multilocularis* in Japan: human cases of alveolar echinococcosis on Rebun Island and Hokkaido[a]**

Period	New cases diagnosed in various regions			Total	Number of years	Average number of cases per year
	Rebun Island	Hokkaido: Nemuro and Kushiro	Hokkaido: other districts			
1937-1964	111	2	4	117	28	4.2
1965-1974	13	40	6	59	10	5.9
1975-1984	5	39	12	56	10	5.6
1985-1994	2[b]	60	50	112	10	11.2
1995-1997	0	5	24	29	3	9.7
Total	131	146	96	373	61	6.1

a) *Source:* (105)
b) last case diagnosed in 1989

4.2.7. *Echinococcus multilocularis* in North America

Geographic range

Echinococcus multilocularis currently occurs in two geographic regions in North America, one in the northern tundra zone of Alaska (USA) and Canada and another further south, in the north central region (Fig. 4.3.) (103, 162, 163).

- **Northern tundra zone:** the range of *E. multilocularis* in this area is roughly equivalent to that of the arctic fox and extends along the coast of Alaska from the mouth of the Kuskokwim River northward and eastward to Canada, and southward along the western shore of Hudson Bay (163). The parasite is also present on sub-Arctic islands, including St Lawrence island, St George Island (Pribilof Group) and Nunivak Island. It is also found on some islands of the Canadian Arctic Archipelago, but is apparently not present on the northernmost islands of Canada. There is no evidence of the presence of *E. multilocularis* within the forested interior (taiga) between the northern tundra and the north central endemic region, although fairly large numbers of foxes and rodents have been examined (162).

- **North central endemic region:** currently, this region includes parts of 3 Canadian provinces (Alberta, Saskatchewan and Manitoba) and 13 contiguous States of the USA. It is assumed that prior to the 1960s *E. multilocularis* spread from the northern tundra zone and became established in central North America, in an endemic area centred in southern Manitoba and North Dakota. Previous surveys of endoparasites of canids and rodents in the central North American region had failed to reveal *E. multilocularis* (103, 163). The first finding of *E. multilocularis* was 1964 in a red fox in North Dakota. Subsequently, the parasite was identified in wild canids and rodents in South Dakota, Iowa, Minnesota, and Montana in 1965-1969, in Wyoming in 1976, in Nebraska and northern Illinois in 1981-1982, and in Wisconsin in 1982-1983. The most recent surveys extended its known distribution to as far east as east-central Illinois, Indiana, Ohio and as far south as Missouri.

Given the abundance of suitable definitive and intermediate hosts throughout the USA, it may be assumed that *E. multilocularis* will continue to spread and become established in contiguous states. Translocation of

foxes and coyotes from endemic states and their release in hunting enclosures of non-endemic areas may contribute to parasite spreading (162).

Definitive and intermediate hosts and transmission cycles

The hosts and the dynamics of transmission of the parasite differ in these two regions (162, 163). Throughout the northern tundra zone *E. multilocularis* occurs in foxes, mainly the arctic fox (*Alopex lagopus*) and the arvicoline rodents that they prey on. The northern vole (*Microtus oeconomus*) is the most important intermediate host in western Alaska and on St Lawrence Island. The brown lemming (*Lemmus sibiricus*) is the only intermediate host on St George Island (Pribilof Islands) and Nunivak Island, and appears to be the only rodent involved in the cycle in northern mainland Alaska. The northern red-backed vole (*Clethrionomys rutilus*) and shrews have also been found to be infected, but do not play an important role in maintenance of the cestode cycle. Investigations undertaken on St Lawrence Island between 1950-1973 revealed average infection rates in arctic foxes of 77% (range: 40% to 100%). Numbers per infected fox ranged from 1 to more than 180,000 *E. multilocularis* specimens. The mean infection rates in northern voles ranged from 2% to 16%, but reached 80% in certain locales. Studies carried out between 1980 and 1989 showed prevalences varying from 42% to 83% in northern voles trapped away from the villages.

In certain villages inhabited by indigenous people of the Arctic, houses were built directly on wet tundra, thus permitting northern voles to occur locally as commensals. Since the voles are easy prey for the numerous dogs, 'synanthropic' hyperendemic foci developed. In one of these foci on St Lawrence Island, 12% of dogs and 22%-35% of voles were infected with *E. multilocularis* (162). Such conditions result in heavy contamination of the environment with *E. multilocularis* eggs and are ideal for disease transmission. The highest rates of human infection in North America have occurred in such villages on St Lawrence Island and elsewhere on the Arctic coast. In other regions of the tundra zone in Alaska and Canada, the life-cycle has not been well defined.

- **Northern central region:** to date, *E. multilocularis* has been documented in foxes, coyotes and several species of rodents in regions of 13 contiguous states and 3 Canadian provinces (Fig. 4.3.). Where surveys have been carried out repeatedly, the prevalence has tended to increase; infection rates in samples of red foxes and coyotes have ranged from 69%-90% in North Dakota and South Dakota to 19%-35% in Illinois, Indiana and Ohio (81, 162, 163).

Echinococcus multilocularis life-cycles in the northern central region involve the red fox (*Vulpes vulpes*) and the coyote (*Canis latrans*) as final hosts (162). The grey fox (*Urocyon cineroargenteus*) has been rarely found to be infected. The deer mouse (*Peromyscus maniculatus*) and the meadow vole (*Microtus pennsylvanicus*) serve as the most important intermediate hosts. Other animals occasionally reported with larval *E. multilocularis* infection in this region include the muskrat (*Ondatra zibethicus*), the woodrat (*Neotoma cinerea*) and the house mouse (*Mus musculus*). Most of the records of *E. multilocularis* in the northern central region are from the prairie (steppe or grassland) biome. This region has been extensively modified for agriculture in ways that favour the increase in populations of foxes and rodents.

Domestic cats (*Felis catus*) have also been found to be infected; *E. multilocularis* was found in previous years in 3 of 131 cats near Saskatoon, Saskatchewan, and in 1%-5% of farm cats in North Dakota in 1971-1976 (162). Of 123 farm dogs from Minnesota, 3 (2.4%) were suspected to be carriers of *E. multilocularis* based on coproantigen detection (103).

Alveolar echinococcosis in humans

Some of the highest rates of human AE have been reported from the northern tundra endemic zone of North America. Despite the widespread occurrence of *E. multilocularis* in animal hosts in the northern zone, almost all cases of AE in humans were diagnosed in Eskimos from a limited number of communities in Alaska. On St Lawrence Island, in the small population of approximately 1,000 inhabitants, 53 cases were diagnosed between 1947 and 1990, corresponding to annual incidence between 7 and 98 per 100,000 population (162, 163). In the extensive Arctic and sub-Arctic areas of Canada where *E. multilocularis* is endemic, cases of human AE have never been recorded, and only two cases of AE were diagnosed to date in the North Central region, one in 1937 and the other in 1977 (163). Between 1990 and 1991, in endemic areas of South Dakota, serum

samples of 115 trappers were evaluated for specific antibodies against several antigens of *E. multilocularis* using the ELISA. Although roughly half of the individuals had trapped more than 50 foxes and almost one-fourth more than 1,000 during their life, all tests were negative (81).

4.3. *Echinococcus vogeli* and *Echinococcus oligarthrus*

The geographic range of these two species, which both cause polycystic echinococcosis (PE) in humans, is restricted to Central and South America, where it overlaps in some regions with that of *E. granulosus*.

Geographic range

Human cases of PE have been recorded from 11 Central and South American countries, including Nicaragua, Costa Rica, Panama, Colombia, Ecuador, Venezuela, Surinam, Brazil, Uruguay, Argentina and Chile (40, 179). Cases of *E. vogeli* infection were recorded from 5 countries (Panama, Colombia, Ecuador, Venezuela and Brazil), and cases caused by *E. oligarthrus* from 3 countries (Venezuela, Surinam and Brazil); in cases from the other countries, the parasite species could not be diagnosed (40). The distribution of *E. vogeli* and *E. oligarthrus* overlaps in some of the areas where suitable hosts are present (40).

Definitive and intermediate hosts, transmission cycles

The life-cycle of *E. vogeli* involves the bush dog (*Speothos venaticus*) and domestic dog (*Canis familiaris*) as major or occasional definitive hosts, and small mammals as intermediate hosts, such as paca (*Cuniculus paca*), agouti (*Dasyprocta* spp.), and spiny rat (*Proechimys* spp.). *Echinococcus oligarthrus* typically uses wild felids as definitive hosts (puma, jaguarundi, jaguar, ocelot, Pampas and Geoffoy's cat), and the same spectrum of intermediate hosts as *E. vogeli* but including a rabbit (*Sylvilagus floridianus*). Studies in Colombia revealed metacestodes of *E. vogeli* in 22% of 325 pacas (40).

Polycystic echinococcosis in humans

Until 1999, at least 96 cases of PE have been diagnosed in humans, probably representing only the tip of the iceberg (41); 37 of these cases (38.5%) were caused by *E. vogeli*, 3 (3.1%) by *E. oligarthrus*, and in 56 (58.3%) hooks were not found or described so that a species diagnosis was not possible (41). In cases of *E. vogeli* infection cysts were most frequently (80%) found in the liver alone or in combination with other organs. In two of the *E. oligarthrus* cases, cysts were located in the orbit and in 1 case in the heart (41).

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Chapter 5

Epidemiology

5.1. Quantitative epidemiology and transmission dynamics with special reference to *Echinococcus granulosus*

M.A. Gemmell, M.G. Roberts, T.C. Beard and J.R. Lawson

Summary

An understanding of factors contributing to the regulation and stability of populations of Echinococcus granulosus *and other Taeniidae is an important basis for planning of control programmes. Of great significance are the following factors:*

a) biotic potential of the parasite in the definitive host

b) acquired immunity as a density-dependent constraint by the intermediate host, and

c) climate as a density-independent constraint in the free-living egg-phase.

Methods of determining endemic, hyperendemic, and extinction steady states empirically and mathematically are described. Further, a brief description is given of successful transfer of eggs of E. granulosus *from dogs leading to cystic echinococcosis in humans.*

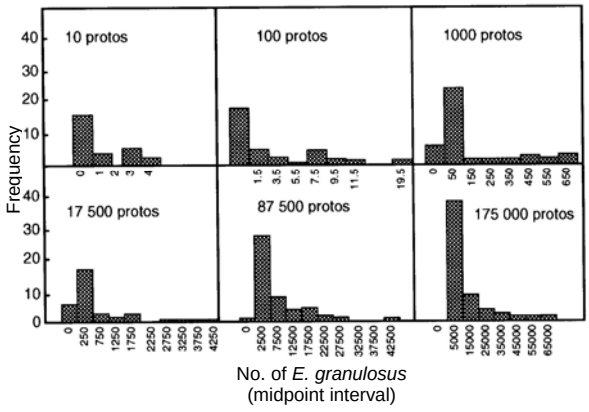
During the past two decades, considerable advances have been made in breaking the 'epidemiological code' of the family Taeniidae with the aid of mathematical modelling. This family contains such zoonotic parasites as *E. granulosus*, *E. multilocularis*, *Taenia solium* and *T. saginata.* From a human health point of view, some of them are difficult to study. Where data cannot readily be obtained, *T. hydatigena* and *T. ovis*, have been used with caution as models to describe the transmission dynamics and compare the stability of each system.

At any one time, the parasite population consists of three sub-populations. These are adults in the definitive host, larvae (metacestodes) in the intermediate host and eggs in the environment. The first step in understanding the transmission dynamics and problems of control of any member of this family is to determine the contributions made by the parasite and each host population to its stability. The second step must be to evaluate the role of intrinsic, extrinsic and socio-economic factors in modifying this stability. The third step involves quantifying the equilibrium steady state of the whole system in each socio-ecological situation. From this, a further step can then be taken to determine effective and cost-effective control options, predict their outcome, and test feasibility by field trial.

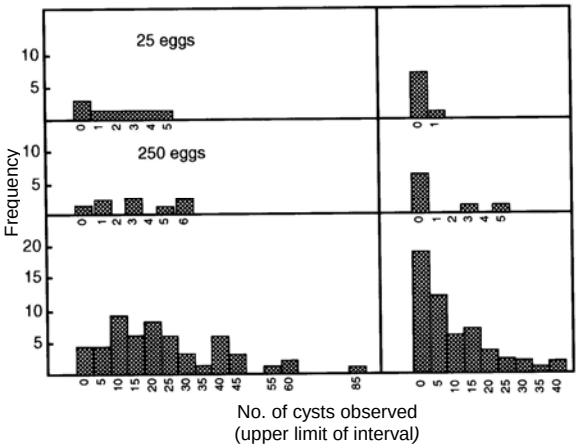
This review describes, and where appropriate, quantifies the contributions made by the parasite, hosts and environmental factors to the stability of the system.

5.1.1. Contributions by the parasite to transmission dynamics

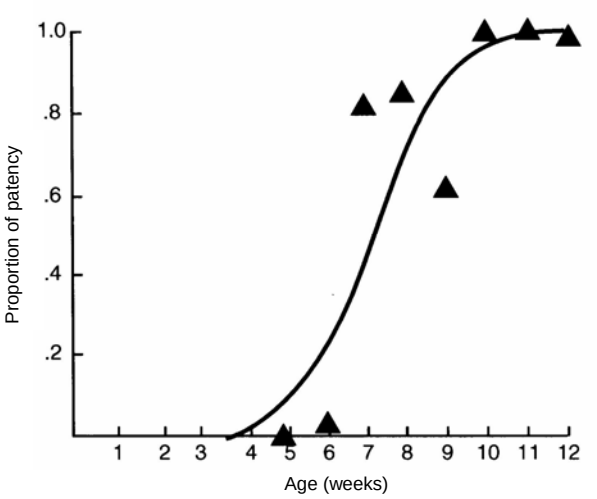
As with other taeniids of dogs and sheep (*T. hydatigena* and *T. ovis*), *E. granulosus* has an over-dispersed distribution that fits a series of negative binomial distributions in both hosts, with only a small number of animals harbouring large numbers of worms or larvae (15, 16, 18, 19, 20, 21, 22, 27, 33, 34, 42, 43, 44). There is neither a 'crowding' effect nor parasite-induced host mortality, and this distribution does not contribute to the regulation of either adult and larval sub-populations. The pre-patent period is similar for all 3 species; patency being reached in dogs between 6 and 12 weeks. The larvae of *E. granulosus* grow slowly in sheep with only 50% reaching fertility by 6.65 years (Fig. 5.1.1.).



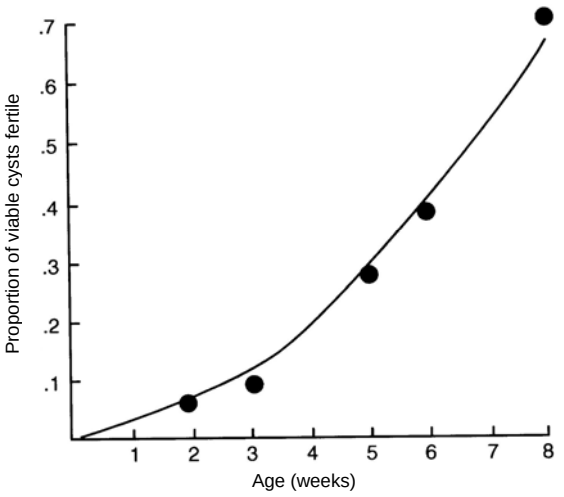
a) Frequency distribution of *E. granulosus* in dogs given specified numbers of protoscoleces



b) Frequency distribution of *E. granulosus* in sheep given specified numbers of eggs
left: total number of cysts; right: viable cysts



c) Relationship between patency and age (weeks) of *E. granulosus* in dogs



d) Relationship between fertility of cysts and age (years) of *E. granulosus* in sheep

Fig. 5.1.1.
Biological parameters of *Echinococcus granulosus* in dogs and sheep (15, 16, 19)
Source: M.A. Gemmell (15)
Reproduced from (15) with kind permission from F.L. Andersen (ed.)

The parasite's major contribution to the transmission dynamics is its biotic potential (Table 5.1.1.). This can be defined as the potential number of viable cysts which can be established in an intermediate host by an individual definitive host per day. Estimates suggest that *E. granulosus* has about 1/100th and 1/30th the biotic potential of *T. hydatigena* and *T. ovis* (Table 5.1.1.). The generally reported mean worm burden for *E. granulosus* in its dog-sheep life-cycle is about 200-400. However, with such highly susceptible animals as Turkana dogs in Kenya and dingoes in Australia, very high worm counts may be present in the majority of animals (16, 31, 36). It follows that the biotic potential may vary widely in different ecological situations and climatic zones.

With *E. multilocularis*, the time required to reach patency is about 28 days and the time taken to reach fertility in some rodents may be only 60 days (38). There are also large variations in worm burdens of *E. multilocularis*. For example, that in arctic foxes (*Alopex lagopus*) in Alaska is two orders of magnitude greater than that in the red fox (*Vulpes vulpes*) in Dakota (10, 21, 39). Low mean worm burdens are the rule in western Europe also (Chapter 5.3.). Although the biotic potential for *E. multilocularis* has not yet been defined in any host in any wildlife situation, it will, as with the dog-sheep taeniids, have a great influence on its stability in the different ecosystems where it exists.

Table 5.1.1.
Estimates of the biotic potential of *Echinococcus granulosus*, *Taenia hydatigena* and *Taenia ovis* (13, 19, 20)

Characteristic	*Echinococcus granulosus*	*Taenia hydatigena*	*Taenia ovis*
Mean number of eggs per proglottid	587	38,000	87,000
Mean number of proglottids shed per worm per day	0.071	1.0	1.0
Mean number of worms per infected host	202	1.0	1.0
Number of eggs shed from average infected dog per day	8,470	38,000	87,000
Proportion of eggs transforming into viable cysts	0.0033	0.071	0.0074
Potential number of viable cysts per infected dog per day = biotic potential	28	2,698	644

5.1.2. Contributions by the hosts to transmission dynamics

Considerable knowledge has now been gained on the protective immune response to adult and larval cestode infections (13, 15, 23, 24, 30, 40, 41). In epidemiological terms, acquired immunity is a negative feedback system operating as a density-dependent constraint to limit population abundance.

With the exception of the family of Taeniidae, Cyclophyllidean systems (Class Eucestoda) usually have arthropods or other invertebrates as intermediate hosts. With these, for example Hymenolepididae, immune regulation usually occurs through the definitive host. For example, with *Hymenolepis diminuta* and *H. microstoma,* resistance to superinfection or reinfection may be manifested by loss of worms, stunting or failure to produce eggs. With *E. granulosus* and *Taenia* spp., however, it is the intermediate host that is the density-dependent regulator of the parasite population. Thus, with any strong infection pressure, density-dependent constraints on unbounded growth of the population must first occur through that host.

- **Definitive hosts:** dogs by their lingual-anal grooming habits have abundant access to tapeworm eggs, but appear only to acquire immunity to *E. granulosus* from the ingestion of protoscoleces. Each dog remains susceptible to infection for varying numbers of challenges with about 50% of the population showing reduced susceptibility by the 6th infection (Fig. 5.1.2.). An extrapolation suggests that 99% may do so by the 12th infection (13, 15, 16, 19, 20).

Immunity acquired by dogs against intestinal stages of *E. granulosus* could act as a density-dependent constraint. However, it has been shown that protective immunity is weak or lacking. Thus acquired immunity by dogs to *E. granulosus* can be ignored in the epidemiological equation (13, 15, 16, 33, 43, 44). This may also turn out to be the case with *E. multilocularis* for both dogs and foxes (Chapter 5.3.).

- **Intermediate hosts:** immunity to superinfection by *E. granulosus*, *T. hydatigena* and *T. ovis* can be acquired or induced in sheep (12, 13, 16, 17, 18, 21, 24, 46). Insufficient studies have been made to quantify the role played by acquired immunity in the epidemiology of *E. multilocularis* infections in rodents. Some protection against superinfection with *E. granulosus* in mice and *E. multilocularis* in cotton rats has been demonstrated (8, 37). Immunity can also be induced to *E. multilocularis* in red-backed voles (*Clethrionomys rutilus)* by an injection of eggs (R.L. Rausch and M.A. Gemmell, unpublished findings). There is good reason to suppose that this also acts as a density-dependent constraint, preventing superinfection in rodents, but its duration in the absence of ingestion of further eggs is not known.

Based on studies with *T. hydatigena* and *T. ovis*, the characteristics of acquired immunity by sheep appear to be:

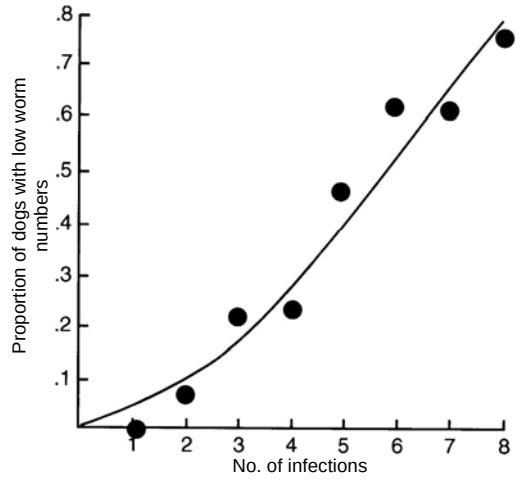
a) acquired within 7 to 14 days by the ingestion of as few as 10 eggs (Fig. 5.1.2.)

b) life-long in the presence of eggs

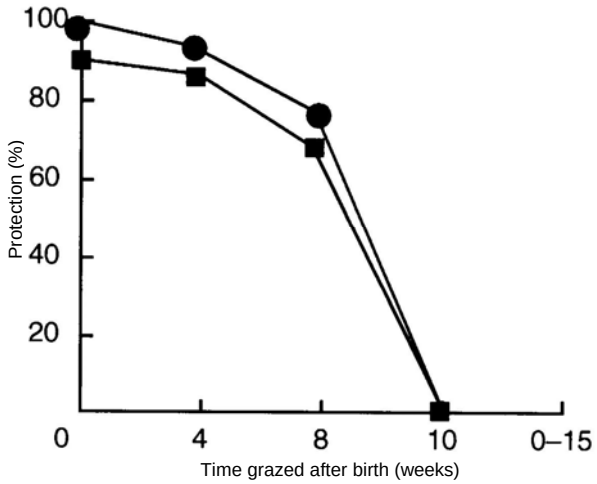
c) lost between 6 and 12 months in the absence of eggs (Fig. 5.1.2.)

d) not dependent on the presence of larvae from a previous infection.

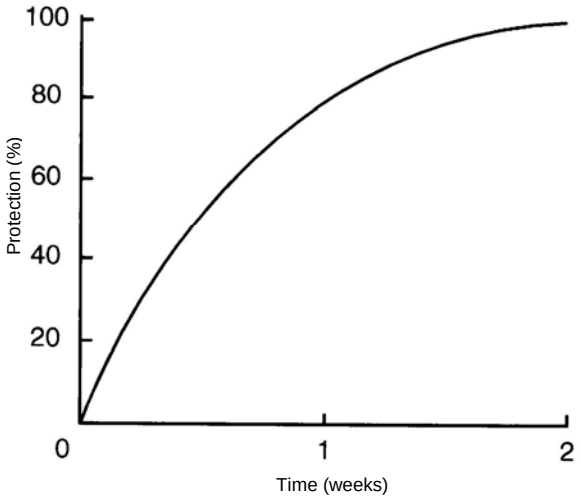
Without doubt, this is the density-dependent constraint that regulates the parasite population, but only under high infection pressures (15).



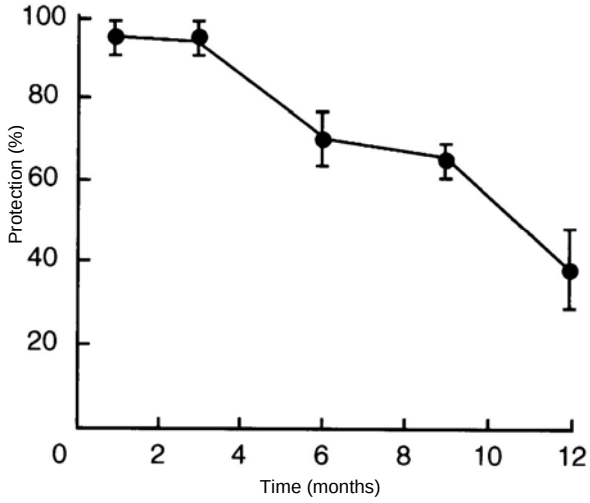
a) Changes in susceptibility of dogs to *E. granulosus* following reinfection



b) Changes in the protection given to lambs against *T. hydatigena* ■ and *T. ovis* ● from passively transferred immunity when grazed for specific periods after birth or from birth for 1-16 weeks on pasture contaminated with eggs



c) Time interval (weeks) between immunisation and acquisition of immunity to *T. hydatigena* by lambs



d) Time interval (weeks) between immunisation and loss of immunity to *T. hydatigena*

Fig. 5.1.2.
Density-dependent constraints imposed by the host on adult and larval Taeniidae
(13, 14, 15, 19, 20, 22)
Source: M.A. Gemmell (15)
Reproduced from (15) with kind permission from F.L. Andersen (ed.)

Passive immunity may also play a role as a density-dependent constraint in some systems. For instance, it operates with *T. ovis*, but not *T. hydatigena* (Fig. 5.1.2.). With the former under a high infection pressure, there is no 'window of susceptibility' as maternally derived intestinal antibodies provide protection until immunity is acquired. In the latter case under a similar high infection pressure, there is a 'window of susceptibility' and infection can occur before immunity is acquired (22).

Little is yet known of the role played by passively transferred immunity with either *E. granulosus* or *E. multilocularis* under a high infection pressure, as the only experiments conducted so far have used donors that were infected but not necessarily immune (24).

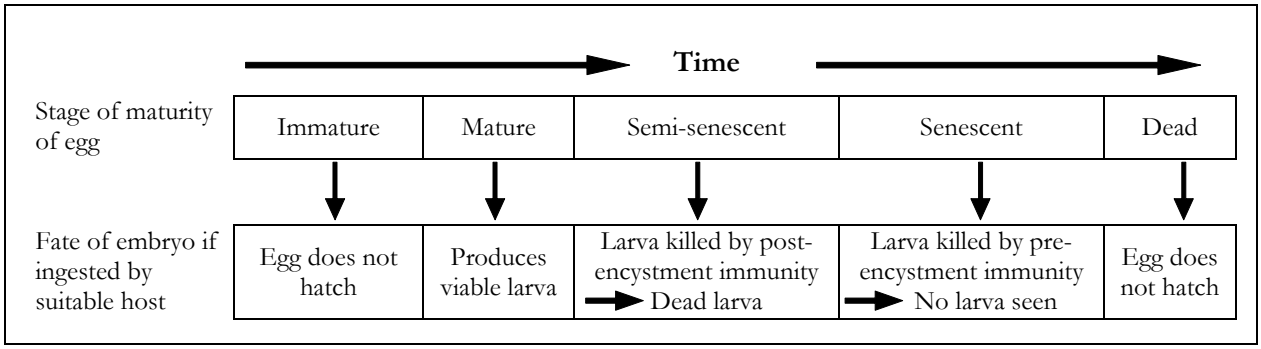
5.1.3. Contribution by the environment to transmission dynamics

Maturation-ageing process of the egg and density-independent constraints

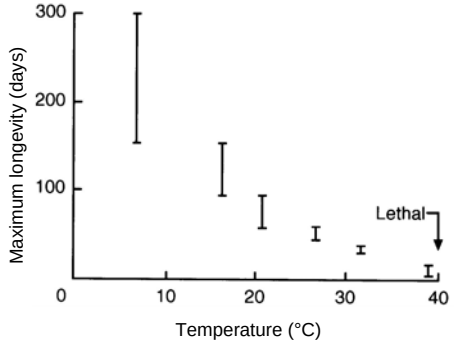
Eggs on expulsion from the proglottid are subject to ageing by environmental effects (Fig. 5.1.3.). Weather and climate are density-independent constraints and contribute to the basic reproduction ratio, but do not regulate the parasite population. Desiccation is lethal and the limits of temperature tolerance are between +40°C and −70°C. Between these two extremes, temperature regulates the maturation-ageing process. For example, longevity of eggs of *T. ovis* was reduced from 150-300 to 2-10 days by raising the temperature from +7°C to +38°C. Similarly, eggs of *E. granulosus* survived for more than 200 days at +7°C but only 50 days at +21°C (Fig. 5.1.3.) (14, 15). It was concluded that eggs of *E. multilocularis* may survive for up to 3 and 8 months in the summer and winter in Europe, respectively (11). It is the duration of this seasonal climatic effect on egg survival that *inter alia* determines the transmission dynamics and geographic prevalence (14, 15, 16, 20, 21, 27) (Chapter 5.3.).

Immigration, emigration and egg-dispersal mechanisms

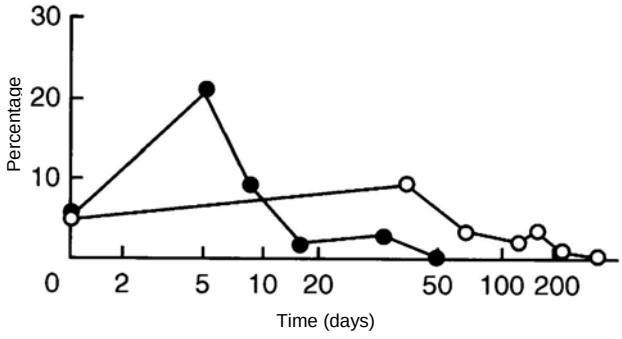
Although most taeniid eggs usually remain within 180 m of the site of deposition, some may rapidly disperse over an area of up to 30,000 ha (Fig. 5.1.4.). Experimental evidence is now available that blowflies (particularly Calliphoridae) are important transport hosts (15, 16, 27, 28, 29). Birds have been reported as potential transmission agents of *T. saginata* (7). More recently, transfer of *T. hydatigena* eggs over a distance of 60 km has been explained by combined activities of birds and insects (47).



a) Sequence of events during the hatching/activation process



b) Effect of temperature on the longevity of eggs of *T. ovis*



c) Longevity of eggs of *E. granulosus* stored at 21°C ( ) and 7°C (O)

Fig. 5.1.3.
Density-independent constraints imposed by climate on taeniid eggs
(13, 14, 15, 16, 27)
Reproduced from (15) with permission from F.L. Andersen (ed.)

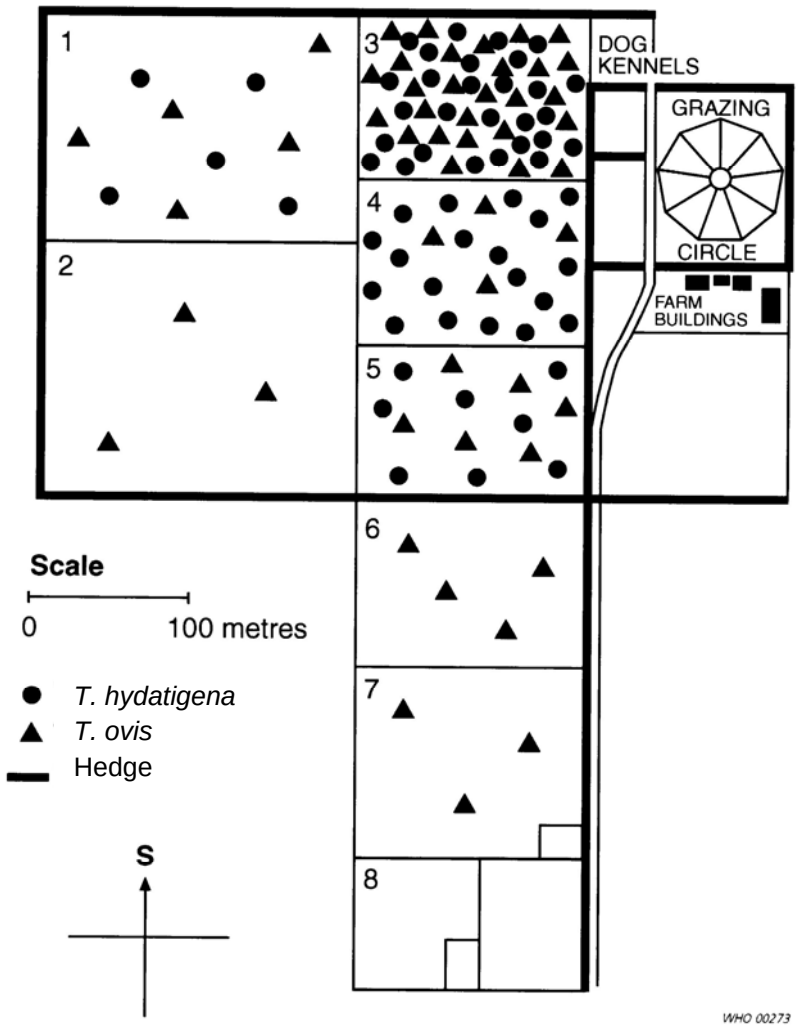


Fig. 5.1.4.
Dispersal of taeniid eggs from the site of deposition
Eggs of *Taenia hydatigena* ( ) were dispersed from an experimental grazing circle in which infected dogs were kept to plots 1, 3, 4 and 5. The eggs of *Taenia ovis* ( ) were dispersed from dog kennels with infected dogs to plots 1-7
Measurement was made by grazing sentinel lambs and counting the larvae in them
(13, 15, 16, 27, 28, 29)
Adapted from (15) with permission from F.L. Andersen (ed.)

Summary of factors determining the numerical distribution of the larval population in animal intermediate hosts

In the natural environment, the numerical distribution and degree of over-dispersion of the larval sub-population in the animal intermediate host population is a product of the following factors:

- spatial distribution of the eggs
- age of the eggs at the time of ingestion
- density-independent constraints on egg viability and infectivity
- proximity to grazing of the egg deposits
- age of the hosts when first exposed to eggs
- heterogeneity within the flock
- density-dependent constraints (15).

5.1.4. Stability and equilibrium steady states

Stability is an essential part of the description of host/parasite systems. It describes the ability of biological systems in equilibrium to withstand perturbation, such as might be encountered in a control programme, and after that perturbation has ceased to return to the previous equilibrium or reach a new one. In general, a parasite system is asymptotically stable if the parasite population returns to that state, following a temporary perturbation away from it. A parasite system is structurally stable if its dynamics are qualitatively unchanged by perturbations in its parameters (42). The overall stability is the product of the complex interactions of stabilising and destabilising forces, such as numerical distribution, biotic potential and immunity.

Basic reproduction ratio

The concept of the basic reproduction ratio (R_0) is central to an understanding of the transmission dynamics, stability in the environment, and control and eradication of parasites (1). The ratio of the number of adult parasites in the 'next generation' to the number of adult parasites in 'this generation' defines the basic reproduction ratio of the parasite population, and is usually denoted by R_0. In the past it has often been called the basic reproductive rate (2), but the former term is now preferred as being more scientifically and grammatically correct (25, 42). Some of the factors that contribute to R_0 are summarised in Figure 5.1.5.

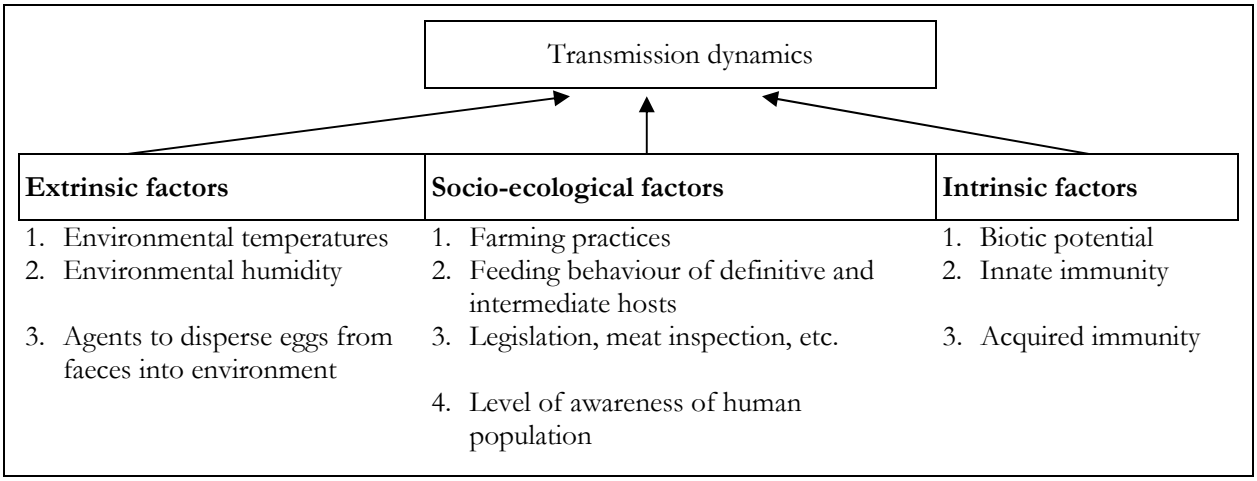


Fig. 5.1.5.
Factors contributing to the transmission dynamics of *Echinococcus granulosus* in the farm situation
(13, 15, 18)
Reproduced from (15) with permission from F.L. Andersen (ed.)

Density-dependent and independent constraints

Parasite populations are subject to two types of constraint. Density-independent constraints, such as the action of weather on free-living stages and the mortality of host animals due to reasons not connected with their parasite burden, do not regulate the parasite population. A density-dependent constraint, which is a constraint whose severity increases as the parasite density increases, does regulate the parasite population. Density-dependent constraints include parasite-induced host mortality (not important for cestodes), and the acquisition of immunity to infection by the host. By definition R_0 is the reproduction ratio in the absence of density-dependent constraints (42).

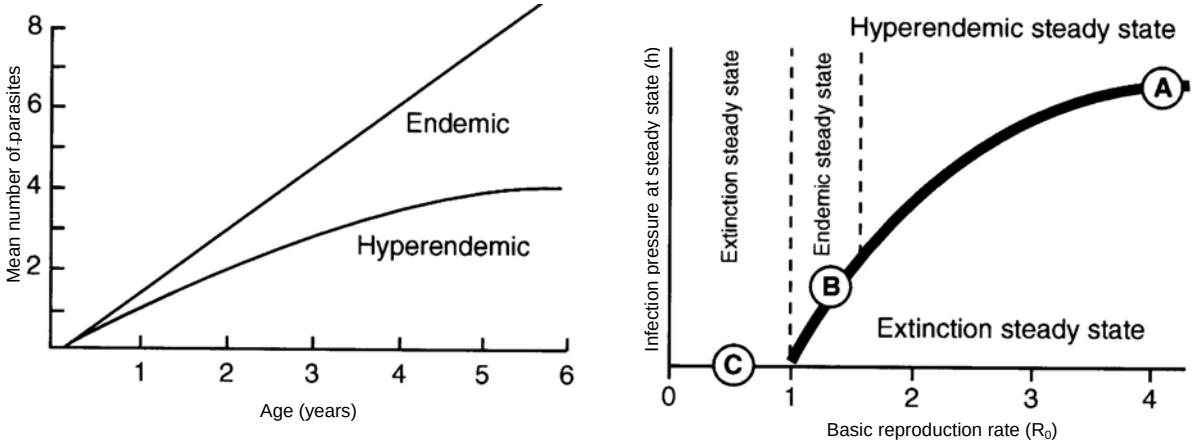
Equilibrium steady states

If a parasite population is neither increasing nor decreasing with time, then it is in a steady state and its effective R_0 is 1. Various epidemiological steady states of cestodes can be distinguished as follows (15):

- **Endemic steady state:** the population size is constant ($R = 1$) and the effects of density-dependent constraints are insignificant ($R_0 > 1$, $R_0 \cong 1$).

- **Hyperendemic steady state:** R = 1 and the population is strongly regulated by density-dependent constraints (R_0 >>1).
- **Extinction steady state:** no parasite is present.

The possible steady states as a function of R_0 are illustrated diagramatically in Figure 5.1.6.



a) Age-intensity prevalence curves in the endemic and hyperendemic steady states

b) Graph showing the existence of steady states for different values of R_0 with 'h' representing the infection pressure

Fig. 5.1.6.
Transmission dynamics of taeniidae
(15, 21, 42, 43, 44)
Reproduced from (15) with permission from F.L. Andersen (ed.)

If R_0 <1, the only possible steady state of the parasite population is extinction steady state (Fig. 5.1.6.). Furthermore, if R_0 is reduced and maintained below one, then the parasite population becomes extinct with time. The extinction steady state is a possible realisation of the dynamics of the parasite population regardless of the value of R_o. However, if a parasite population is near to extinction but R_0 >1, then the population will increase in size. Thus, if R_0 >1, the extinction steady state is unstable.

A steady state of a parasite population is said to be globally asymptotically stable if the population will tend to that steady state over time, regardless of the initial parasite abundance. A steady state is said to be locally asymptotically stable if a population will return to that state over time and if it were originally in that state, but has been perturbed by a small amount. The threshold theorem (26) says that if R_0 <1 the extinction steady state is globally asymptotically stable, and if R_0 >1 it is unstable. If R_0 >1 the (hyper)endemic steady state may be locally or globally asymptotically stable, or even unstable, depending on the possibilities for long-term non-steady behaviour. A cestode population is said to have extinction, endemic or hyperendemic status depending on whether R_0 <1; R_0 >1 and $R_0 \cong 1$; or R_0 >>1 respectively (42). Clearly, the objective of any parasite eradication campaign must be to reduce the parasite population to extinction status, and maintain this until no parasites remain. It should be noted that if conditions are then relaxed and once again R_0 >1, then the extinction steady state becomes unstable, and if the parasite is reintroduced it will re-establish. On the other hand, the objective of a control programme may be to reduce some measure of the parasite abundance to an acceptable level, and to maintain that level. A control programme, therefore, is not time-limited (13, 18, 42, 43, 44, 45) (Chapter 6.1.).

Empirical method of determining the equilibrium steady state

Provided that the infection pressure has remained constant throughout the lifetime of the host animals, such as sheep, the equilibrium steady state can be defined by determining the intensity of infection in relation to their age. A linearly increasing age-intensity curve indicates endemicity, but if the curve is depressed below the straight line, hyperendemicity (Fig. 5.1.6.).

Mathematical method of determining the basic reproduction ratio

It has been shown by Roberts *et al.* (18, 42, 43, 44, 45) that the life-cycles of cestodes can be modelled by non-linear integrodifferential equations of the form:

$h' = -\mu h + \lambda f * (Sh)$

h : infection pressure on intermediate host

μ : rate of loss of parasites from the system

λ : rate of transmission of parasites through the system in the absence of density-dependent constraints, $R_0 = \lambda/\mu$

f : probability density function for delays

S : proportion of intermediate hosts which are susceptible to infection

$*$: denotes convolution representing delays in the system.

A non-linear form is used for parasites with high biotic potentials such as the ovine cysticercoses (*T. hydatigena* and *T. ovis*) and a linear form (with $S = 1$) is used for *E. granulosus*. If the parasite is in a steady state, R_0 can be estimated from:

$$R_0 = 1 + \frac{\text{mean duration of immunity in the host population}}{\text{mean time to immunity in the population}}$$

If the infection pressure is so high that acquired immunity lasts for life, then this formula is equivalent to:

$$R_0 = \frac{\text{mean life expectency of the host}}{\text{mean age at which immunity is acquired}}$$

Where age-intensity prevalence surveys have been made (namely: in New Zealand, the People's Republic of China and Uruguay), *E. granulosus* has been found to be endemic (13, 15, 42, 44).

5.1.5. Transmission dynamics of human cystic echinococcosis

Results from experimental studies and control programmes shed considerable light on the transmission dynamics of infection in human beings.

Experimental evidence for egg-transmission to humans

There are several documented studies associating various risk factors with human infections (Chapters 5.2. and 5.3.). Man can contract the *Echinococcus* infection by direct contact with infected definitive hosts, or indirectly through food, water and objects, contaminated with eggs of the parasite. However, exact information on the actual significance of the direct and indirect ways of transmission is scarce because studies on this topic are difficult to perform and have been hampered by the fact that the eggs of *Echinococcus* and *Taenia* species cannot be distinguished, except for *E. multilocularis* with recently developed PCR techniques (32, 35).

Coprophagic flies and other animals may serve as mechanical vectors of the eggs. Experiments exposing blowflies first to dog faeces containing proglottids of *T. pisiformis* or *T. hydatigena* and then to grass and cooked meat that were then fed to rabbits and pigs appropriately, demonstrated that:

a) taeniid eggs remain viable after passage through the gut of flies, and also

b) blowflies transmit them indirectly to these hosts by their normal activities of vomiting and defecation.

If it is assumed that the taeniid eggs used represent *Echinococcus* eggs, then these experiments do suggest that where there is a natural abundance of blowflies together with unlimited opportunities for contacting both dog

and fox faeces and human foodstuffs, blowflies would provide one practical way for *E. granulosus* and *E. multilocularis* infections to occur (15, 27, 28, 29).

Susceptibility of humans to infection with *Echinococcus granulosus* in the endemic steady state involving dogs and sheep

Intensive studies were made of the changes in the age incidence of CE that occurred in the human population during the control programmes in Tasmania and New Zealand (3, 4, 5, 6). In both programmes, *E. granulosus* was regarded as being in the endemic steady state prior to control.

The control campaigns in Tasmania and New Zealand were officially started in 1965 and 1959, respectively. All hospitals in Tasmania performing hydatid surgery agreed to make quarterly returns of CE. Diagnostic criteria were:

a) a cyst confirmed at operation, or

b) a cyst confirmed at necropsy as a cause of symptoms or death, not as an incidental finding.

Only new cases were collected for surgical incidence. The New Zealand data were collected only from the public hospitals, but the relative proportion of public and private surgical patients was stable over the whole period.

In Tasmania, incidence data were collected annually from 1966, and the most important were those of the first two 5-year periods, 1966-1970 and 1971-1975, during which incidence halved. Age-specific rates were calculated from the estimated populations in each age group at the mid-point in each period.

Of the 87 new patients, 77 (89%) were born in Tasmania, 6 in other States and 3 overseas. Only one of the latter came from an endemic area for hydatid disease (Greece) and only 22 (25%) lived in a Tasmanian city at the time of admission. The birthplace of one patient was not recorded. The age distribution of age-specific incidence for the two periods are shown in Tables 5.1.2. and 5.1.3., respectively. The total incidence in the second period was reduced to less than half, due to a substantial fall in all age groups. Assuming that the numbers of cases showed a Poisson distribution, a test for a decline in the number of patients aged 25 years and over was significant at the 5% level. However, Table 5.1.2. shows that the age distribution was unchanged according to the chi-square test. Thus, the incidence had been halved without significantly altering the age distribution. The same applies when the age groups under 15 years and over 45 years were combined to give larger numbers for the chi-square test (6).

Table 5.1.2.
Age distribution of new cases of human cystic echinococcosis: Tasmania, 1966-1970 and 1971-1975

Age group (years)	1966-1970	1971-1975	Total
0-4	3	0	3
5-14	11	4	15
15-24	9	7	16
25-44	15	7	22
45-64	16	8	24
65+	5	2	7
Total	**59**	**28**	**87**

The chi-square test shows that the age distribution is not significantly different in the two five-year periods (Table 5.1.3.).

This and the other data from the two campaigns are incompatible with Dew's theory that most infections occur in childhood. Dew (9) believed that a case diagnosed at age 45 years usually resulted from an infection at least 30 years earlier. According to that hypothesis, a control programme starting in 1965 would not affect the incidence in the 45 year-old cohort very much until about the year 2000 (6). The only interpretation that these data will allow is that about half of all the new adult cases had been infected less than a decade earlier, so these people must have been susceptible in adult life.

Table 5.1.3.
Age-specific annual surgical incidence (per 100,000 person per year) of human cystic echinococcosis in Tasmania, 1966-1970 and 1971-1975, and percentage reduction in the second five-year period

Period	All ages	0-4	5-14	15-24	25-44	45-64	65+
1960-1970	3.1	1.5	2.7	2.9	3.2	4.4	3.4
1971-1975	1.4	0	1.0	2.0	1.5	2.0	1.3
Percentage reduction	55	100	63	31	53	55	62

Source: T.C. Beard (4, 5, 6)

The incidence of CE changed rapidly during the Tasmanian control programme, and was halved in the second five-year period, without a statistically significant change in age distribution. Data from New Zealand (Table 5.1.4.) show that incidence was also halved there, without significantly altering the age distribution. This means that adults are susceptible and that cases with short latency are common. The discovery of adult susceptibility and short latency (4, 5, 6) is still compatible with the existence of long latency in certain individuals. What the data show is that childhood infection can no longer be regarded as the rule. They cast no doubt on the existence of exceptions to authentic cases of long-term latency before clinical symptoms occur and there is no inconsistency with the fact that silent infections can still be discovered at necropsy.

Table 5.1.4.
Age distribution of new public hospital cases of human cystic echinococcosis, New Zealand, 1951-1955 and 1963-1967

Age groups (years)	1951-1955	1963-1967	Total
0-4	19	3	22
5-14	98	35	133
15-24	82	46	128
25-44	112	64	176
45-64	98	48	146
65 +	44	25	69
Total	**453**	**221**	**674**

Source: T.C. Beard (4, 5, 6)

Changes in the transmission dynamics of *Echinococcus granulosus* in animals and humans during control

The changes in the transmission dynamics for human CE during successful control are similar to those observed in sheep (Table 5.1.5.).

In the dog-sheep life-cycle, echinococcosis is usually endemic in the absence of control. This was shown to be due to the low biotic potential in the dog and more particularly to the low proportion of eggs that develop into cysts in sheep (Table 5.1.1.). In the endemic steady state, there is no density-dependent constraint in the

form of acquired immunity and the sheep remains susceptible to infection throughout its lifetime. Once the infection pressure is reduced by treating dogs, the prevalence declines rapidly in both young and old sheep. This is also the case with *E. granulosus* in children and adults. In practical terms, it seems that in the endemic state, there is neither age nor acquired resistance in animals and humans and a vigorous control effort should benefit the whole community including the middle aged and elderly (4, 5). Little is known of the transmission dynamics in human infection with CE under very high infection pressures, such as may occur in parts of the African Continent.

Table 5.1.5.
Changes in the prevalence of *Echinococcus granulosus* in dogs, sheep and humans during the hydatid control programme in Tasmania

Year	Dogs infected (percentage)	Sheep infected		Human cases	
		<1 year (percentage)	>3 years (percentage)	1-19 years (number)	>19 years (number)
1965-1966	12.7	–	–	–	–
1966-1967	5.5	11.5	52.2	11	8
1967-1968	2.6	19.9	49.7	5	13
1968-1969	1.6	9.8	43.4	0	6
1969-1970	1.2	13.4	36.3	2	6
1970-1971	1.1	4.5	17.7	3	6
1971-1972	0.8	1.8	13.5	1	6
1972-1973	1.1	0.6	8.4	1	6
1973-1974	0.9	0.2	7.0	2	4
1974-1975	0.4	0.1	6.5	2	3
1975-1976	0.4	0	7.9	0	3
1976-1977	0.3	0	3.9	1	4
1977-1978	0.2	0.1	3.4	0	8
1978-1979	0.2	0	2.1	0	3
1979-1980	0.2	0	1.6	0	3
1980-1981	0.1	0.1	1.1	0	5
1981-1982	0.1	0	0.7	0	3

Source: T.C. Beard (4, 5, 6)

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5.2. Epidemiology of *Echinococcus granulosus* in transhumant situations

C.N.L. Macpherson

Summary

Transhumance, the seasonal movement of people and their livestock to regions of different climate, is a specialised life-style which permits the utilisation of vast tracts of seasonally productive land. This chapter deals with factors contributing to the epidemiology of the E. granulosus *infection in transhumant situations. Socio-economic conditions, particularly low levels of education, lack of knowledge about the disease and its transmission, poor sanitary conditions, the sharing of water sources with dogs and home slaughter, human behaviour, migrations and climate all contribute to high prevalences of cystic echinococcosis (CE) in transhumant communities throughout the world. Control methods rely on provision of appropriate education through traditional communication methods and the control and treatment of dogs.*

5.2.1. Definition and general aspects

Transhumance, the seasonal movement of people and their livestock to regions of different climate, is a specialised life-style which permits the utilisation of vast tracts of seasonally productive land. Transhumance, nomadism and semi-nomadism are ways of life for between 50-100 million people (36), who herd more than 120 million cattle or cattle equivalent units of livestock (31). Contemporary transhumant pastoralists live either in cold and temperate areas or in the hot, arid and semi-arid regions of the world. In the cold or seasonally cold areas, livestock are moved from south to north in the summer, returning south in the winter: in the cold mountainous areas, livestock are moved successively from plains, through hills and eventually on to mountainous pastures in the summer months, returning, often to more permanent housing, to the lower, warmer latitudes when autumn approaches. Such peoples live in northern and central Asia, Europe, north-west Africa, Greenland, Canada, parts of the south-western USA and along the western part of South America. In the hot desert and semi-desert parts of the world, mainly in Africa, the Middle East and Asia,

transhumant pastoralists move in response to seasonal rainfall patterns. Animals are grazed on the plains during the wet season and move to hilly areas during the dry season. A north/south migration pattern is also seen among pastoralists living in the Sahel who move in response to the dry monsoon, the *harmatan*, and the wet monsoon which moves up from the Gulf of Guinea (44).

Transhumant pastoralists maintain a range of different livestock species: sheep, goats, cattle, dromedaries and donkeys are kept in Africa; water buffalo are maintained by transhumant pastoralists living in Rajasthan; Kazak and Tibetan peoples herd bacterin camels, horses, mules, pigs and yaks; llamas and alpacas are herded by South Americans. Reindeer are herded in northern Norway, Sweden and Finland. Dogs are almost universally maintained by transhumant peoples and are valued for a variety of reasons: for herding, hunting, guarding, transportation, and food; as bed-warmers and as sanitation animals and companions.

Transhumant pastoralists have one of the lowest socio-economic levels in the world in terms of education, income and standard of living. This, coupled with their herding occupation and close association with their animals, the almost complete lack of piped water, abattoirs and the poor sanitary conditions, provide ideal conditions for parasitic diseases, including CE. Until relatively recently, this was largely unrecorded in these peoples because transhumant peoples spend most of the time in remote areas where, prior to the 1950s, there were invariably no veterinary, medical or educational facilities nor trained personnel. During the past 40 years with the introduction of primary care and hospital facilities into areas inhabited by transhumant peoples, the perception of the public health importance of CE has been radically revised. In parts of Africa and in Xinjiang Uygur Autonomous region in the north-west of the People's Republic of China, CE was unrecorded until the introduction of improved medical services and it is now acknowledged that CE is highly endemic in such areas (Figs 5.2.1.a. and 5.2.1.b.).

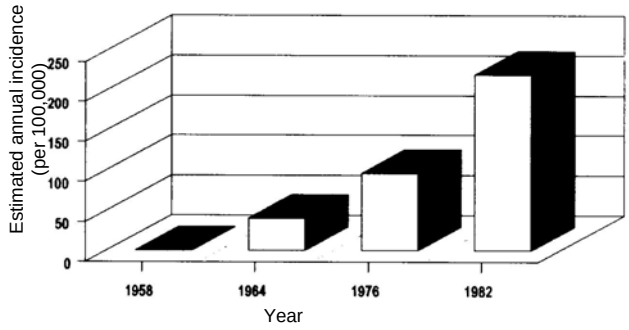

Fig. 5.2.1.a.
Changes in the annual surgical incidence rate of cystic echinococcosis in Turkana, Kenya
(11, 35, 43)

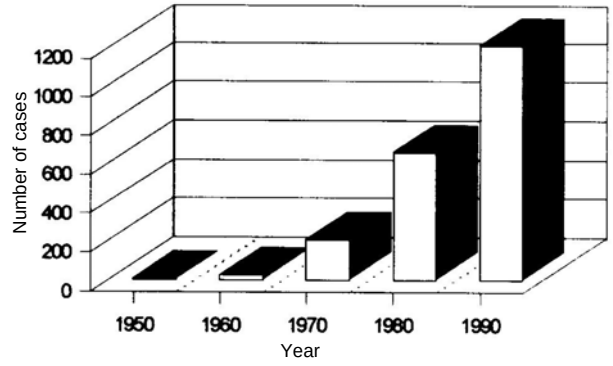

Fig. 5.2.1.b.
Annual number of surgical cases of cystic echinococcosis in Xinjiang between 1950 and 1990
Reproduced and adapted from (32) with kind permission from F.L. Andersen (ed.)

5.2.2. Cystic echinococcosis among transhumant pastoralists in the arid and semi-arid areas of Africa

In East Africa, CE is prevalent among the Masai (10, 30), Turkana (11, 30, 35) Boran and Pokot (30) in Kenya, the Maasai in Tanzania (10, 29), the Karamajong, Lango and Acholi in Uganda (37), the Nyangatom, Dassanetch, Boran and Hamar in south-western Ethiopia (12, 14, 16, 30) and the Toposa in southern Sudan (9). In this region, females had almost 3 times the prevalence of CE compared to males (Fig. 5.2.2.).

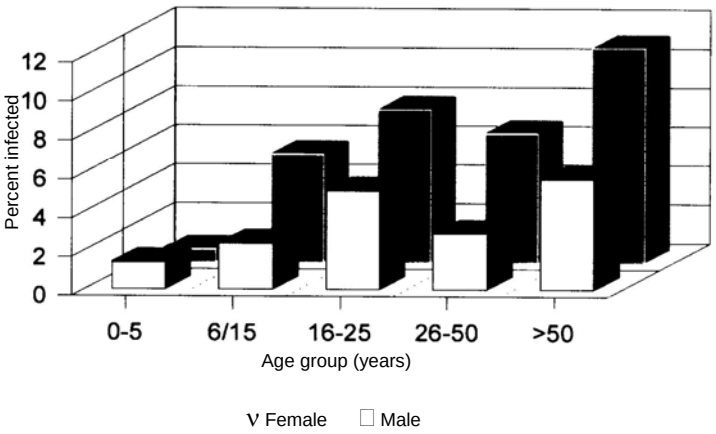

Fig. 5.2.2.
Prevalence of cystic echinococcosis in male and female Turkana as determined by a cross-sectional ultrasound survey
Adapted and reproduced from (30) with kind permission from C.N.L. Macpherson

There have been no reported human infections among Somali transhumant pastoralists (30). This is probably due to the fact that the Somali are Moslems, who do not keep many dogs and have little direct contact with them.

Domestic intermediate hosts in Africa

Most of the livestock in North Africa (Algeria, Morocco, Libya, Tunisia, Egypt and Niger), West Africa (Nigeria and Senegal) and East Africa (Sudan, Somalia, Kenya and Tanzania) are owned by transhumant pastoralists. In Nigeria, the Fulani produce more than 90% of the animal protein generated in that country (34). In East Africa, transhumant pastoralists maintain livestock for cementing friendships, marriage and for milk, but not for meat production, so few animals are slaughtered. Most animals are slaughtered at home and little is known about the prevalence of CE in livestock owned by many of the transhumant peoples. Sensitive, specific ante mortem diagnostic methods for CE in livestock have yet to be developed (40). Available abattoir records of animals owned by the transhumant Maasai of southern Kenya indicate that sheep and goats are the main domestic intermediate hosts (10, 21), whilst in Turkana, camels are also important (19).

Humans as intermediate hosts in Africa

Many transhumant peoples in Africa do not bury their dead and dogs and wild carnivores are able to scavenge from cadavers. Humans in the hyperendemic region of eastern Africa harbour large, single, unilocular cysts which are usually fertile (20, 30). Protoscoleces removed from human cysts at surgery have been shown to be infective to dogs and to silver-backed jackals (*Canis mesomelas*) (25) and humans can act as intermediate hosts in these parts of the world.

Domestic definitive hosts in Africa

The domestic dog is the main definitive host of *E. granulosus* among transhumant pastoralists. Autopsy surveys consistently demonstrate high rates of infection: 39%-70% in Turkana (26, 33), 27%-50% in Maasailand, Kenya (10, 29, 33) and southern Sudan (9).

Domestic wildlife interactions in Africa

Transhumant pastoralists, particularly in eastern Africa, live in close proximity to wild animals. This facilitates exchange of many diseases. The most prevalent and economically important in Africa include viruses (rinderpest, malignant catarrhal fever, bluetongue, ephemeral fever, Rift Valley fever, African horse sickness, foot and mouth disease and rabies), bacterial and rickettsial infections (brucellosis, tuberculosis and bovine petechial fever), protozoan diseases (trypanosomosis and theileriosis) and numerous arthropod and helminth parasites (24). More than 18 species of wild herbivores and 6 species of wild carnivores, including the lion (*Panthera leo*), silver-backed jackal, golden jackal (*Canis aureus*), Cape hunting dog (*Lycaon pictus*), hyena (*Hyena* spp.) and African wild cat (*Felis lybica*), have been found infected with *E. granulosus* (22). The Turkana eat jackals and hyenas (25) which could place such people at risk of exposure to *E. granulosus* infection from these wild carnivores. Wild carnivores predate on domestic livestock in some pastoral areas, such as in Maasailand and in Samburu in northern Kenya, but the significance of wildlife in the domestic life-cycle of the parasite is unknown.

5.2.3. Cystic echinococcosis in transhumant communities in cool and seasonally cold climates

The People's Republic of China

General aspects

In the People's Republic of China, CE is prevalent among the Kazak, Mongolian and Kergez transhumant pastoralists living in the vast desert-steppe pastures of the Xinjiang Uygur Autonomous region, in the north-west of the People's Republic of China (17, 32). Between 1951 and 1990, 16,663 surgical cases of CE were recorded in this region (most reported in the 1980s) with 42% of cases occurring in peasants and herdsmen: nomadic pastoralists were at highest risk (32). Males and females were at equal risk of contracting CE and peaks of infection were observed in the 6-10 and 36-40 year old age groups. Transhumant Mongolians in the Bayanbluk prairie had almost twice (31.4% compared with 17.5%) the prevalence of CE as settled Mongolians in Tekes County of Xinjiang (32). Cystic echinococcosis is also prevalent among transhumant pastoralists in Tibet. During a US and chest X-ray survey in Guoluo, Qinghai, 18 of 423 (4.3%) cases of CE were recorded (32) and 43 (4%) cases were detected in Chayu (15). In the Xia He Town and Hezou hospitals in Gannan Prefecture, most of the surgical cases of CE were Tibetans (6).

Domestic hosts in the People's Republic of China

Dogs are the main definitive hosts in the highly endemic regions of northern the People's Republic of China with prevalences varying between 7.1% and 71.4% in rural areas (17). The highest prevalences were found in dogs owned by transhumant pastoralists. In Hutubi County in Xinjiang, 87 (10.3%) of 848 dogs autopsied in agricultural areas harboured *E. granulosus* infection compared with 4 (25%) of dogs owned by transhumant pastoralists (17). A similar situation exists between agricultural and pastoral areas throughout Xinjiang. In the highly endemic areas of Xinjiang, 1-2 year-old dogs comprised 70%-80% of the dog population and so transmission infection pressure was high (17). Although yaks, pigs, goats and cattle are commonly found infected with CE, the principal animal intermediate host in the highly endemic regions of the People's Republic of China are sheep (6, 18, 42). Other animals found infected among the transhumant herds in Xinjiang include the *pien-niu* (offspring of bull and female yak), camels, buffalo, horses, donkeys and mules (5). Sheep have the highest infection rates and are slaughtered in greater number than other livestock species. In transhumant communities, between 31% and 54% of one year old sheep are infected, indicating an intense transmission infection pressure (18).

Cystic echinococcosis in transhumant peoples in the United States of America and Sicily

Cystic echinococcosis is more prevalent among the transhumant Basque-Americans who live in California (3) and among the Navajo and other native American tribes in New Mexico and Arizona (41), than among non-transhumant groups in the same area. Dogs are used for herding and have access to sheep that die in the field and to offal, after slaughter, as there are no meat inspection facilities. The sheep strain of the parasite is also responsible for a high CE infection rate in transhumant peoples in Sicily (45).

5.2.4. Factors affecting the epidemiology of cystic echinococcosis in transhumant situations

Socio-economic conditions, particularly low levels of education, lack of knowledge about the disease and its transmission, poor sanitary conditions, the sharing of water sources with dogs and home slaughter all contribute to high prevalences of CE in transhumant communities throughout the world. Human behaviour, migrations and climate also contribute to the epidemiology of CE in transhumant communities.

Small, scattered populations reduce the transmission infection pressure of many parasitic and infectious diseases (2). Periodic movements further reduce the risk of the build-up of faecally transmitted parasites, particularly where no other sanitary methods exist (24). In Xinjiang, the People's Republic of China, winter and summer pastures may be over 200 km apart and pastoralists may move their temporary camps more than 36 times in a year (18). It is estimated that the harsh conditions endured by livestock, particularly over winter, result in the premature death of approximately 10% of the livestock (about 2 million animals in Xinjiang annually) (18). These deaths occur primarily during the spring migrations when weak overwintered animals die along the migratory routes leading to the summer pastures in the mountainous areas (18). Such a situation provides scavenging opportunities for the numerous dogs (typically two to four shepherd dogs per household) that are used by the herdsmen to guard the flocks.

The authorities in the People's Republic of China, as elsewhere where transhumants live, are encouraging the nomads to settle. This has resulted in increased rates of CE in previously nomadic communities. In Alaska, an increased rate of CE was observed in the Nunamiut Eskimos once they made the transition from a nomadic to a sedentary lifestyle (38). Here, once the sanitary effect of frequent migrations was lost, the new towns soon became fouled by dog faeces. Forced sedenterisation, due to prolonged droughts, is thought to be important in contributing to the periodic increase in infection pressure of *E. granulosus* amongst the Turkana of Kenya (46).

The occurrence of seasonal and prolonged droughts in parts of Africa result in profound socio-economic and socio-cultural changes. A prolonged drought in Turkana, Kenya, between 1978 and 1981, killed more than 70% of the livestock (46), causing large numbers of people to enter crowded famine relief camps. Such prolonged drought cycles occur every 10 years and appear to increase the prevalence of many diseases. The death of livestock and the lack of meat inspection, led to heavy *E. granulosus* infections in dogs (Fig. 5.2.3.). The dog population increased and parasite transmission to susceptible intermediate hosts was facilitated by the change from small, widely dispersed mobile populations, to large sedentary populations concentrated around water points. Following the drought years, the dog population dropped dramatically (Fig. 5.2.3.), largely due to the lack of available food. Camels, which may survive droughts in greater number than other stock, consequently experienced a higher CE prevalence than other hosts (19).

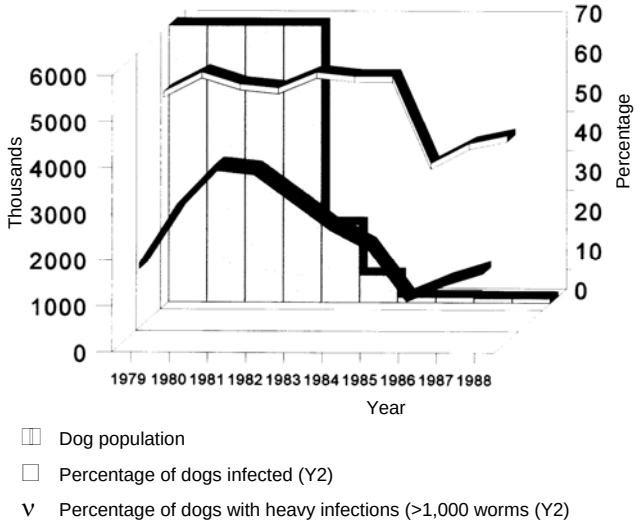

Fig. 5.2.3.
Change in the dog population and number of dogs harbouring heavy *Echinococcus granulosus*

worm burdens during and after the 1979-1981 drought in Turkana, Kenya
Adapted from Wachira *et al.* (46)

In Xinjiang and in Gansu Prefecture, the People's Republic of China, the severe winters result in little grass cover on the winter pastures and livestock are left in poor condition by the arrival of spring (6, 18). The livestock deaths provide scavenging opportunities for dogs during the spring migrations.

5.2.5. Climate and human behaviour

Hot and dry environmental conditions are inimical to the survival of infective free-living cestode stages. *Echinococcus granulosus* eggs in Turkana perish within a few hours in hot, dry conditions (47). Rapid transfer of eggs from dogs to humans is facilitated by close contact between humans and dogs. A quantitative study of contact between humans and dogs in regions of high, medium and low CE infection rates demonstrated that the amount of contact reflected different infection rates (48). Women had significantly more contact with dogs than men due to the dogs being concentrated around the home where women spend most of the day. Additionally, dogs are used to clean babies when they defecate, are always in attendance when a small baby is present and dogs are not used by the Turkana for herding. In contrast, the Maasai use dogs for herding and there is a greater dog:livestock contact in Maasailand than among Turkana which coupled with the cooler conditions, may partly explain the higher CE rate in their domestic animals (19).

5.2.6. Surveys, surveillance and control

The establishment of appropriate, sustainable, communication systems and veterinary, medical and educational infrastructure and trained personnel in regions that are seasonally occupied by transhumant pastoralists remains unfulfilled (1, 13). Methods of diagnosis, surveillance and control in such regions have to be adapted to the prevailing conditions. Portable US and rapid dot-ELISA (7, 39) have been demonstrated to be applicable for CE surveys (4, 28, 30). The use of US provides useful prevalence data on different parasite infections but care must be exercised to differentiate CE cysts from other space occupying lesions in all organs in which US can be used (23).

Control methods rely on provision of appropriate education through traditional communication methods (songs and role plays) and the control and treatment of dogs. Longitudinally collected prevalence data in livestock, following the implementation of control measures, is traditionally used to monitor control attempts (42). In the transhumant communities in eastern Africa this is not possible and surveillance is carried out through monitoring changes in the prevalence of the disease in dogs, through autopsy surveys and in humans using US and seroepidemiological surveys (27).

In Xinjiang, the People's Republic of China, a National Hydatid Disease Center has been established which administers field stations in the remote mountainous areas. Local control programmes have been implemented using 'bare-foot' vets and qualified veterinarians, who concentrate on the treatment of dogs with praziquantel (5, 6). As in other transhumant areas, education forms the cornerstone of the local control programmes (8).

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5.3. Epidemiology of *Echinococcus multilocularis*, *Echinococcus vogeli* and *Echinococcus oligarthrus*

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Summary

Echinococcus multilocularis *is essentially perpetuated in a sylvatic cycle with wild carnivores (mainly foxes of the genera* Vulpes *and* Alopex*) as definitive hosts and several species of small mammals (mainly Arvicolidae and Cricetidae) as intermediate hosts. Domestic dogs and cats may be involved in a synanthropic cycle. Evidence suggests that in most of the endemic regions the sylvatic cycle of* E. multilocularis *is the predominant source of infection for humans and for other aberrant hosts. However, in certain circumstances dogs have been shown to play a role as source for human infection. The role of cats needs further clarification. The potential ways of egg transmission to humans are discussed. The sylvatic cycle can persist with low (<2%) or high (>60%) prevalence rates of* E. multilocularis *in foxes and with variable infection rates of rodents (<1% to >80%). Transmission dynamics are influenced by many factors which are described. Recent epidemiological data suggest that dynamics of rodent populations may have a significant impact on transmission parameters of the parasite. Some data on the epidemiology of* E. vogeli *and* E. oligarthrus *are presented.*

Annexes contain biostatistical hints for the study of the E. multilocularis *infection in foxes, and descriptions of methods for sampling of small rodents and for age determination of foxes.*

The forms of echinococcosis caused by *E. multilocularis*, *E. vogeli* and *E. oligarthrus* are essentially natural-focal diseases. That is, the assemblages consisting of the *Echinococcus* parasites and their natural definitive and intermediate hosts exist independently in nature, and the involvement of domestic animals or humans in the cycle is incidental. Anthropogenic factors may influence and modify the natural-focal cycles leading to synanthropic cycles (see below). Several anthropogenic factors relevant to epidemiology of the above mentioned *Echinococcus* species can be discerned, among which the following are important:

- The modification of ecosystems by people, which results in conditions that enhance completion of the cycles of *Echinococcus* spp. Large-scale alterations, such as deforestation and agricultural land use, have brought about qualitative and quantitative changes in the composition of the mammalian fauna which induced modifications of intensities of predator-prey interactions, and expansion of geographic ranges of the cestodes has followed.
- The natural or artificial introduction of the cestodes into ecosystems in which they previously did not occur. Such introductions may result in new foci of endemicity, from which dispersal may follow.
- The ubiquity of the domestic dog, *Canis lupus* f. familiaris, is of special epidemiological significance. That canid takes the place of natural final hosts and can be involved in the cycles of *E. multilocularis* and *E. vogeli*.

These factors are discussed with special reference to *E. multilocularis*, the epidemiology of which is comparatively well known but not yet completely understood.

5.3.1. Epidemiology of *Echinococcus multilocularis*

The following chapter is focused on some key issues in the epidemiology of *E. multilocularis* and is partially based on recent reviews (23, 26, 70, 73, 84). These key issues include the life-cycle patterns and various factors of transmission dynamics. More details on geographic epidemiology are presented in Chapter 4.

5.3.1.1. Life-cycle patterns

General aspects

The natural life-cycle of *E. multilocularis* is based upon the predator-prey relationship that exists between carnivores (definitive hosts) and small mammals (intermediate hosts). The natural definitive hosts are wild carnivores, mainly foxes of the genera *Vulpes* and *Alopex*. In some regions, other wild canids, such as coyotes,

raccoon-dogs, wolves, etc., or domestic dogs and cats may also serve as definitive hosts. Metacestodes of *E. multilocularis* have been reported from mammals representing 8 families, but genera and species of the family Arvicolidae (voles and lemmings) (seven genera) and Cricetidae (hamsters, gerbils, and related rodents) (six genera) are the most important intermediate hosts (73). Definitive host species and particularly intermediate host species involved in the cycle may differ in various endemic regions and even within smaller areas or foci (73). Examples of different definitive and intermediate host assemblages are presented in Table 5.3.1.

Table 5.3.1.
***Echinococcus multilocularis*: selected examples of definitive and intermediate host animals in various geographical regions and of parasite prevalence in some regions**
Adapted from (23)

Region	Definitive hosts	Intermediate hosts
Western and central Europe	Red fox (*Vulpes vulpes*)* Domestic dog (*Canis lupus* f. familiaris) Domestic cat (*Felis silvestris* f. catus) Prevalence: foxes: <1%->60%; dogs and cats: generally low, <1%, rarely higher	Common vole (*Microtus arvalis*)* Snow vole (*Microtus nivalis*) Earth vole (*Pitymys subterraneus*) Bank vole (*Clethrionomys glareolus*) Water vole (*Arvicola terrestris*)* Muskrat (*Ondatra zibethicus*)* House mouse (*Mus musculus*) Prevalence: generally low, <1% to 6%, rarely higher, notably in muskrats
States of former Soviet Union	Arctic fox (*Alopex lagopus*)* Red fox (*Vulpes vulpes*)* Corsac fox (*Vulpes corsac*) Wolf (*Canis lupus*) Wildcat (*Felis silvestris*) Domestic dog (*Canis lupus* f. familiaris)	Northern vole (*Microtus oeconomus*) Common vole (*Microtus arvalis*) Voles (*Microtus* spp.) Brown lemming (*Lemmus sibiricus*) Red-backed voles (*Clethrionomys* spp.) Gerbils (*Meriones* spp.) Muskrat (*Ondatra zibethicus*) and others
China	Red fox (*Vulpes vulpes*)* Wolf (*Canis lupus*) Domestic dog (*Canis lupus* f. familiaris)* and others Prevalence: dogs in some regions up to 25%	Brandt's vole (*Microtus brandti*)* Pika (*Ochotona* spp.)* Gerbils (*Meriones unguiculatus*) Common Chinese zokor (*Myospalax fontanieri*) Red-cheeked souslik (*Spermophilus erythrogenys*) and others
Japan	Red fox (*Vulpes vulpes*)* Domestic dog (*Canis lupus* f. familiaris) Domestic cat (*Felis silvestris* f. catus) Raccoon-dog (*Nyctereutes procyonoides*) Prevalence (averages 1965-1991): foxes: 14% (locally >40%); dogs: 1%; cats: up to 5.5%	Red-backed voles (*Clethrionomys rufocanus bedfordiae*, *C. rutilus mikado*, *C. rex*)* Small Japanese field mouse (*Apodemus argenteus*) Norway rat (*Rattus norvegicus*) and others Prevalence (averages 1965-1991): generally low with average around 1%, locally up to 20%
North America		
Northern tundra zone	Arctic fox (*Alopex lagopus*)* Domestic dog (*Canis lupus* f. familiaris)* Prevalence: arctic foxes: 40%-100%; dogs: 12%	Northern vole (*Microtus oeconomus*)* Brown lemming (*Lemmus sibiricus*)* Northern red-backed vole (*Clethrionomys rutilus*) and others Prevalence: generally high, average ≈ 25% (1%->80%)
Central North America	Red fox (*Vulpes vulpes*)* Coyote (*Canis latrans*)* Grey fox (*Urocyon cinereoargenteus*) Prevalence: foxes: <1%->65%; coyotes: 6%-35%; cats; focally 1%-5%	Deer mouse (*Peromyscus maniculatus*)* Meadow vole (*Microtus pennsylvanicus*)* Muskrat (*Ondatra zibethicus*) Woodrat (*Neotoma cinerea*) House mouse (*Mus musculus*) Prevalence: generally low, about 0.5%-6%, rarely higher

* Animals known to be of special significance in the cycle

Sources: (21, 69, 70, 73, 75, 83, 84, 85, 95, 96, 113; F.-J. Liu, personal communication, 1998)

Epidemiologically, two types of cycles of *E. multilocularis* are of practical importance, namely the sylvatic and the synanthropic cycles (Fig. 5.3.1.)

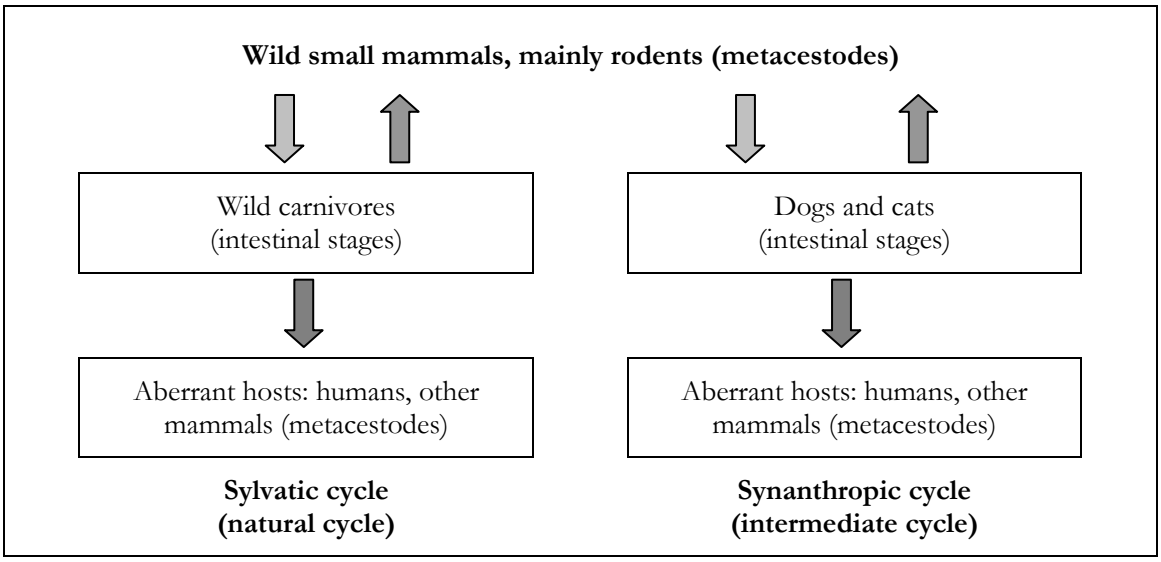


Fig. 5.3.1.
Epidemiologically relevant cycles of *Echinococcus multilocularis*

Sylvatic cycle

The sylvatic cycle of *E. multilocularis* is restricted to wild animal hosts, predominantly to foxes (*Alopex*, *Vulpes*) as definitive hosts and small mammals, mainly rodents, as intermediate hosts. *Echinococcus multilocularis* eggs excreted by definitive hosts to the environment are ingested by intermediate hosts in which the metacestode stage with protoscoleces develops. Infected intermediate hosts are prey of wild carnivores.

This cycle is of further significance in the epidemiology of *E. multilocularis* as it is the source of infection for:

a) aberrant hosts (humans and synanthropic animals, Chapters 2 and 3) that ingest eggs, and
b) for domestic carnivores which acquire an intestinal infection by ingestion of metacestode-infected small mammals thus becoming part of the synanthropic cycle (Fig. 5.3.1.).

Evidence suggests that the sylvatic cycle of *E. multilocularis* is the predominant source of infection for humans and for other aberrant hosts in most of the endemic regions (Chapter 3).

For example, in western and central Europe red foxes (*Vulpes vulpes*) have to be regarded as predominant definitive hosts, as prevalence rates of *E. multilocularis* in these hosts are high in wide areas, other wild carnivores do not play a role, and domestic dogs and cats are less frequently infected. Considering the infection rates with *E. multilocularis* and the population sizes of foxes, dogs and cats in the Canton of Zurich, Switzerland, a model calculation has shown that foxes are the largest group of *E. multilocularis* carriers (22, 26). Therefore, in this epidemiological situation foxes have to be regarded as main contaminators of the environment with *E. multilocularis* eggs (Chapter 6.2.).

In Hokkaido, Japan, with a similar epidemiological situation as in western and central Europe, the dominant role of foxes is well documented: during 1965-1991 the average prevalence of *E. multilocularis* was 14% in 18,073 foxes and only 1% in 9,742 dogs (69). Furthermore, the occurrence of human AE on Rebun Island and Hokkaido Island was closely associated with the spread of the parasite by foxes (92, 93) (Chapter 6.2.).

Other aberrant hosts may also acquire the infection from the sylvatic cycle. For example, in Japan, *E. multilocularis* metacestodes were found in 0.14 of approximately 1.1 million pigs, and in 0.81% of approximately 1,100 horses (70) (Chapter 3).

Synanthropic cycle

Domestic dogs and cats may be involved in a synanthropic cycle (intermediate cycle). They acquire the intestinal infection by preying on small mammals infected with fertile metacestodes of *E. multilocularis* (Fig. 5.3.1.).

Dogs having regular access to metacestode-infected rodents may frequently become infected with *E. multilocularis*. Under these special circumstances, they may represent a major source of infection for humans. For example, on St Lawrence Island, Alaska, numerous infected voles are an easy prey for dogs maintained in the villages. In 1951, 12% of the dogs in one of the villages were infected with *E. multilocularis*, and 22%-35% of the voles trapped in the years 1980-1982 harboured metacestodes (84). A study in north-western Alaska, with a similar epidemiological situation as on St Lawrence Island, revealed that Eskimo patients with AE were more likely than the controls to have owned dogs for their entire lives, tethered their dogs near their house, and lived in houses built directly on the tundra. Interestingly, trapping or skinning of foxes was not associated with a higher infection risk (91).

High prevalence rates of *E. multilocularis* in dogs were also reported from other areas, for example from the People's Republic of China with 14% (4/28) infected dogs in Sichuan (59) and 10% (6/58) in Gansu (8) and from a highly endemic focus in Switzerland with 12% (5/41) infected dogs in a rural area (44) (Chapter 6.2.). In Ganze County, the People's Republic of China, prevalences of *E. multilocularis* in stray dogs remained on high levels (23%-26%) during 1985-1998; the infection intensities ranged between 7 and 36,850 per dog (F.-J. Liu, personal communication, 1998). In Shiqu County, Sichuan, stray dogs in the same town were frequently infected with *E. multilocularis* (11%, 24/209), and in the same population the prevalence of *E. granulosus* was also high (13%, 28/209) (F.-J. Liu, personal communication, 1998).

It has to be stressed, however, that according to the present (incomplete) knowledge infection rates of domestic dogs and cats in various endemic areas are normally low. For example, in a recent study carried out in an endemic area of eastern Switzerland, where in average approximately 33% of the foxes are infected with *E. multilocularis*, 0.30% of 660 dogs and 0.38% of 263 cats were identified as carriers of the parasite by coproantigen detection in combination with PCR (19).

It is well documented that dogs and cats become infected with *E. multilocularis* by ingestion of metacestode-infected small mammals (Fig. 5.3.1.) that acquire the infection from the sylvatic cycle. On the other hand, intermediate hosts may also get the infection from eggs excreted by infected dogs and cats. This is evidenced by studies on St Lawrence Island, Alaska, where during a period of regular dog treatments with praziquantel the prevalence of *E. multilocularis* in locally captured rodents declined from 29% at the beginning to 5% at the end of campaign (76).

The synanthropic cycle can also serve as an infection source for humans. It has been shown that in special epidemiological circumstances in Alaska, *E. multilocularis* infected dogs have to be regarded as an important source of infection for humans (76, 91). It has to be underlined, however, that the role of domestic carnivores as infection source for humans may differ in various epidemiological situations (Chapter 6.2.).

Domestic cycle

Infrequently metacestodes of *E. multilocularis* have been found in house mice, and the possibility of a 'domestic' cycle involving domestic cats and house mice has been considered by various authors (57, 102). In Japan, there is a potential for the Norway rat to be involved in such a cycle. However, to date, there is no evidence for any epidemiological significance of such a cycle.

5.3.1.2. Transmission dynamics

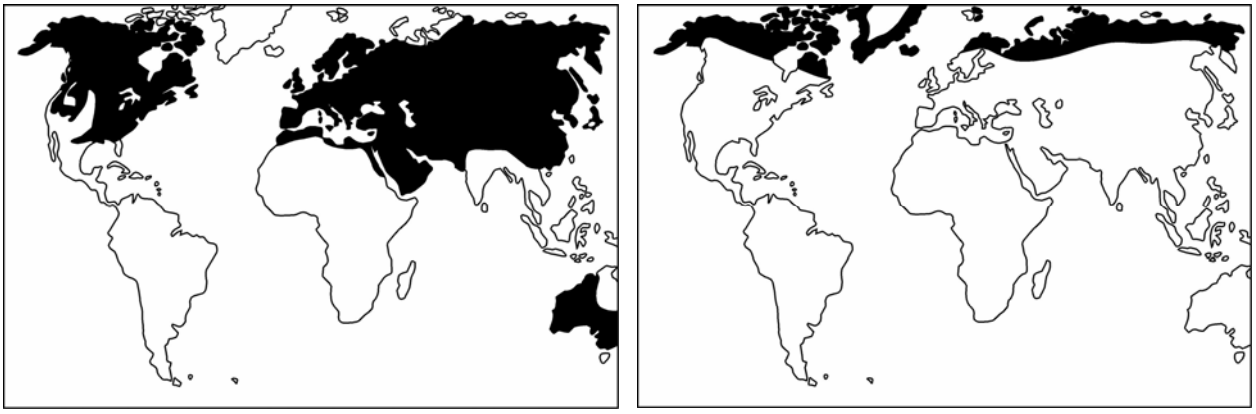
In this section, it is discussed how final hosts, the parasite, intermediate hosts, population dynamics and some other factors contribute to the transmission dynamics of *E. multilocularis* (for comparison see *E. granulosus,* Chapter 5.1.). In some fields the current knowledge is insufficient.

5.3.1.2.1. Contributions of final hosts

Many published records document the wide-spread occurrence of *E. multilocularis* in the arctic fox (*Alopex lagopus*) and the red fox (*Vulpes vulpes*) which have to be regarded as the most important definitive hosts of the parasite. A variety of other carnivores may also act as definitive hosts (see below). Details on the infection rates of various species of definitive hosts were reported by R.L. Rausch (72, 73).

- Arctic fox

The arctic fox or polar fox (*Alopex lagopus*) inhabits the Eurasian and North American tundra zone, including the Arctic islands (Fig. 5.3.2.). In many areas, the arctic foxes principally depend on arvicolid rodents as prey, especially on northern voles (*Microtus oeconomus*) and lemmings (*Lemmus* spp. and *Dicrostonyx* spp.), but they also use a wide variety of nesting birds and marine invertebrates in summer (75, 105). The primary source of food in winter is carrion, mainly consisting of beach-cast marine mammals, but voles and birds captured in summer can be apparently stored in large quantities for using during the winter (75). In Alaska, the young foxes are borne in early summer, become independent in autumn and reach sexual maturity in next spring (75). The fox populations fluctuate in size from year to year, and population dynamics are influenced by emigration or immigration. Arctic foxes are known as long-distance travellers over the pack ice, as well as over land, sometimes passing thousands of kilometres from their point of origin (30, 31). Such dispersing foxes may harbour parasites, including *E. multilocularis* (75). In Alaska, arctic foxes may be attracted by garbage that is discarded at places rather distant from villages, but normally they do not enter villages (R.L. Rausch, personal communication, 1998).



a) Red fox (*Vulpes vulpes*) (55) **b) Arctic fox (*Alopex lagopus*)** (114)

Fig. 5.3.2.
Approximate geographic distribution of the red fox (*Vulpes vulpes*) and the arctic fox (*Alopex lagopus*) (55, 114)

- Red fox

The red fox (*Vulpes vulpes*) has a wide range of distribution in the northern hemisphere, including parts of North America, Eurasia and North Africa; in the southern hemisphere it has been introduced to Australia. In the north, the ranges of the arctic fox and the red fox overlap in the same regions (Fig. 5.3.2.).

Red foxes are the principal definitive hosts for *E. multilocularis* in sub-Arctic regions of North America and Eurasia. It has to be noted, however, that *E. multilocularis* has not been reported from foxes in all areas of its distribution. Red foxes are essentially carnivorous, but omnivorous feeding habits are quite common. They

are scavenging and preying on small vertebrates and invertebrates (especially earthworms, insects), but eat also a wide variety of plants (fruits, seeds), and can survive on refuse provided by humans (55, 105). This generalist foraging behaviour enables the red fox to adapt to a wide variety of habitats, and to reach high population densities around and in human habitations (105). The sizes of territories of fox pairs or families may vary widely, for example in Europe between <0.04 and 16 km², depending on landscape factors and the availability of food sources (55). The average fox densities per km² are difficult to measure. Normally, they are estimated based on the Hunting Index (HI) which is an indicator for the number of foxes sampled per year and km² by hunting. In previous years (1974-1976) the Hunting Indices in large parts of central Europe ranged approximately between 1 and 1.4 foxes (60), and between 0.9 and 1.2 according to another source (6). In 1990-1991, the average HI in 13 Cantons of Switzerland was 1.7, with ranges between 0.1 and 2.5 (28). Artois (2) has provided evidence of an increase of fox densities in continental Europe and in the UK since the 1960s, notwithstanding a stabilisation or a temporary decrease on the continent in the late 1970s and early 1980s due to rabies epidemics.

Female foxes give birth to one litter per year in spring with an average size of approximately 5 cubs, which reach sexual maturity at about 9 months of age (28, 105). Surveys in Europe, the USA and Japan have shown that approximately up to 60% of a fox population may consist of animals up to one year of age (55). The high reproductive capacity of mature foxes ensures rapid recovery of the population reduced by hunting, trapping, traffic accidents or disease, such as rabies and mange (rabies may eliminate up to 60% of a fox population) (55). Young foxes may leave the family territory at about 6 months of age, with usual dispersal distances of between 10 km and 50 km (105).

In the UK, during the mid-1980s, an increasing invasion of cities by red foxes was noticed, and a few years later they were established in approximately 200 cities (55). In the city and in suburbs of Oxford, population densities of between 2.7 and 10 foxes per km² have been determined by radiotelemetry (55). The same phenomenon is now also recognised in other regions, such as central Europe (20, 45) and Japan (49, 100), and this can be relevant for the epidemiology of *E. multilocularis*. An urban cycle of the parasite was described in Zurich, Switzerland, with 67% of 123 foxes infected during the winter period in suburban areas, and 47% of 129 infected in the urban area; furthermore 14% of 135 water voles (*Arvicola terrestris*) from the city harboured the metacestode stage (20, 45).

- Other wild carnivores

Regionally or locally other wild carnivores may be involved in the cycle of *E. multilocularis*, such as the corsac fox (*Vulpes corsac*), the coyote (*Canis latrans*), the wolf (*Canis lupus*), the raccoon-dog (*Nyctereutes procyonoides*), and the wildcat (*Felis silvestris*). Also, captive wild carnivores may be involved, for example wolves in Hokkaido, Japan (Chapter 3 and Table 3.2.).

- Domestic carnivores

Domestic dogs and cats can act as definitive hosts for *E. multilocularis*. Their epidemiological role will be discussed below (Chapter 6.2.).

5.3.1.2.2. Contributions of the parasite

Echinococcus multilocularis in dogs and foxes

- Parasite biology

Relatively little knowledge exists on the natural history of *E. multilocularis* in definitive hosts. Data of experimental infections of dogs and red foxes have shown that the prepatent period of *E. multilocularis* can be as short as 26 to 29 days (67, 98, 109). These findings are supported by studies on the development of *E. multilocularis* in experimentally infected golden hamsters (50, 51). Egg excretion in faeces of foxes persisted for about 1 to 4 months (109), and egg counts per gram are variable from day to day and may reach values as high as 100,000 (67, 108). The mean number of eggs per proglottid of the mature *E. multilocularis* isolated from dogs or foxes is approximately 300 (98), as compared to approximately 600 in *E. granulosus* (Chapter 5). The number of proglottids produced per *E. multilocularis* specimen per day is estimated to 0.08 to 0.14 (18).

Thus, a fox infected with 10,000 mature *E. multilocularis* stages could theoretically excrete 800 to 1,400 proglottids per day, corresponding to approximately 240,000 to 420,000 eggs.

- Susceptibility of hosts and worm burdens

Experimental infections have shown that dogs of various age groups are highly susceptible to *E. multilocularis* (25, 80, 98, 109). After experimental application of high doses of protoscoleces of *E. multilocularis* normally all animals of a group acquire the infection with worm burdens of >100,000 per animal (25, 80). In naturally infected dogs, worm burdens of 45,000 have been found (24).

Also foxes are highly susceptible, and this is evidenced by the fact that in some regions of central Europe approximately 40%-75% of the red fox populations are infected with *E. multilocularis* (1, 4, 5, 26, 28, 62, 79, 89). On St Lawrence Island, Alaska, the overall mean rate of infection of arctic foxes with this parasite was 77% in 1,579 animals, but prevalences ranged up close to 100% seasonally (75).

Under natural conditions, infection intensities with *E. multilocularis* in red foxes vary in wide ranges. In central Europe, most of the foxes carry low to medium worm burdens. Examples are presented in Table 5.3.2. The data based on estimated worm burdens indicate that the percentages of foxes with high worm burdens (>1,000 per animal) are relatively low, but variable. This may depend on many factors, including differences in the subjective estimation method. Basically, these data are confirmed by a recent study from Switzerland in which worm burdens of 36 foxes were counted (18). As indicated in Table 5.3.2., 25% of the foxes had worm burdens >1,000, but 75% had lower burdens. The 36 foxes of this study harboured a total biomass of 115,200 parasites, and only 2 animals harboured 78% of the total biomass. Maximum worm burdens were approximately 60,000 per animal (18). This finding could indicate that transmission may predominantly depend on a small percentage of foxes with high worm burdens. However, the egg production of *E. multilocularis* in relation to the intensity of infection has never been determined. It could well be that egg production in foxes with low or medium worm burdens may be relatively high in comparison to heavily infected animals.

Table 5.3.2.
Burdens of *Echinococcus multilocularis* in naturally infected foxes

Country	Number of foxes examined	Percentage of foxes with worm burdens of: 1-10	11-100	101-1,000	>1,000	References
		Estimated worm burdens[a]				
Germany	397	18.5	23.1	24.6	33.8	4
	801	17.2	41.9	24.7	16.2	104
	304	64.8[b]		25.0[c]	10.2	5
Switzerland	3,048	–	67.1	23.5	9.4	28
Japan	32	10	10	25	55	107
	42	11.9	26.2	33.3	28.6	66
		Worm burdens counted[d]				
Switzerland	36		38.9[e]	36.1	25.0	18

a) foxes examined by intestinal scraping technique and worm burdens estimated (Chapter 3)
b) percentage of foxes with worm burdens of 1-50
c) percentage of foxes with worm burdens of 50-1,000
d) foxes examined by the sedimentation and counting technique (Chapter 3)
e) percentage of foxes with worm burdens of <20-100

In Alaska, burdens of *E. multilocularis* in 138 arctic foxes were generally high, with 57% of the foxes harbouring an average of 7,399 worms (range: 1-60,350), 29% an average of 43,750 (range: 120-184,200) and 14% and average of 58,975 (range: 966-157,150) (75).

- Immunity and reinfection

Infection with *E. multilocularis* induces certain immune responses, as evidenced by production of circulating antibodies (Chapter 3). In four foxes experimentally infected with *E. multilocularis*, it was observed that a distinct reduction of coproantigen excretion occurred beginning around 3 to 4 weeks post infection which may have indicated that a large number of the parasites were expelled at this time (67). Moreover, it has been shown that an intestinal infection of golden hamsters with *E. multilocularis* which was terminated by chemotherapy with praziquantel at 23 and 25 days post infection induced a significant degree of resistance against homologous reinfection as indicated by a 95% reduced worm burden as compared to controls (48).

In several surveys in Europe, juvenile red foxes (up to one year) (except cubs) had higher prevalence rates of *E. multilocularis* and higher worm burdens than adult foxes (4, 28, 104), but such differences could not be found in other studies (94). In a recent survey, carried out in an endemic area of Germany, prevalences of *E. multilocularis* in juvenile foxes were significantly higher than in adult foxes between July and September, and this was proven by sound statistical methods (94). This phenomenon could be due to differences between juvenile and adult foxes in exposure and susceptibility. While young foxes may be less exposed to the infection through their food until July, their diet is similar to that of the adults from August onwards. Therefore, the findings suggest that young foxes are more susceptible than older foxes and that the latter may acquire partial immunity (94). To date, it is unknown whether or not protective immunity plays a significant role in the regulation of *E. multilocularis* populations in foxes and other definitive hosts. It is assumed that reinfection is likely to occur after elimination of the parasites. According to Rausch (R.L. Rausch, personal communication, 1998) experimental superinfection of dogs with *E. multilocularis* was possible, resulting in two cohorts of parasites, the first consisting of fully developed cestodes, the second of uniformly small, immature states. In discussions of these matters, it has to be considered that many questions are still open, and very little is known on the intestinal immune responses of canids against *Echinococcus* spp. (18).

- Other factors

Some other factors which may influence *E. multilocularis* populations in final hosts are discussed below (see population dynamics).

Echinococcus multilocularis in domestic cats

Cats naturally infected with *E. multilocularis* have been found in various endemic regions, including North America, Europe, and Asia. Some examples are listed in Table 5.3.3. (1, 98). The findings show that cats may harbour mature *E. multilocularis* with thick-shelled eggs so that they have to be regarded as potential sources of infection for intermediate hosts and humans.

Table 5.3.3.
Examples of natural infections of domestic cats with *Echinococcus multilocularis*

Country	Period	Number of cases	Percentage infected	References
France	1987-1996	3 of 81	3.7	71
Germany (Baden-Württemberg)	1974	1 of 207	0.5	27
	1988	4 of 316	1.3	111
	1989	5 of 170	2.9	32
Germany (Thuringia)	1992	2 of 58	3.4	106
Switzerland (eastern area)	1995	1 of 263	0.38	19
Japan (Hokkaido)	1965-1991	5 of 91	5.5	69, 110

However, there is experimental evidence that susceptibility of cats is more variable than that of foxes and dogs, and worm burdens may be retarded in development in comparison to dogs. For example, Zeyhle and Bosch (112) inoculated 10 cats and 2 red foxes with 100,000 to 400,000 protoscoleces of *E. multilocularis* in southern Germany. Two of the cats had high worm burdens (figures not given), in 6 other animals the establishment rate was below 1%, and 2 further cats were free of cestodes. An average of 106 eggs was counted per proglottid of worms from cats compared to a mean of 300 in worms from foxes (112). Retarded development of the parasite in cats after experimental infection was observed by Thompson and Eckert (98) and Kamiya *et al.* (53). It was also observed that a sudden decrease in the recovery rate of *E. multilocularis* from cats occurred after 10 days of infection (52). These and other data suggest that cats may have a lower capacity of egg excretion to the environment than foxes and dogs. However, they still may have significance as sources of infection due to their close association to humans (see below).

5.3.1.2.3. Contributions of eggs

- Dispersal of eggs

How eggs of *E. multilocularis* are dispersed has not been adequately investigated. Foxes infected with *E. multilocularis* may disperse eggs with their faeces anywhere within their individual areas of activity, in rural and in urban regions. In Europe, it has been observed that red foxes tend to deposit faeces on field borders, road and path verges, molehills and *Arvicola terrestris* tumuli more frequently than on meadows, on fields or other landscape structures (35, 55). Foxes living in urban, suburban and rural situations (e.g., in Hokkaido, Japan, or central Europe) may contaminate plants and soil in gardens and yards. Since red foxes are using faeces and urine as olfactory markers, they tend to deposit faeces also on garbage material, such as glass bottles, plastic material and others (55). Foxes may also contaminate vegetation used for food by people. For example, in Alaska several plants species on the tundra are collected by the Eskimos and others, and stored for later consumption. Voles, in which rates of infection are often high, also utilise such plants (Lin Yugang and R.L. Rausch, personal communication, 1998). Dandelions (*Taraxacum officinale*) are frequently collected and eaten as raw salad in Franche-Comté, France, in grassland areas where over 90% of fox faeces are scattered (35).

The contamination of water-supplies by eggs of *E. multilocularis* has been considered as a source of infection by several investigators, but documentation has been inadequate. In Iakutia, the incidence of hydatid disease (both cystic and alveolar) was three times greater in populations supplied with water from certain lakes as compared with those using water from rivers (63).

Eggs of *Taenia* species from dogs spread up to 80 m from the site of deposition within 10 days (33). *Taenia* and *Echinococcus* eggs can be dispersed by flies which may travel several kilometres (33). In Alaska, blowflies (*Phormia regina*) have been shown experimentally to be capable of transporting eggs from faeces of infected foxes, but their epidemiological role is not yet known (87). Evidence from an island off the west coast of Scotland suggests that eggs of *Taenia hydatigena* may have been transported by birds over 60 km (99). It is assumed that eggs of *E. multilocularis* can be dispersed with plants contaminated with droppings of infected definitive hosts. In Switzerland, *E. multilocularis* infections were observed in pigs and monkey colonies in a zoo after feeding of grass harvested from meadows accessible to infected foxes (23).

- Resistance of eggs to environmental factors

Echinococcus eggs are highly resistant, and may remain infective for approximately one year in a suitable, moist environment at lower temperatures. For example, eggs of *E. multilocularis* remained viable for about 16 months at +4°C in water in the laboratory (101). Under natural climatic conditions of southern Germany, the maximal survival time of *E. multilocularis* eggs was 8 months in an experiment performed in autumn and winter and almost 3 months (78 days) in summer (101). Eggs may be well preserved in moist soil. In France, higher prevalence rates of *E. multilocularis* in rodents have been observed in places where fox faeces were at higher density and could be washed by rain into the soil (14, 15, 38). On the other hand, desiccation and high temperatures are the two most important factors reducing the longevity of the eggs (24, 33, 101).

At a relative humidity of 25% eggs of *E. granulosus* were killed within 4 days and at 0% within 1 day (56). Eggs of *E. multilocularis* lost infectivity to rodents after exposure at +25°C and a relative humidity (RH) of 27% for 2 days, at +43°C and 15% RH for 2 h, and at +45°C and 85%-95% RH for 3 h (101) (Chapter 7).

5.3.1.2.4. Contributions of intermediate hosts

The contributions of intermediate hosts to transmission dynamics of *E. multilocularis* have not yet been clearly defined because their role in epidemiology is interrelated with a complicated network of variable factors, including the following:

a) in an endemic region several intermediate host species may be involved in the cycle of *E. multilocularis*

b) the involved intermediate host species may differ in several variables, such as habitat, behaviour, population dynamics, seasonal prevalence, life span, etc.

c) various intermediate host species may differ in their susceptibility to *E. multilocularis*, and the capacity of the metacestode to produce protoscoleces may also differ

d) definitive hosts may have preying preferences for certain intermediate host species, and these may be influenced by habitat factors, season, availability of other food sources, etc.

Therefore, only preliminary considerations can be presented here.

- Intermediate host species

Under natural conditions many species of small mammals have been found infected with the metacestode stage of *E. multilocularis* (Chapter 3, 3.3.2. and Table 5.3.1.), but the epidemiological role of the various species differs (72, 73).

For example, the northern vole, *Microtus oeconomus*, is the most important intermediate host in western Alaska and on St Lawrence Island. On this island, the infection rates of the voles with metacestodes of *E. multilocularis* may be less than 10%, but can locally exceed 80% in overwintered intermediate hosts (75). The northern red-backed vole, *Clethrionomys rutilus*, and shrews have also been found infected, but they appear to have low significance in the cycle (75). In the northern mainland of Alaska and on some islands (St George Island and Nunivak Island), the brown lemming, *Lemmus sibiricus*, appears to be the only rodent to play a role in parasite transmission (75, 84).

In central North America, the deer mouse (*Peromyscus maniculatus*) and the meadow vole (*Microtus pennsylvanicus*) serve as the most important intermediate hosts, but the infection occasionally occurs also in other rodents (84) (Table 5.3.1.). A similar situation exists in western and central Europe, where at least 7 species of rodents have been reported with larval *E. multilocularis* infection, but only the common vole (*Microtus arvalis*), the water vole (*Arvicola terrestris*) and the muskrat (*Ondatra zibethicus*) are regarded as principle intermediate hosts which differ in their significance in various countries and regions (21, 23, 37, 84). It appears that the water vole is a suboptimal intermediate host as metacestodes do not form protoscoleces in a rather high proportion of infected animals (88).

In Japan (Hokkaido), the grey red-backed vole (*Clethrionomys rufocanus*), the red-backed vole (*Clethrionomys rutilus*) and *Clethrionomys rex* appear to play a role in transmission (70, 95, 97). Further examples are presented in Table 5.3.1.

- Susceptibility and immunity of intermediate hosts

In the epidemiology of *E. granulosus* acquired immunity of the intermediate host population (sheep, cattle, etc.) represents an important density-dependent constraint for transmission. On the other hand, parasite-induced mortality in intermediate host populations does not play a role in the regulation of the cycle (34). The intermediate hosts of *E. granulosus* are long-living animals in which the infection with *E. granulosus* eggs can provoke a high degree of protective immunity (Chapter 3).

In contrast, the natural intermediate hosts of *E. multilocularis* have a short life expectation (lasting some months and rarely exceeding 1 year in most species of small mammals), the metacestode evades immune

responses and proliferates progressively, and is capable to produce large numbers of protoscoleces within approximately 40 to 60 days. However, protoscolex formation can be retarded under natural conditions (see below). Experimental studies have shown that various rodent species and strains vary in susceptibility to larval *E. multilocularis* infection, and this factor may also play a role in natural intermediate hosts. A rapid proliferation of the parasite in certain intermediate hosts may inhibit their mobility and increase their vulnerability to predation by foxes. On the other hand, about 5% of *Microtus oeconomus* survive over 2 winters; in them *E. multilocularis* infection is especially massive (F.H. Fay and R.L. Rausch, personal communication, 1998). Whether acquired immunity of the intermediate host population has any effect in regulating the cycle of *E. multilocularis* is still an open question.

5.3.1.2.5. Contribution of population dynamics

A comparatively clear situation exists on St Lawrence Island, Alaska, characterised by uniform biotope conditions, high population densities of voles (*Microtus oeconomus*), and relatively high prevalence rates of *E. multilocularis* both in arctic foxes and in voles (72). The transmission cycle is clearly influenced by seasonal factors and population dynamics of the hosts.

Northern voles are typically present in large numbers, and their populations exhibit low-amplitude fluctuations in density. The natural life span of the voles is usually less than a year, and their populations in spring consist mainly of animals born during the previous spring and summer. Reproduction by the voles on the tundra becomes general in early June, and the population consists predominantly of young-of-the-year by autumn, when in them the rate of infection by the larval *E. multilocularis* (the metacestode), is at the lowest level for the year.

The prevalence of adult *E. multilocularis* in arctic foxes varies seasonally. The mean annual rate on the island has been uniformly about 77%, with the maximum of approximately 100% attained in early autumn (75). The foxes gradually expel the strobilae of the cestode over winter, and by late May, when the melting of snow again makes the voles vulnerable to predation, only about 30% of the foxes are infected. The rate of infection increases rapidly thereafter. Since a high proportion of the diet of young foxes after weaning consists of voles, nearly all pups have become infected by the time they leave the dens in late summer.

The interactions involved in the natural cycle of *E. multilocularis* on St Lawrence Island are such that maximal numbers of its eggs are being expelled by the foxes at the time of year when the population of voles consists mainly of non-infected young. Thereafter, the rate of infection in the rodents increases until spring and attains the maximal level by late May to early June when, at some localities, more than 80% may be infected (31). Fay (29) determined that less than 10% of larval cestodes in young voles on tundra contained infective protoscoleces by autumn of the year of birth, and it was not until early spring, coinciding with the period of their rapid physical and sexual maturation that the rate of infectivity increased abruptly to about 40%. Voles living as commensals within the villages harboured larval cestodes with a high proportion of infective protoscoleces by early winter, coinciding with the earlier sexual maturation of those hosts. In villages, consequently, voles with infective larvae may be consumed by dogs during much of the winter, to some extent offsetting the loss of cestodes such as occurs naturally in foxes during that period.

In other regions, such as Europe and Japan, the situation appears to be more complex as the biotopes of foxes and several species of intermediate hosts, feeding habits of foxes, macro- and microclimatic conditions, and other factors may vary from region to region, and even within smaller areas (23). In central Europe, the cycle of *E. multilocularis* persists in endemic areas with prevalence rates of the parasites in foxes as low as 2% (28) and as high as >60% (79, 89). Infection rates of rodents with larval *E. multilocularis* are generally low (<1% to 6%) (23), but they may reach higher levels locally, for example up to 39% in water voles (*Arvicola terrestris*) in a hyperendemic focus in Switzerland (88). Some seasonal variation in prevalence rates of *E. multilocularis* in foxes occurs, but this is not pronounced. There are some indications that prevalence rates of *E. multilocularis* in foxes are associated with high fox densities, but this is not yet substantiated (Chapter 4).

Ecological studies in France by Giraudoux (35) have lead to the conclusion that the *E. multilocularis* infection in foxes and rodents exists in 'patches' and persists in a region by shifting to non-endemic patches with foxes being the main vector. This view is supported by the fact that prevalence rates of *E. multilocularis* in foxes have been found to differ significantly from patch to patch within a larger endemic area (94).

In Franche-Comté, France, a fair overdispersion of *E. multilocularis* in its hosts was recorded with microfoci overrepresented on ploughed field borders, in an area where ploughed fields are less than 2% of the farmland (14, 15). In such microfoci of some 10 m², prevalence rates of *E. multilocularis* (corrected for age of the hosts) reached 12%-15% in *Microtus arvalis* (38), whilst the average prevalence rate was 0.3% in this area. Seasonal patterns of infection were also documented on the basis of an analysis of the age-structure of populations of *Microtus arvalis*. From spring to summer, metacestodes were recorded only in animals having overwintered, and the first infection of a young animal was detected in October (15). The age-structure of small mammal populations changes largely over seasons and years, and estimating of prevalence rate should always refer to this important parameter in population dynamics and epidemiology (Annex 5.3.2.) (58).

Population dynamics of rodents are influenced by landscape characters (17). Giraudoux (35) pointed out that the numerical increase of common vole (*Microtus arvalis*) and water vole (*Arvicola terrestris scherman*) populations in Franche-Comté (France) is related to the ratio of permanent grassland (16, 39) and that this relation may influence the pattern of transmission of *E. multilocularis* on a regional scale. Further studies in Franche-Comté and in Zhang County, the People's Republic of China, have shown that prevalences of human AE are correlated with land use variables (37). Land use variables determine the risk of long periods of high rodent density (16, 39). During 'outbreaks' of *M. arvalis* and *A. terrestris* populations, the rodent biomass on grassland is over 100 times higher than in woody areas, the habitat of other rodent species (36, 37). During this time red foxes feed almost exclusively on *M. arvalis* or *A. terrestris* (35, 103). On the other hand, numbers of foxes can decrease during periods of low rodent density (numerical response). In these periods, fox diet is diversified, and includes more fruits, insects and rodents of woody habitats (*Clethrionomys glareolus*, *Apodemus* sp., etc.) (functional response) (37).

The water vole (*A. terrestris*) has been identified as intermediate host of *E. multilocularis* in various European countries, namely in France (13, 46), Switzerland (82, 88) and Germany (64). From the UK to Siberia, 35 subspecies of *A. terrestris* have been described (65, 81) whose behaviour and population dynamics can differ. Two main ecological groups are distinguished: aquatic subspecies, which are the most frequent, living along streams, and terrestrial subspecies thriving in grasslands. *Arvicola terrestris scherman*, the fossorial water vole, belongs to the terrestrial group and is present, for example, in mountainous regions north of the Alps and in the Massif Central, France. It shows multiannual cycles of 4-8 years with population outbreaks during which densities can exceed 1,400 voles per hectare (39, 103). In the period between spring 1988 and spring 1991 annual and seasonal variations in population densities between 160 and 980 per hectare were observed in Switzerland (103). During this period, *A. terrestris* was the main prey of the red foxes representing 54% of all items found in stomachs of 1,213 animals. It was further shown that a statistically significant correlation exists between the average seasonal availability of *A. terrestris* and their consumption by foxes (103). Other studies in several European countries revealed that the percentage of water voles in the diet of red foxes is generally low (103). In these countries, the behaviour and population dynamics of this species could limit predation by foxes.

Rodent population dynamics could be a key factor in transmission dynamics of *E. multilocularis*, as they may influence the prevalence of *E. multilocularis* in foxes, which determines the degree of environmental contamination with eggs. Recently, Giraudoux *et al.* (37) formulated the hypothesis that transmission of *E. multilocularis* might be more dependent on the dynamics of one or two species of rodents which reach high densities for long periods, rather than on the presence of a number of rodent species which are continuously on low or medium population densities.

5.3.1.3. Mathematical model

A mathematical model of the life-cycle of *E. multilocularis* has been recently proposed (34, 78). As the knowledge on the epidemiology of *E. multilocularis* and the life histories of its hosts is rather limited, such models remain on an uncertain basis.

5.3.1.4. Potential transmission routes to humans and infection and risk

- Potential transmission routes

It is generally assumed that humans can become exposed to the eggs of *E. multilocularis* by handling of infected definitive hosts, or by ingestion of food contaminated with eggs. Some reports suggested that egg transmission may occur by waterborne routes (84) (Chapter 5.3.1.2.3.). However, studies on the epidemiological significance of the various potential ways of transmission are lacking.

In relation to the high prevalence rates of *E. multilocularis* in definitive hosts, the incidence rates of AE in humans are low in most of the endemic areas (Chapter 4). This discrepancy is still unexplained. Several aspects have to be considered and should be further studied, including exposure of humans to eggs of *E. multilocularis* and the resistance/immunity of humans to infection.

- Exposure of humans to eggs of *Echinococcus multilocularis*

Echinococcus multilocularis is mainly restricted to the sylvatic cycle and thereby to some degree ecologically separated from humans. However, the degree of separation may vary from region to region from high in isolated and sparsely populated areas to moderate or low where infected foxes or other definitive hosts live in close proximity or even within villages and urban areas, for example, in Europe or in Hokkaido, Japan. Ecological separation does not exist if infected foxes, dogs or cats live in close association with humans (see above).

Exposure to eggs may be influenced by occupational and behavioural factors. Hunters, trappers and persons who work with fur may frequently be exposed to eggs of *E. multilocularis*, but there is little evidence that these groups are at increased risk (22, 84). However, in a recent Austrian study, the habits and activities of 21 patients with AE were retrospectively (1967-1997) compared with those of 84 control persons matched by sex, age and residence (54). Cat ownership and hunting were found as independent risk factors. On the other hand, data from Austria (3), Germany (68), France (7) and Switzerland (40) indicate that persons working in agriculture are at increased risk of infection. Living in the country-side in close proximity to infected foxes, dogs or cats and/or frequent contacts with egg-contaminated food or soil may be the reasons for higher infection risk, but specific risk behaviours are not fully understood. In Bavaria, Germany, high prevalence rates of *E. multilocularis* in the fox population were correlated with high incidence of AE in humans (68) but this needs further evaluation. The wide distribution and high prevalence rates of *E. multilocularis* in foxes on the one hand and the low incidence of human AE on the other suggest that the infection risk for humans is limited by certain factors. Immunogenetic predisposition for susceptibility or resistance has been discussed in this context (41, 42, 43).

- Resistance and immunity to infection

Apparently, humans have a relatively high degree of innate resistance to infection with eggs of *E. multilocularis* as indicated by the slow development of the metacestode stage in the liver and other organs, the reduced capacity of protoscolex formation and the degree and type of histopathological reaction. The reasons for this resistance are not well understood, but recent preliminary studies have shown that in patients with AE the frequency of certain HLA-antigens was increased (42, 86), implying the possibility of a immunogenetic predisposition for susceptibility or resistance to AE (43). The potential role of acquired immunity for the regulation of the metacestode population in humans is still obscure, but cases of self-cure from the infection indicate that immunity may play a role (Chapter 2).

5.3.2. Epidemiology of *Echinococcus vogeli*

- Definitive hosts and egg dispersal

The bush dog, *Speothos venaticus,* appears to be the only natural definitive host of *E. vogeli.* Although it has an extensive geographic range in the northern half of South America (south at least to Bolivia and southern Brazil), the bush dog is rarely observed. The dogs hunt in packs, and their characteristic prey is a large rodent, the paca, *Cuniculus paca.* In oriental plains of Colombia, bush dogs and pacas inhabit gallery forest, within

which the cycle of *E. vogeli* is completed. Natural infection with *E. vogeli* was also found in a hunter's dog in Brazil (72).

- Intermediate hosts

Of 325 pacas collected in Colombia over the period 1962-1979, 96 (29.5%) harboured the larval stage of *E. vogeli* (11). In Colombia also, 6 (0.5%) of 1,168 spiny rats, *Proechimys* spp., were infected.

The larval stage of *E. vogeli* typically develops in the liver of pacas, where usually one to a few large, fluid-filled cysts, or vesicles, are produced. The vesicles exhibit internal trabeculae, forming chambers in which large, but relatively few, brood capsules are produced (74). Invasiveness takes place by means of a unique mode of exogenous proliferation often leading to formation of disseminated lesions (77).

- Transmission to humans and primate animals

Domestic dogs, in expelling eggs of *E. vogeli,* appear to be the sole source of risk to people. The intermediate host, the paca, is much hunted for food among rural villages in northern South America, and the viscera from animals killed are regularly fed by the hunters to their dogs, which become infected and live in close association with their owners' families (10). A captive bush dog was the source of infection for several higher primates in the Los Angeles Zoo, of which at least 15 died as a direct consequence (47). Information on the incidence of PE in humans is presented in Chapter 2.

5.3.3. Epidemiology of *Echinococcus oligarthrus*

- Definitive hosts and egg dispersal

The adult is host-specific for carnivores of the family Felidae in Central America and South America. It has been reported from wild cats of the following species: cougar (*Felis concolor*), jaguar (*Panthera onca*), ocelot (*F. pardalis*), jaguarundi (*F. yaguaroundi*), Geoffroy's cat (*F. geoffroyi*), and pampas cat (*F. pajeros*) (74). Findings in the few animals examined indicate that the rates of infection are relatively high. Four domestic cats were experimentally infected with cysts from rodents which had received eggs from strobilae from a naturally infected cougar in Panama (90).

The means by which eggs of *E. oligarthrus* are dispersed have not been defined. The wild felids mainly inhabit forest, and may cover their excrement. The behavioural characteristics of rodents that lead to their ingesting the eggs of *E. oligarthrus* are unknown.

- Intermediate hosts

In Colombia, the metacestode stage of *E. oligarthrus* was found in 3 (0.9%) of 325 pacas, in one agouti, (*Dasyprocta fuliginosa*), and in 2 of 11,68 spiny rats (*Proechimys* spp.) (74). Three (8%) of 39 brown agoutis, (*D. punctata*) collected in Panama, were infected (90). Rodents infected experimentally were spiny rat (*Proechimys semispinosus*), climbing rat (*Tylomys panamensis*), brown agouti (*D. punctata*), cotton rat (*Sigmodon hispidus*) and Mongolian gerbil (*Meriones unguiculatus*) (90). Larger rodents, especially agoutis, are probably the usual source of infection for cats of large size, such as the cougar and jaguar, but much more investigation in the field is required to define the range of predator-prey interactions.

The metacestode has been reported also from rabbits (*Sylvilagus brasiliensis*) in Venezuela, and has been studied in experimentally infected rodents (90). In naturally and experimentally infected agoutis, usually a few cysts were found in subcutaneous muscle or in muscle of the extremities. No indication of proliferation was noted. Structurally, the cyst of *E. oligarthrus* resembles that of *E. vogeli,* but internal trabeculae are lacking, and large numbers of brood capsules are produced peripherally.

- Transmission to humans

Hydatid disease caused by *E. oligarthrus* appears to be rare. In view of the selective habits of the final hosts of that cestode, the probability of contact with eggs must be low. The first known case of hydatid disease caused

by *E. oligarthrus* was reported in 1989 from a patient in Venezuela (61). Two further cases were diagnosed in Brazil and Surinam (9, 12) (Chapters 2 and 4).

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Annex 5.3.1.

Epidemiological approaches in the study of the *Echinococcus multilocularis* infection in foxes

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5.3.1.1. Factors influencing epidemiological analyses

Epidemiological analyses of the sylvatic cycle of *Echinococcus multilocularis* can be influenced by the following factors:

a) sampling
b) space
c) age of the foxes
d) time.

A. Sampling

Conclusions drawn from the analysis of a sample can only be transferred to the entire population if:

a) each member of the sample has the same chance to enter the sample (random sample)
b) the sample is representative with respect to all epidemiologically relevant conditions existing in the population
c) the sample is large enough to allow precise estimates of the prevalence of the infection and of associations of epidemiologically relevant factors.

For the determination of the epidemiological status in a region where the fox is the main definitive host, these criteria can only partially be fulfilled in cross-sectional studies. As far as the infection status of individual foxes with *E. multilocularis* is concerned, infected and uninfected animals have the same chance to enter a sample because the infection status of a fox cannot be determined by clinical signs, behaviour, etc. (Chapter 3). In contrast, a rabid fox may have a higher chance of entering a sample because symptoms of the infection may be a reason for eliminating (by shooting) the animal from the population. Clearly, this 'selection' can lead to an over-estimation of the real prevalence of rabies in the fox population.

With respect to regional origin, however, samples of foxes obtained by hunting are usually heterogeneous, because the places where the animals are shot are not randomly distributed, but follow certain rules. This heterogeneity at the local level may be reinforced by variations in the entire study area due to varying possibilities and differences in the readiness to submit foxes to the investigating laboratories at the regional level. This may lead to an under-representation of parts of the study area in the sample, while other regions may be over-represented at the same time. Moreover, whole fox families may enter the sample between February and June (period valid for central Europe), when these families live close together. The members of such families cannot be considered as independent members of the sample, because a familiar exposure or

lack of exposure to the infection, respectively, must be assumed. If the bitch is exposed to *E. multilocularis*-infected rodents, it can be expected that she will feed her progeny with infected intermediate hosts. By contrast, unweaned cubs are not exposed, even if their mother is.

Finally, since the spatial distribution of *E. multilocularis*-infected foxes is often heterogeneous at the population level, this is also true for samples obtained in such a scenario (8).

B. Spatial distribution

Generally, the spatial representation of the sample has be taken into account in the interpretation of the data. Prevalences can only be reliably estimated in a spatial raster where the sample as a whole and also the infected foxes are nearly homogeneously distributed. In all other cases, endemic foci can be overlooked or supposed changes of the prevalence may be caused by spatial shifts in the tested sample (confounding by the variable 'space'). It should be noted that the home range of foxes is much smaller than previously thought, at least under central European conditions. Moreover, habitat factors which may have a limiting influence on the life-cycle of the parasite, also seem to be effective at the local level. Therefore, temporary stable endemic foci of less than 400 km² are possible.

C. Age of foxes

Unweaned cubs are of course not exposed to infection with *E. multilocularis*. In central Europe, the first infections in juvenile animals are usually observed in the second half of May. Then the exposure increases gradually, until it reaches a level comparable with adult animals in the course of the following months.

Since the risk of contracting an infection with *E. multilocularis* is related to the age of a fox, the age structure of the sample can influence the result of the analysis. In some, but not all studies, it has been observed that juvenile foxes were more frequently infected than adults. Therefore, an over-representation of juvenile foxes in the sample would inevitably lead to an over-estimation of the prevalence, while an over-representation of adult foxes would lead to underestimation. Furthermore, differences in the age structure of the sample can pretend temporal and spatial changes of the prevalence. It has to be stressed, however, that a higher prevalence of *E. multilocularis* in juvenile foxes, as compared with adults, cannot always be expected. In areas with sporadic occurrence of *E. multilocularis*, the time of exposure seems to be a limiting factor for infections, with the effect, that statistically significant differences between juvenile and adult foxes are not observed in a period of equal exposure for the age groups (in central Europe approximately after June). By contrast, under endemic conditions adult foxes are less frequently infected than juveniles during the same period of time. This phenomenon may be explained by a partial immunity to the parasite in older animals, which seems to be restricted to endemic situations where the immune system may be better stimulated by repeated exposures to the parasite (methods for age determination of foxes: Annex 5.3.3.).

D. Time

The term prevalence is commonly used to describe the number of infected individuals in a population at a designated time point (point prevalence). Since the number of foxes available for examinations is limited, comprehensive samples can often only be collected over a certain period of time. This requires a modified definition of the term prevalence in the sense of 'period prevalence' (number of infected individuals in a population during a specified period of time). The period for examinations should, therefore, be chosen in a way that no prevalence changes are to be expected in that time period.

It should also be noted that, strictly speaking, an unbiased observation of the population in time is not possible, because infections with *E. multilocularis* are diagnosed post mortem, i.e. the animals are irreversibly removed from the population, and therefore, also from the life-cycle of the parasite. On the other hand, the post mortem investigations make sure that a single individual can enter the sample only once.

5.3.1.2. Data and analysis

The interpretation of the available data should always rely on a spatial analysis, and the exclusion/detection of the presence of the parasite should be based on a predetermined prevalence threshold. For data analysis adequate statistical methods have to be used (5, 7, 9).

- Sampling strategy

When epidemiological studies on the *E. multilocularis* infection in foxes are designed, the variables 'space', 'age' of the animal and 'time' must be taken into account. Therefore, the parameters place of origin (e.g. municipality or precise location marked on map), age (juvenile vs. adult), time (day, month and year of sampling), and the infection status (yes/no; perhaps intensity of infection) should be recorded. If a heterogeneous distribution of data regarding the first 3 variables is expected or observed, provisions should be made that these data can be analysed in the respective intervals or strata where they were collected (e.g. month, municipality, village, juvenile vs. adult, etc.).

The examined sample should contain specimens from all parts of the study area. Ideally, these samples should reflect a homogeneous distribution in the investigated region. Since infected animals may cluster in some areas independently of the regional investigation density, the regional raster to be analysed should not be too large, especially when nothing is known about the prevalence of *E. multilocularis* in this particular area. With relation to the age of the animals to be examined and the investigation period, sampling should be performed when a high prevalence can be expected. Even if this may initially lead to a 'controlled' over-estimation of the prevalence, it will help to identify endemic foci which otherwise can easily be overlooked.

In central Europe, it would be advisable to examine juvenile foxes between July and September, because they can clearly be distinguished from adult foxes during these months, and it can be expected under endemic conditions that they will be more frequently infected than the older animals. If there are only sporadic cases in the study area, this sampling procedure still leads to a valid analysis of the situation, since the representation of infected foxes in both age groups is not different. Furthermore, during July and September, the importance of familiar clustering of the juvenile foxes has decreased. At the same time, the intensity of infection seems to be significantly higher in juvenile than in adult foxes. This sampling strategy may help to increase the chances of parasite detection.

Alternatively, juvenile and adult foxes could be examined during winter when, according to some investigators, prevalences tend to be higher as compared to the total summer population. Since exact age determination in foxes with routine methods is difficult during winter, it is also difficult to estimate the age dependence of the parasite prevalence. This may become a problem if prevalences of different years are to be compared in an endemic area and if the age structure of the samples varies over the years (the age determination of foxes is given in Annex 5.3.3.).

Finally, the number of animals to be sampled has to be determined. This decision often requires to compromise between a high reliability of the results and the feasible extent of the investigations. Any sampling strategy is based on statistical principles, which require independent random samples and a homogeneous distribution of the studied properties. Since these prerequisites are only partially fulfilled in samples obtained from fox populations, the validity of estimates based on these sampling strategies is limited.

- Sample size

The reliable detection/exclusion of the *E. multilocularis* infection in a population at a certain confidence level (e.g. 95% or 99%) depends on the sample size. The latter is determined by the size of the fox population on the one hand, and the suspected prevalence of the parasite. Since the real size of the fox populations in a given area can usually not be determined, the number of individuals can only be estimated. In epidemiological studies, the population size should be over-estimated for safety reasons. If a population consists of more than 10,000 individuals, the sample size becomes independent of the size of the total population. In areas where pre-information on the prevalence of *E. multilocularis* does not exist, it is advisable to anticipate a low prevalence, at least for an initial study.

If the population size and a prevalence threshold are selected, the required sample size can be read from Table 5.3.1.1. (3). If at least one animal in this sample is infected, the real prevalence is equal or higher than the selected prevalence threshold at the selected confidence level. If no infected animal is found in the sample, the real prevalence is lower than the selected prevalence threshold.

Table 5.3.1.1.
Sample sizes required for the detection/exclusion of infections at expected prevalence thresholds and 99% confidence level
Adapted from R.M. Cannon and R.T. Roe (3)

	Expected percentage of infected animals in the population (prevalence)											
Population size	50	40	30	25	20	15	10	5	2	1	0.5	0.1
	Upper limits for the number of infected animals in the population											
10	5	6	7	10	10	10	10	10	10	10	10	10
50	7	9	12	14	17	22	29	42	50	50	50	50
100	7	9	13	15	19	25	36	59	90	100	100	100
200	7	9	13	16	20	27	40	73	136	180	198	200
500	7	9	13	16	21	28	42	83	183	300	421	500
1,000	7	9	13	16	21	28	43	86	204	368	601	990
2,000	7	9	13	16	21	29	44	88	216	410	737	1,800
5,000	7	10	13	16	21	29	44	89	223	438	840	3,009
10,000	7	10	13	16	21	29	44	90	226	448	878	3,689
>10,000	7	10	13	16	21	29	44	90	228	459	919	4,603

Example 1

A study area of 500 km² harbours an estimated fox population of >10,000 animals. In this population, a prevalence of 5% is to be excluded or confirmed at the 99% confidence level. A sample of 90 foxes has to be collected, and this will contain at least one infected fox, if the real prevalence is equal or higher than 5% (Table 5.3.1.1.). If no infected fox is found in this sample of 90 animals, the real prevalence in the population is below 5% with 99% probability. If a similar study is conducted in a country of 500,000 km² with regional units of each 500 km², 90 foxes will have to be examined in each unit to obtain the same information for the entire country. The required sample size can be reduced if the confidence level is set to 95% or 90% (Example 2 and Table 5.3.1.2.). This affects of course the reliability of the conclusions drawn from the data.

It should be noted that the samples sizes presented in Table 5.3.1.1. and 5.3.1.2. are based on a high sensitivity (close to 100%) of the diagnostic procedure used for the identification of infected individuals. However, techniques used in the field may have lower sensitivities (see Chapter 3). Therefore, the real precision of the estimates may be lower than expected.

- Prevalence estimates in relation to confidence levels and precision

If the prevalence of *E. multilocularis* is to be determined with a predetermined degree of precision, then sample sizes have to be modified as described in Example 2.

Example 2

In an area with an expected prevalence of 50%, an estimate with 10% precision (corresponding to 40%-60% true prevalence) is to be performed at the 95% confidence level. In this case, 96 foxes have to be sampled as indicated in Table 5.3.1.2. If the prevalence is to be estimated with a precision of 5% (corresponding to 45%-55% true prevalence), 384 animals have to be examined. The choice of precision should take the

epidemiological relevance into account. For example, differences between 45% and 55% prevalence may not be relevant in most circumstances. However, it may be relevant whether the prevalence ranges between 0% and 20% (10% expected prevalence; 10% precision; 95% confidence level; required sample size: 35 animals). A computer programme for the determination of sample sizes for prevalence estimates has been developed by de Blas *et al.* (4).

Table 5.3.1.2.
Required sample sizes for prevalence estimates in relation to confidence levels and precision
Adapted from R.M. Cannon and R.T. Roe (3)

Expected prevalence (percentage)	Confidence level 90% Desired precision			95% Desired precision			99% Desired precision		
	10	5	1	10	5	1	10	5	1
1	*	*	268	*	*	381	*	*	657
2			531			753			1,301
3			788			1,118			1,931
4			1,039			1,476			2,548
5		52	1,286		73	1,825		127	3,152
6		62	1,526		87	2,167		150	3,742
7		71	1,762		101	2,501		173	4,320
8		80	1,992		114	2,828		196	4,884
9		89	2,216		126	3,147		218	5,434
10	24	97	2,435	35	138	3,457	60	239	5,971
20	43	173	4,329	61	246	6,147	106	425	10,616
30	57	227	5,682	81	323	8,067	139	557	13,933
40	65	260	6,494	92	369	9,220	159	637	15,923
50	68	271	6,764	96	384	9,604	166	663	16,587
60	65	260	6,494	92	369	9,220	159	637	15,923
70	57	227	5,682	81	323	8,067	139	557	13,933
80	43	173	4,329	61	246	6,147	106	425	10,616
90	24	97	2,435	35	138	3,457	60	239	5,971

* Required sample sizes are only listed when the precision percentage is higher than the expected prevalence

- Stratification of sampling in relation to investigation periods

As already pointed out, estimates of the *E. multilocularis* prevalence in foxes usually represent 'period prevalence' estimates. Thus, the shorter the observation interval is, the smaller the influence of potential prevalence changes in time will be. It is therefore desirable to minimise investigation periods. If it is necessary to obtain samples over longer periods, they should be temporally stratified, i.e. divided into smaller intervals. It is important to note, however, that a representative number of individuals must remain in each period (stratum). If it is unclear whether the sample in a stratum is still representative, care should be taken with the interpretation of statistical results. If the statistical analysis of the prevalence estimates for individual intervals (strata) does not provide evidence for significant differences, and if the prevalence can be estimated in the individual intervals with the required fidelity, it is possible to estimate the prevalence over the entire study period.

With respect to the determination of the frequency of an infection in a population, it is important to stress that the value determined in a sample represents an estimated prevalence. The probability that the estimated prevalence approaches the true prevalence, depends largely on the sample size. In addition to the point estimate calculated from the examined sample (number of infected foxes divided by the number of examined

animals), it is convenient to quote a range within one is reasonably confident that the true prevalence will lie. This range is known as the confidence interval. Usually, prevalence estimates are supplemented with the 95% confidence interval. Small sample sizes lead to large confidence intervals.

Example 3

In a sample of 20 foxes, one infected individual was detected. While the estimated prevalence is 5%, the confidence interval, i.e. the range within which the true prevalence lies with 95% probability, extends from 0.1% to 24.9%. By contrast, if 5 infected foxes are detected in a sample of 100 animals taken from the same population, the estimated prevalence will still be 5%, but the confidence interval (1.6%-11.3%) is much smaller.

These examples illustrate that confidence intervals provide a more valid basis for the interpretation of prevalence data than estimated prevalences alone. Confidence intervals may be read from tables (1, 2, 6) or determined using biostatistical formulas (5, 7). It should be noted, however, that some tables are based on different distribution functions. However, the differences in the confidence limits resulting from the use of these functions are usually small and therefore not relevant for prevalence estimates.

- Spatial analysis

Plotting of all examined animals on a map of the investigation area using the municipalities where the foxes were shot as a raster represents a useful descriptive technique, which allows the identification of heterogeneous distribution patterns in the total sample (infected and uninfected animals) and among the infected animals (Fig. 5.3.1.1.). In this way, it is possible to recognise regions in the study area where the sampling may have been insufficient for conclusions on the prevalence of *E. multilocularis*. Mapping of the results also provides a first impression about regional clusters of infected animals, which may indicate endemic foci. It must be emphasised, however, that this method of explorative data analysis only allows the formulation of hypotheses which have to be evaluated by biostatistical procedures (5, 7, 9).

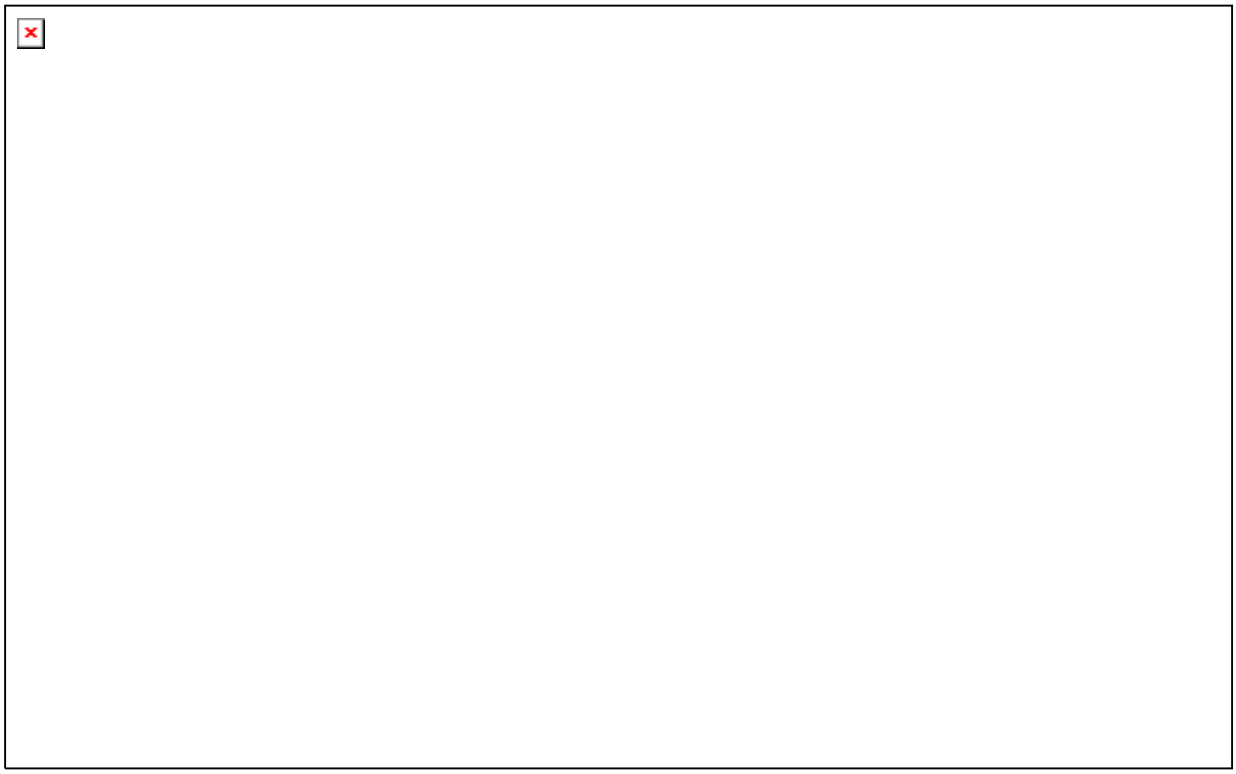

Fig. 5.3.1.1.
An example of heterogeneous spatial distribution patterns of a random sample of red foxes (*Vulpes vulpes*) and of foxes infected with *Echinococcus multilocularis*
The municipality where the foxes were shot is known. Positions within the municipalities of origin are randomly chosen

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Annex 5.3.2.

Sampling of rodents for epidemiological studies on *Echinococcus multilocularis*

P. Giraudoux

General aspects

For any sound study on rodent population dynamics or on *E. multilocularis* prevalence rates it is essential to consider the following key-points (2):

a) sampling of small mammals has to be based on a reasonable strategy. In any case a specialist should be consulted already in the planning phase of a project

b) the diversity of habitats and of species and the age-structure (which is variable within a year) of rodent populations have to be taken into account (3)

c) for data analysis, adequate statistical methods have to be used (5) (Annex 5.3.1.)

d) in each country the legal regulations on protected animal species (Berne Convention) and the basic norms of humane handling of animals should be observed (Chapter 3).

Trapping

For trapping small mammals suitable traps have to be used considering:

a) the species to be sampled

b) the aim of sampling (estimation of rodent densities, qualitative search for parasites, estimation of parasite prevalence rates, etc.).

Strategies and the selection of traps become more complicated when samples have to be collected from small mammal communities which may include 10 or more species. Various types of traps can be used, and a few of them are briefly described here.

- Door-inside live traps

They include INRA traps, Longworth traps (e.g. for small rodents <50 g bw), and Sherman traps (e.g. for *Arvicola terrestris* [4], and for other species of similar size). They can be set on the surface of the ground or underground, inside rodent galleries. They can be baited or not, depending on the species to be trapped. For example, baits are not necessary for trapping European species, but is was almost impossible to trap *Microtus limnophilus* in the People's Republic of China without baiting (2). The INRA traps (Fig. 5.3.2.1.) are small boxes (5 cm × 5 cm × 15 cm) with a paddle to trigger door closing when a small mammal enters (1). They have the advantage of being less expensive than Longworth traps.

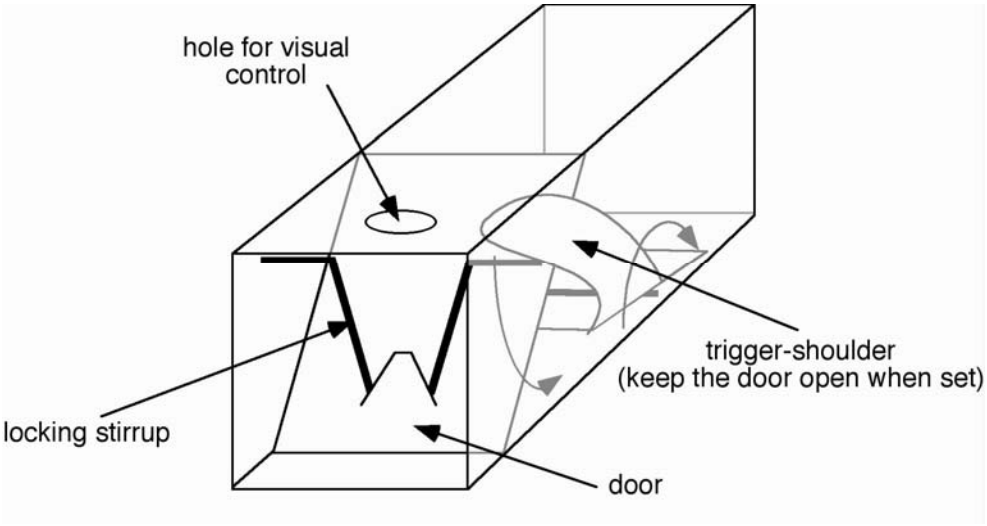

Fig. 5.3.2.1.
Trap used by *Institut national de la recherche agronomique* (INRA)
Courtesy: P. Giraudoux

Animals can be collected alive in these types of traps, if they are checked every 2 h to 4 h, according to temperature. However, rodents rarely can survive one night, unless some straw and food is provided in the traps. Some species cannot be trapped on the surface of the ground, and holes have to be made to set the traps in the galleries (e.g. for *A. terrestris* and *Ellobius talpinus).*

- Grid traps

Grid traps (Fig. 5.3.2.2.) are also live traps, and are available in various sizes for trapping small to medium sized animals, such as mice, muskrat (*Ondatra*), coypus (*Myocastor*) or even larger animals, such as paca (*Cuniculus paca*). They all need baiting and must be set on the surface of the ground.

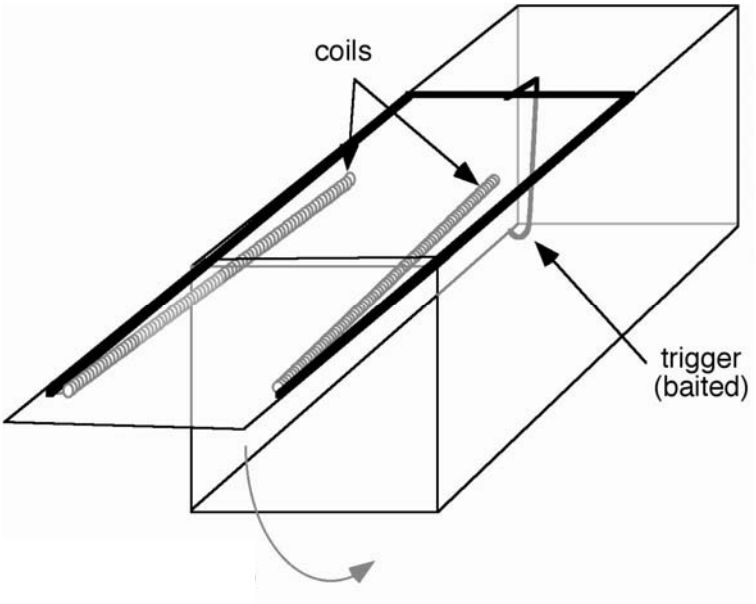

Fig. 5.3.2.2.
Grid trap
Courtesy: P. Giraudoux

- Break-back traps and tong traps

These traps are supposed to kill the animals instantly when trapped (Figs 5.3.2.3. and 5.3.2.4.). Different sizes exist, and they have to be adapted to the target species. Tong traps are always set in galleries. Such traps must be used with caution, as they are not selective. They have to be controlled frequently, and they should only be used if they do not represent a risk for protected species. They are not recommended; in some countries their use is prohibited.

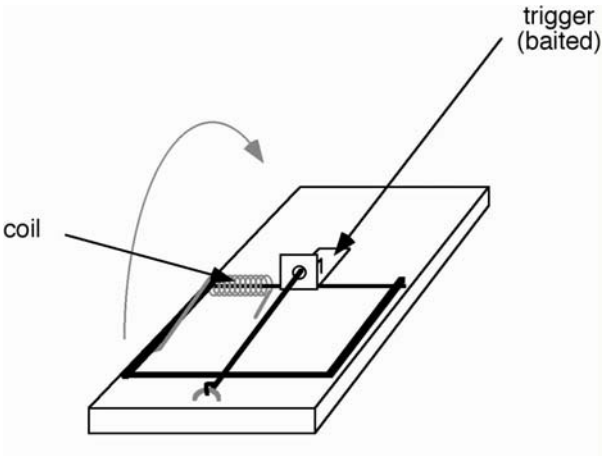

Fig. 5.3.2.3.
Break-back trap
Courtesy: P. Giraudoux

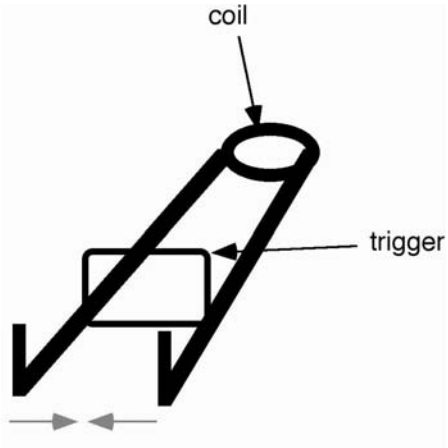

Fig. 5.3.2.4.
Tong trap
Courtesy: P. Giraudoux

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Annex 5.3.3.

Age determination in foxes

P. Giraudoux, T. Romig and J. Eckert

1. Red foxes (*Vulpes vulpes*)

- General aspects

Young red foxes (<6 months of age) can easily be distinguished from older foxes (>6 months) by size and weight of the body and general morphology (1). These general features are irrelevant for other age categories. Therefore, dental characteristics are commonly used for estimating the age of carcasses of red foxes in epidemiological studies on *E. multilocularis* (5). Dentition age and morphological changes of teeth can be used to distinguish foxes under and above one year of age (5), but the gold standard for age determination in older animals is by counting cement lines in tooth sections (10, 12).

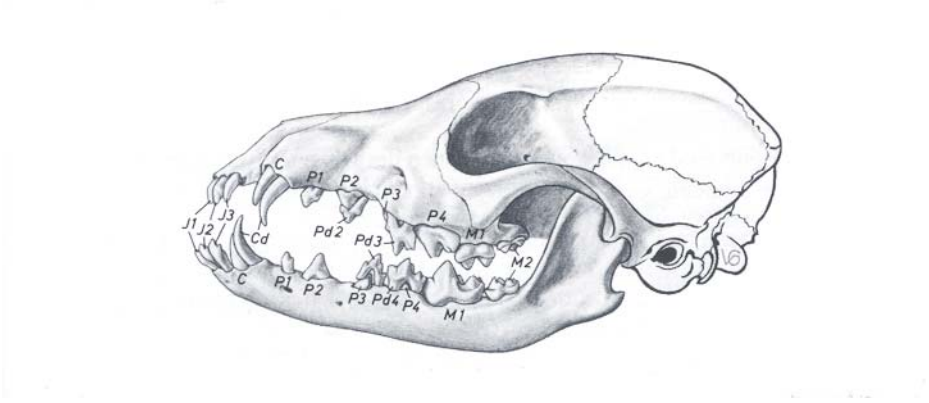
- Dentition age and changes

In young foxes, the complete set of deciduous teeth has penetrated the gingiva at an age of 3 to 4 weeks and complete eruption of teeth has occurred at 4 to 5 weeks (10). Temporary dentition consists of 28 teeth (Formula A and Fig. 5.3.3.1.). Permanent dentition is complete after 5 to 6 months with a total number of 42 teeth and types according to formula B and Figure 5.3.3.2.

Formula A: deciduous teeth of 3-5 week-old foxes (10): $\frac{3\,In\ 1C\ 3\,PM}{3\,In\ 1C\ 3PM}$; total 28 teeth

Formula B: permanent dentition of foxes over 5-6 months old (10): $\frac{3\,In\ 1C\ 4\,PM\ 2\,M}{3\,In\ 1C\ 4\,PM\ 3\,M}$; total: 42 teeth

C	canine teeth	In	incisors
PM	premolar teeth	M	molar teeth

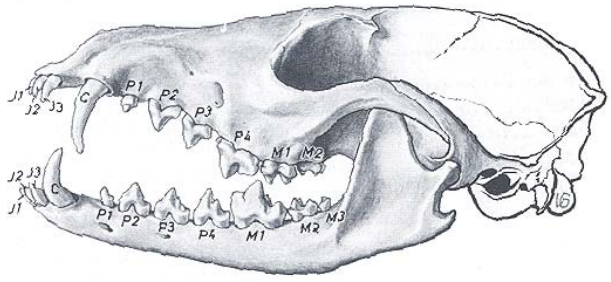


Jd1-Jd3 : incisors

Cd : canine teeth

Pd2-PD4 : premolar teeth

Fig. 5.3.3.1.
Skull of three-week-old cub of a red fox (*Vulpes vulpes*) with complete deciduous dentition
Courtesy: K.-H. Habermehl (10)



J1-3 : incisors

C : canine teeth

P1-4 : premolar teeth

M1-3 : molar teeth

Fig. 5.3.3.2.
Skull of adult red fox with complete permanent dentition
Courtesy: K.-H. Habermehl (10)

Young foxes in their first year of life and older foxes can be distinguished by changes occurring in the incisors of the upper and lower jaw (Fig. 5.3.3.3.). In juvenile foxes below 12 month of age, the incisors are three-lobed and the occlusal surfaces normally do not show distinct signs of wear. From the second year onwards, incisors are losing lobation and show oval occlusal surfaces with a brown dentin spot, starting in the lower jaw (5, 10, 15). It has to be underlined that these changes in tooth characteristics are dependent on the diet of the foxes. Therefore, the method allows only to distinguish between foxes under and over 12 months.

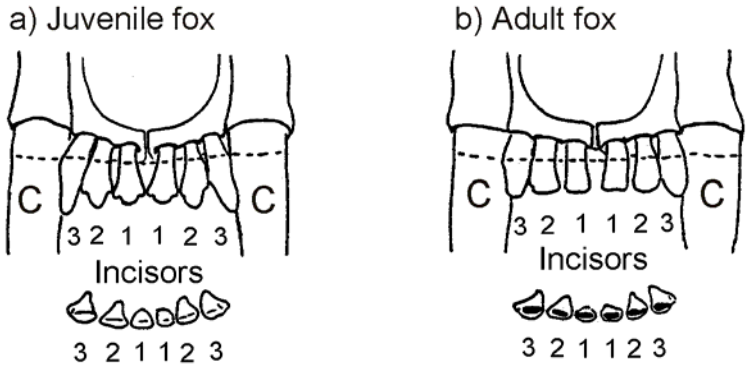
- Volume of pulp chamber

Young foxes of the year have a pulp chamber with thin walls and a large pulp volume. The age dependent changes in the volume of the pulp chamber can be determined by X-ray examination of the canine teeth (7) and are correlated to the age of the animals by using standard radiographs for comparison (15). This method has a quite high precision (15).

- Counting of annual layers of cementum

The most reliable method for age determination in foxes is counting the number of annual layers of cementum in sections of canine (C) teeth or premolars (P 2 and P 3) under × 10-30 magnification (10). Some authors use horizontal sections through the lower third of the teeth roots (10), others recommend longitudinal sections parallel to the symmetrical plane (8, 9, 12). The advantage of longitudinal sections is that one and the same section may show zones in the tip of the root and in the walls of the pulpa which can be of interest for comparison (12). The dense zones of cementum in the tip of the root are used for counting the layers (12).

For preparing the sections (30 μm thickness, about 15-20 sections are needed) one canine tooth (another serves as a reserve) is decalcified in 5% HNO_3 for approximately 24 h-48 h, thoroughly washed in running water for 24 h and then sectioned by means of a freezing microtome. The sections are then mounted on slides and stained with haematoxilin (haematoxilin: 1.0 g, sodium iodate [$NaIO_3$]: 0.2 g, potassium aluminium sulphate [$KAl(SO_4)_2$], citric acid: 1.0 g, distilled water: 1,000 ml). Details of the technique have been described by Jensen and Brunberg Nielsen (12).



a) dentition of a juvenile fox without signs of wear

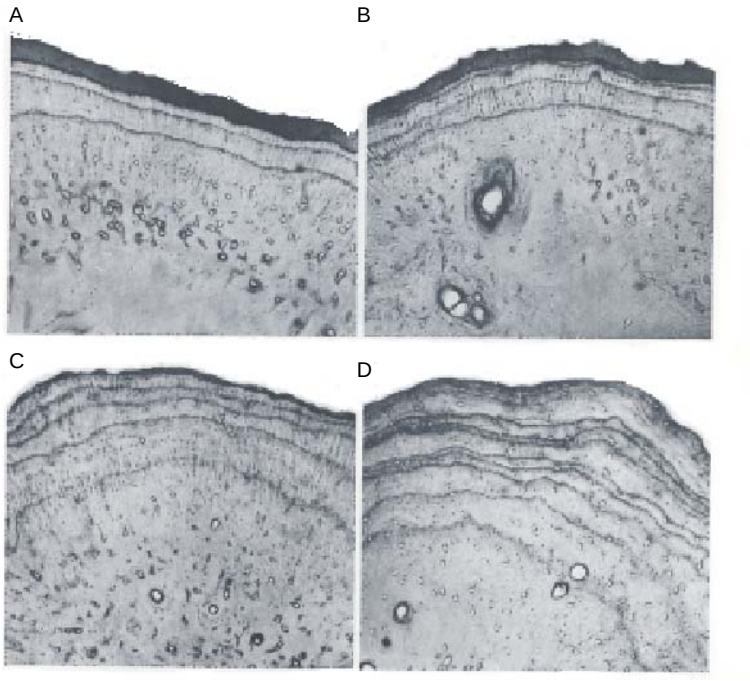
b) dentition of adult fox with signs of wear

I^{1-3} incisors

C canine teeth

Fig. 5.3.3.3.
Schematic presentation of signs of wear in dentition of foxes
Adapted and reproduced from from (5)

Some examples of age determination by counting the cementum layers are presented in Figure 5.3.3.4. Both the counting of the cementum layers and the determination of the pulp volume require high expenditure and experience (2, 4, 11, 13, 14).



Presumed age of foxes: A: 2 years; B: 3 years; C: 4 years; D: 10 years

Fig. 5.3.3.4.
Longitudinal sections through canine teeth of red foxes, showing annual layers of cementum
Adapted and reproduced from (10) with kind permission from K.-H. Habermehl

2. Arctic foxes (*Alopex lagopus*)

Methods for age determination in arctic foxes were described by Bradley *et al.* (3). In studies on dynamics of the arctic fox population, young foxes of the year were identified by the large volume and thin walls of the pulp chamber, and ages of the adults were determined by counting of the annual layer of cementum (6).

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Annex 5.3.4.

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Chapter 6

Control of echinococcosis

6.1. Control of *Echinococcus granulosus*

M.A. Gemmell, M.G. Roberts, T.C. Beard, S. Campano Diaz, J.R. Lawson and J.M. Nonnemaker

Summary

In the present chapter, a review is given of the methods used and results obtained from field trials and control programmes during the last half of the current century to drive endemic E. granulosus *towards extinction status. Control can be divided into four phases:*

a) planning
b) attack
c) consolidation
d) maintenance of eradication.

Options for control include horizontal and vertical approaches. The former emphasises long-term primary health care (education, sanitation, upgrading of meat inspection, etc.) with the aim to reduce disease transmission. However, this may not result in control of E. granulosus. *The vertical approach is targeted to the parasite, it is based on specific control measures (dog population control, dog-dosing, etc.) and must include a base-line survey and surveillance of intermediate animal hosts to monitor progress. Various options of the vertical approach are described. The effectiveness of various control options are discussed using examples from both continental and island control programmes.*

6.1.1. Strategies for control and evaluation of control programmes

Definitions

It is essential to differentiate between control and eradication which are defined as:

Control: active implementation of a programme to limit the prevalence of a specific disease by a recognised authority on an instruction from the legislature.

Eradication: the purposeful reduction of a specific disease prevalence to the point of continued absence of transmission within a specific area by means of a time-limited campaign.

Any reduction in the basic reproduction ratio (R_0) (Chapter 5.1.) may be helpful, as it may reduce transmission to humans, but unless R_0 is reduced and maintained below unity until transmission has ceased, eradication will not be achieved. These two goals are not the same.

Preconditions for eradication are:

- absence of adverse ecological factors
- adequate administration, operational and financial resources
- availability of effective tools
- favourable epidemiological features
- socio-economic importance
- specific reasons for preferring eradication over control.

These preconditions invariably preclude consideration of eradication for continental control programmes.

6.1.1.1. Strategies for control

Over the past thirty years, considerable experience has been gained on the transmission dynamics and ways to control *E. granulosus*. Two philosophies have been applied to determine strategies. The first, a horizontal approach, emphasises long-term primary health care to enhance socio-economic advancement of the population; thereby improving their lives and lifestyles. This includes education, sanitation, upgrading of meat inspection and safe water supplies. This, however, may not result in a specific attempt to control *E. granulosus* or achieve a reduction in prevalence (15, 16, 19, 29, 43, 44).

In contrast, it seems that a specific control programme, if it is to be successful, must be targeted to the parasite and this must include a vertical approach with the use of, for example, arecoline hydrobromide as a diagnostic agent to test dogs or the regular dosing of dogs to eliminate *E. granulosus*. This emphasises an active intervention by drugs. These two approaches are not, of course, mutually exclusive, but the vertical approach must include a base-line survey and surveillance of intermediate animal hosts to monitor progress (13, 19, 21, 22, 43, 44).

- Phases of a control programme

Based on control programmes undertaken during the second half of the 19th Century, it seems that control can be divided into 4 phases, namely preparatory or planning, attack, consolidation and, if appropriate, the maintenance of eradication phase. A similar concept was expressed in 1979 by Todorov in an unpublished WHO document (45).

During the attack phase, control measures are applied non-discriminately to the entire host population at risk. Examples of this are mass dog-dosing campaigns and the introduction of restrictive regulations on dog-feeding practices.

In the consolidation phase, 'at risk' areas or farms are identified through surveillance and control measures are targeted at these only. Here meat inspection and legislation to quarantine infected premises are essential.

The maintenance of eradication phase can be entered once the parasite has possibly been eliminated.

In the last named phase specific activities are disbanded and vigilance is employed, mainly through the normal meat inspection services together with border controls to prevent reintroduction. The major objective, where control is feasible – but not eradication – is to transform permanently from the costly 'attack' to the less costly 'consolidation' phase, as soon as it is technically possible to do so (13, 18, 19, 20, 21, 22, 48).

- Options for control

Based on experience gained in these control programmes 5 options can be discerned. These need to be considered when benefit-cost ratios are being compared in the planning phase (Chapter 6.1.2.)

Option 1 (no control): the first option involves a decision not to proceed with control for a variety of reasons, such as lack of resources.

Option 2 (horizontal approach): the second involves the horizontal approach; namely the upgrading of veterinary public health activities, such as improving hygiene at abattoirs, increasing dog control and registration, and the introduction of an educational programme directed principally at schools. It is pointed out here that the provision of tablets for the owners to treat their dogs, as part of the horizontal approach, was applied in two countries without effect on the prevalence of echinococcosis in humans and animals.

Option 3 (slow attack option): this is orientated towards prevention of dogs gaining access to raw sheep offal and uses arecoline hydrobromide as a diagnostic agent in an educational approach to control. With this slow track option, the duration of the attack phase may last for more than 30 years, although it is possible to transfer to the less costly consolidation phase earlier, provided legislation can be applied for quarantining

sheep flocks still harbouring infected animals. The smaller the number of farms in quarantine, the lower will be the prevalence for which they are quarantined. In the case of permanent control, this must form part of the responsibilities of the meat inspection and animal field services. If eradication is feasible, the programme may be terminated by a purchase and compulsory slaughter of those few flocks still remaining with some infected sheep, but vigilance will still be needed.

Option 4 (fast track option A): this is a fast track approach and includes the application of arecoline in an educational approach, legislation and the surveillance of the human and animal populations as in option 3. In addition, however, to achieve this rapid decline in the attack phase, the dog population may have to be drastically reduced. The duration of this phase may be as little as 10-15 years.

Option 5 (fast track option B): this is also a fast track, but in this case, all dogs are treated with praziquantel at predetermined intervals, for example every 6 weeks. The duration of the attack phase may also be as short as 10 to 15 years, provided that there is no premature reduction in the dog-dosing programme, permitting a plateau in the prevalence of echinococcosis in aged sheep to develop.

6.1.1.2. Testing the feasibility of control and stability of taeniid systems by field trials

The transmission dynamics of the family Taeniidae have been described in Chapter 5. Several field control trials have been undertaken to test stability and they provide information on the events that may occur during control programmes.

- Endemic and potentially hyperendemic echinococcosis

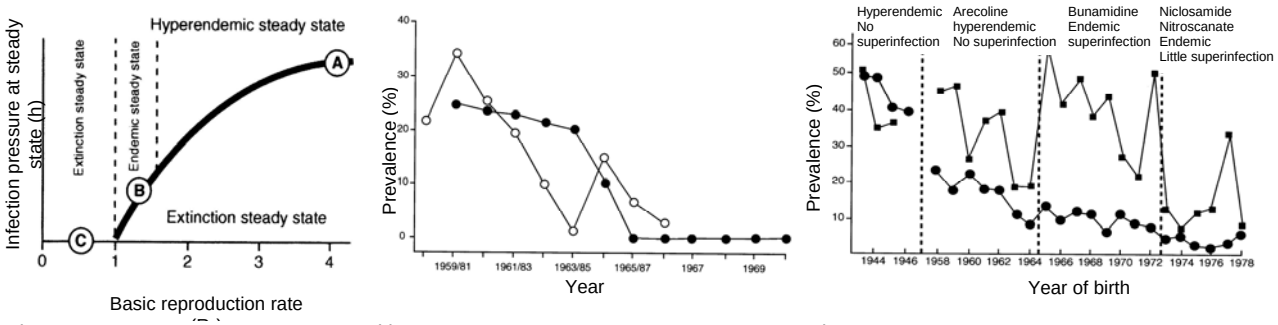
The first trial evaluated the stability of *E. granulosus* with its low biotic potential by applying a 3-monthly dog-testing programme with arecoline hydrobromide (option 3) (12, 17, 25) in an educational approach to control in an isolated valley in New Zealand, the Styx field-trial. The second trial was undertaken to test the stability of *E. multilocularis*, with its potentially high biotic potential, by treating all dogs every 4 weeks with praziquantel (option 5) in a village on St Lawrence Island, Alaska. Stability was measured by changes in the prevalence of hydatid cysts in sheep and rodents, respectively (Fig. 6.1.1.) (12, 14, 17, 18, 25, 40).

The results obtained from the application of option 3 demonstrated that the relatively weak force using arecoline surveillance for educational purposes was sufficient to drive *E. granulosus* from the endemic state to extinction status (R_0 = 1.6 to R_0 = 0.4 from B to C in Fig. 6.1.1.). The strong force (option 5) used was sufficient to drive *E. multilocularis* from the potentially hyperendemic state to extinction status (from A to C in Fig. 6.1.1.) (while it is not possible to measure R_0 due to difficulties in counting individual larvae in age-intensity studies, the high potential to rapid return to high prevalence levels on cessation of dog dosing suggests that *E. multilocularis* in this ecological environment, was in the hyperendemic steady state prior to control). However, in this case, cessation of treatment caused this system to return rapidly to hyperendemic status. This means that this parasite, when it is in this steady state, is globally asymptotically stable and can only be maintained in extinction status by a permanent monthly, or perhaps 6-weekly, dog-dosing programme. To date, little is known of the force needed to drive endemic *E. multilocularis* to extinction status (18, 41, 42).

- Hyperendemic cysticercosis

Little is known of the transmission dynamics of *E. granulosus* in the hyperendemic steady state and *T. hydatigena* has been used as a potential model. When *T. hydatigena* was tested for stability with option 3 (3-monthly arecoline) in the Styx field-trial, there was no effect on its stability and the prevalence remained the same in the lambs and in adult sheep of the same age cohort, but killed 5 years later and the parasite remained at status A (Fig. 6.1.1.a). When, however, the control force was increased using a 4-weekly dog-dosing programme with bunamidine hydrochloride (option 5), there was paradoxically an unexpected increase in the larval population in the adult sheep. This was accounted for by a less than perfect capture rate of dogs for treatment and the parasite was only driven from hyperendemic to endemic status (from A to B in Fig. 6.1.1.a) with a loss of immunity and increase in the larval population through superinfection. A similar increase was observed for *T. ovis* in the national flock in New Zealand during the echinococcosis control programme when a 6-weekly dog-dosing programme with praziquantel (option 5) was introduced to control it (12, 15, 17, 21,

25, 42). Just how *E. granulosus* in the hyperendemic steady state would respond to control using option 5 remains to be defined. It is pointed out, however, that no reports have yet been recorded demonstrating that this parasite exists in the hyperendemic steady state.



a) Diagram showing the possible steady state values of h, the measure of parasite abundance for different values of R_0. To drive the system from endemic (R_0>1) or hyperendemic steady state (R_0>>1) to extinction status (R_0 >1), the force applied must be strong enough to drive it from B to C, or from A to C, respectively
Source: (42)

b) Changes in prevalence of *E. granulosus* in adult sheep (•) in the Styx field-trial in New Zealand from 1959 following the use of 3-monthly arecoline testing of dogs to drive the system from endemic to extinction status (B → C) and a 4-weekly dog-dosing programme with praziquantel to drive *E. multilocularis* in a dog/rodent cycle (o) in Alaska from hyperendemic towards extinction status (A → C). Interruption to this force to *E. multilocularis* resulted in a rapid return towards hyperendemic status (C to A)
Source: (15)

c) Changes in the prevalence of *Taenia hydatigena* in adult sheep ( ) and in lambs (•) in the Styx field trial. A 3-monthly dog testing programme with arecoline had little effect as the prevalence remained similar in the lambs and adult sheep (1944-1964). The introduction of a 4-weekly dog-dosing programme with bunamidine drove the system from hyperendemic to endemic status (A → B) with an increase in the larval population
Source: (15)

Fig. 6.1.1.
Transmission dynamics of the family Taeniidae
Redrawn from (15), with permission from F.L. Andersen (ed.)

6.1.1.3. Analysis of some national and regional control programmes in the attack phase

The control programmes described in this section, differ from one another in administration, resources used, methods applied or rate of decline in transmission. The changes that occurred in the prevalence of *E. granulosus* in adult sheep are illustrated in Figure 6.1.2. Both island and continental situations are reviewed.

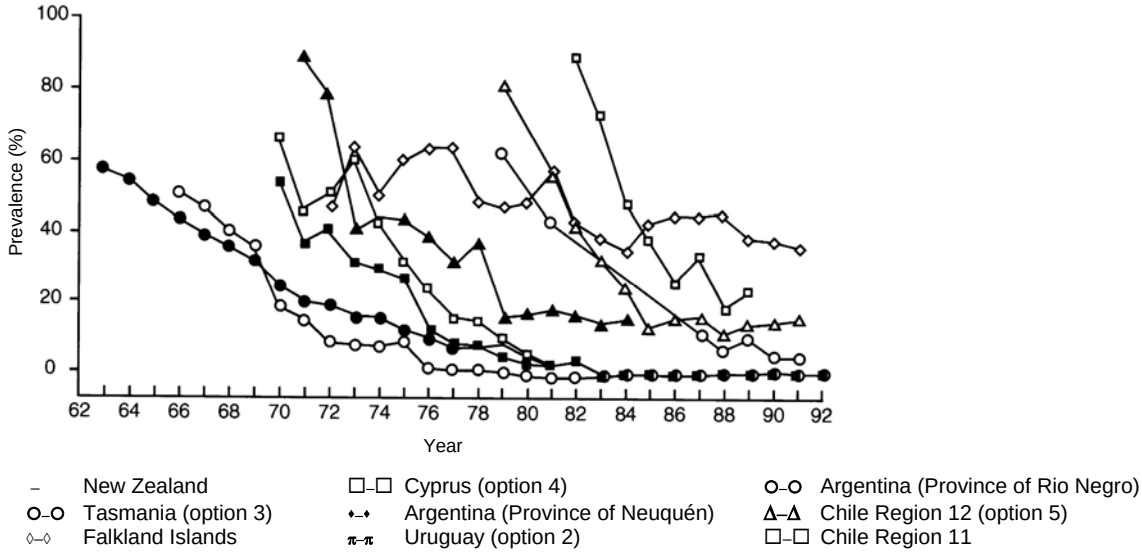


Fig. 6.1.2.
Prevalence of *Echinococcus granulosus* in adult sheep during control in Uruguay (option 2), New Zealand and Tasmania (option 3), Cyprus (option 4), and the Falkland Islands, Argentina and Chile during a thirty-year period (option 5)
Redrawn from (19), with permission from F.L. Andersen (ed.)

- Legislation, administration and funding

There are two models. The first creates, through specific legislation, a national and/or regional executive with responsibility for the control programme. The second utilises an existing government organisation (such as an animal or human health authority), and is directed by the legislature to proceed with surveillance and control. To an extent the former is likely to be funded through a dog tax and the latter through the legislature (19).

Depending on the programme to be adopted, areas in which legislation may be needed include:

a) meat inspection and effective disposal of offal at abattoirs and prevention of clandestine leakage of offal
b) banning dogs from abattoirs and closure if necessary
c) prevention of feeding raw offal to dogs including inspection of offal disposal facilities on farms or other premises where sheep are killed
d) control of dogs including registration, submission for dosing and elimination of unwanted dogs
e) quarantine of premises with infected livestock.

- Comparison of the effectiveness of policies applied in control programmes

Where control programmes directed against *E. granulosus* have been adequately monitored, evidence has been obtained that the methods used in options 3, 4 and 5, but not 2, have been successful in driving the parasite towards extinction, as was observed with endemic echinococcosis in the Styx field-trial. The most frequently used index determining progress has been the reduction in prevalence of echinococcosis in aged sheep (Fig. 6.1.2.). With this index, the slope of the decline in prevalence is dependent *inter alia* on the speed with which all the sheep infected prior to control are removed.

Effectiveness of option 2 (horizontal approach)

With option 2, education forms an important aspect with the introduction of posters and pamphlets together with general upgrading of facilities and hygiene at abattoirs, not specifically for echinococcosis control (Chapter 6.1.3.). This may also involve a specific programme to provide those owners who register their dogs with drugs to treat them. This was attempted for 20 years from 1937 in New Zealand without any noticeable change in prevalence of *E. granulosus* in humans and animals (data not shown). A similar finding was reported from Uruguay between 1970 and 1990 (Fig. 6.1.2.). In other words, no evidence for a decline in prevalence of *E. granulosus* in animal hosts could be discerned in these two endemic countries that applied a horizontal approach using option 2.

It was also found that long-term education, as applied in New Zealand from 1937 to 1959 (option 2), was not needed in order to initiate a control programme. Based on the subsequent New Zealand and Tasmanian experiences, it was found that only a short-term intensive educational programme (community participation) was required in order for the control authority to gain the acceptance and support of dog owners to proceed with a planned control programme using option 3 (5, 6, 7).

Effectiveness of option 3 (slow track option)

New Zealand and Tasmania adopted a slow track with an arecoline-based dog-testing programme in the attack phase. These programmes were almost completed within 30 years (Fig. 6.1.2.). In New Zealand, the programme commenced in 1959 with an attempt to test all dogs four times each year with arecoline hydrobromide, but this was changed in 1972 to a 6-weekly non-discriminatory dog-dosing programme to control *T. ovis* (option 5). The programme was not changed to the less costly consolidation phase until it was decided in 1990 not to continue controlling this parasite by dog-dosing. By that time, *E. granulosus* had been almost eliminated. This programme was funded primarily by a levy on dog licences and undertaken by a National Hydatids Council under an Act of Parliament (Hydatids Act 1959). Following transfer to the Ministry of Agriculture and Fisheries, surveillance and supervision of infested farms lead to the 'maintenance of eradication' phase (1, 14, 25, 28, 31).

In Tasmania from 1964, those dogs that were likely to be at risk were tested. Owners of infected dogs were penalised. Within 10 years, this attack phase was changed to the consolidation phase with a targeted approach using surveillance of sheep and subsequently quarantine of farms and finally purchase and slaughter. This programme was undertaken by the Department of Agriculture with departmental funding using existing legislation relevant to animal health (2, 5, 6, 26, 27).

In both campaigns, transmission to humans almost ceased within about 10-12 years. There was a reduction in all age groups including the elderly, demonstrating for the first time that CE can occur at any age, and that a vertical approach to control, funded either through a dog tax or through legislature, can almost immediately benefit all age groups in a community (Chapter 5).

Effectiveness of option 4 (fast track option A)

Cyprus adopted a similar approach in 1970 to that of New Zealand and Tasmania with dog testing 3 times yearly. However, from 1971 the fast track was achieved by drastically reducing the dog population by about 85,000 animals in the attack phase, thereby removing the habitat for the parasite (Fig. 6.1.2.). The programme was organised and funded by the Department of Agriculture (32, 33, 34, 35, 36, 37, 38, 39). From 1994, the programme entered the 'consolidation' phase with no evidence of transmission to humans in the Greek-administered zone of the island (10, 11).

Effectiveness of option 5 (fast track option B)

The Falkland Islands, Argentina (Neuquen Province and Rio Negro Province) and Chile (Regions XI and XII) achieved the fast track initially by applying a 6-weekly dog-dosing programme in the attack phase, but without reducing the dog population. All programmes substantially reduced the prevalence of *E. granulosus* in animals (Fig. 6.1.2.). In some programmes, a plateau in prevalence of echinococcosis in adult sheep developed. Both Chilean programmes used the Ministry of Agriculture with funding from that Ministry; whereas those in Argentina were administered by the Ministries of Health with funding from that source (3, 4, 8, 9, 23, 24, 46, 47). Similarly with Uruguay, the programme administered by the *Comisión Honoraria de Lucha Contra la Hidatidosis* operated option 2 from 1970 to 1991, and then transformed to option 5 with a rapid decline in the prevalence of *E. granulosus* in adult sheep (data not shown) (30).

6.1.1.4. Dog control policies

All three successful island control programmes, namely, New Zealand, Tasmania and Cyprus, used a vertical approach. This included the application of arecoline in an educational approach and a positive reinforcement of dog registration to achieve eradication. They differed in tactics to achieve effective control of the dog population. The possible numbers of registered and unregistered dogs prior to control were 500,000, 100,000 and 50,000 for New Zealand, Cyprus and Tasmania, respectively.

In all three programmes, registration of dogs was mandatory and enforced. In the case of New Zealand and Tasmania, ownerless dogs were collected by the technicians of the control authority and were, where possible, impounded for reallocation to new owners, and where this was not possible, were euthanised. Owners of registered dogs found wandering were financially penalised. Euthanasia, as a positive policy for dogs, was only undertaken at the request of the owner. In contrast in Cyprus, due to the high number of ownerless wandering dogs, a positive policy for euthanasia was adopted. This policy included all wandering dogs, irrespective of ownership and registration. The real difference between policies adopted in Cyprus and Australasia was in the force used to eliminate all wandering dogs, irrespective of ownership and registration status.

The results showed that within about 10 years of adopting this policy, transmission of *E. granulosus* almost ceased in animals in Cyprus. In contrast, the duration of this transmission was almost twice as long in New Zealand and Tasmania (Fig. 6.1.2.). However, irrespective of the difference in the dog control policies, transmission to humans ceased in all 3 programmes within about 10-12 years (1, 2, 5, 6, 7, 10, 11).

Comparing the decline in prevalence of echinococcosis in sheep in Cyprus (with its positive euthanasia policy) with campaigns that used a 6-weekly dog-dosing programme without a positive euthanasia policy (namely the

Falkland Islands, Neuquen Province, Rio Negro Province in Argentina and Regions XI and XII in Chile) it seems that the slopes of the decline in the prevalence in sheep were similar, except that in Cyprus, no plateau effects were apparent. The policy in Cyprus simply removed the habitat for the parasite, and was almost independent of dog-treatment schedules.

6.1.1.5. Transformation from attack to consolidation phase

As previously implied, transfer from the costly attack phase to the less costly consolidation phase requires effective meat inspection as well as quarantine of premises with infected livestock.

New Zealand

By the early 1990s, the Ministry of Agriculture considered that the attack phase had progressed to the stage that warranted a review of administrative structure so as to enter the consolidation phase. In 1991, the Council was disbanded and slaughterhouse surveillance with trace-back was applied as the dominant method for control by the Ministry.

The last major outbreaks occurred in 1990. In 1995, degenerated cysts were found in livestock on two farms and fertile cysts in sheep on one farm on Arapawa Island in the Marlborough Sounds. As a result of this finding, livestock on all farms on the island were placed under movement control restrictions and routine treatment of all dogs on the island was reintroduced. In 1996, slaughterhouse surveillance confirmed that infection was restricted to a number of sheep from a single farm on the island, while degenerate cysts were found in livestock from only one other farm elsewhere in New Zealand. The programme can now be regarded as in the maintenance of eradication phase with permanent surveillance of livestock (28, 31).

Tasmania

The formal control programme was initiated by the State Department of Agriculture (now named Department of Primary Industry) with funding supplied by the Legislature in 1964. The educational programme was similar to that in New Zealand using option 3 in the attack phase. Unlike New Zealand, transformation from the attack to the consolidation phase was introduced in 1975, only about 10 years after the initiation of the programme. The dog testing was then almost confined to farms with infected sheep flocks. These premises were quarantined and food animals could only be sold to official abattoirs.

In 1996, some 36 years from the initiation of the programme, Tasmania was provisionally declared free from *E. granulosus* and entered the maintenance of eradication phase. At the time of this declaration, the last infected dog had been detected 10 years previously. In 1997, a flock of 4,500 sheep was quarantined following the detection of a cyst in a young cow. This led to the finding that an infected dog had been introduced from the mainland of Australia 18 months previously. As in New Zealand, dogs entering the control area must be treated with praziquantel.

The major difference between the programmes in New Zealand and Tasmania was in the length of time control remained in the attack phase. This was accounted for by differences in administration.

Cyprus

The control programme in Cyprus was introduced into the Republic in 1971. In 1974, this was restricted to the southern area controlled by the government of the island. The force used in the attack phase (option 4) included large-scale dog euthanasia. This rapidly reduced prevalence, and the parasite was considered to have been eradicated and control was terminated in 1985. However, subsequent studies during 1993-1996, revealed that the parasite was present in 82 (20%) of villages in either dogs or food animals and control was reintroduced in the consolidation phase with emphasis on surveillance of the parasite in intermediate hosts, animal movement control and treatment of dogs in 'infected' villages. During these surveys, a major difficulty was found to be in determining whether transmission was autochthonous or introduced from animals from areas not controlled by the government (10, 11). Due to this, Cyprus may at the present time be regarded as a continental model permanently in the consolidation phase.

6.1.1.6. Conclusions

Based on the examination of both island and continental programmes, the following conclusions can be drawn:

- there are four phases of a control programme, namely: planning, attack, consolidation and maintenance of eradication
- from the New Zealand and the Uruguayan programmes, a reduction in prevalence in animals may not be achieved by applying a programme using only an horizontal approach (option 2), but must also include a vertical approach using either options 3, 4 or 5
- using option 5, a successful conclusion to the attack phase directed against *E. granulosus* can be achieved in less than 15 years in livestock, provided that methods needed, such as meat inspection and quarantine, can be applied
- from a study of the New Zealand and Tasmanian island models, it seems that it is very difficult to determine during the consolidation phase when eradication has been achieved
- once the maintenance of eradication phase has been initiated, surveillance through the normal meat inspection services must be regarded as permanent
- the case of Cyprus must be regarded as a continental model, as it is difficult, if not impossible, to prevent reintroduction across even supervised boundaries.

The evidence implies that once the attack phase has been introduced with its emphasis on surveillance and dog dosing, it should not be relaxed until the consolidation phase can be introduced. Continental programmes will need to remain in this phase permanently; whereas island programmes can be transformed from that phase to the maintenance of eradication phase. However, these transformations can only be made if the control authority is able to provide effective livestock surveillance and quarantine programmes. At the present time, no continental programme has reached the consolidation phase and one of the most important investigations still needed will include a determination of the methods required to enter and maintain that phase.

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Annex 6.1.1.

Evolution of programmes for control of *Echinococcus granulosus* (examples)

P. Economides, E.J. Larrieu and D. Orlando

The basic strategies for control of the *E. granulosus* infection and the evaluation of some of the programmes are described in Chapters 6.1.1.-6.1.3. In this annex, examples from 3 countries (Cyprus, Argentina and Uruguay) are presented in order to demonstrate various approaches and problems of control programmes in different areas and epidemiological situations. The authors are aware of the fact that these examples cannot be representative for all control programmes implemented world-wide. Therefore, the reader should consult the recent literature, where more information on programmes in various countries is described, including reports on control programmes in Chile (2, 21), Brazil (19, 20) and Spain (9).

Cyprus

Base-line data and evolution of control

Cystic echinococcosis was a severe public health problem in Cyprus before the 1970s, and *E. granulosus* was present in 40%-100% of adult sheep, in 4%-50% of lambs, in 27%-93% of goats, in 20%-50% of cattle and in 5%-22% of pigs. The average surgical incidence rate in humans was 12.9 per 100,000 inhabitants (5). A programme for *E. granulosus* control in Cyprus, implemented by the Department of Veterinary Services in 1971, was mainly based on:

a) stray dog control
b) registration of all owned dogs
c) spaying of bitches
d) slaughter control
e) arecoline testing of all dogs
f) education of the public (4, 18).

Due to the division of the island in 1974, the control programme was terminated in the northern part under Turkish administration. The actively enforced measures in the southern part (government controlled area [GCA]) have led to a very rapid reduction of transmission, and by 1985, it was considered that the parasite had been eradicated from both food animals and dogs. According to Polydorou (18), the prevalence of *E. granulosus* in dogs decreased from 6.8% (N: 12,213 dogs tested) in 1972 to 0.02% (N: 15,118) in 1982, and to zero (N: 19,955) in 1984. Since 1977-1987, sheep, goats and cattle under 2 years old and pigs were free of *E. granulosus* cysts, and only a low level of infection persisted until 1983-1984 in sheep, goats and cattle over 2 years old (0.92%, 0.01% and 0.01%, respectively). Therefore, the campaign was officially terminated in that year (17, 18). It has to be stressed that the division of the country in 1974 had modified the original 'island situation' to a 'continental-like' situation with a persisting endemic area in the northern part of Cyprus.

For the first few years after 1985, sporadic cases of CE detected in the GCA upon slaughter of food animals were considered to have been in animals smuggled from the northern part. However, studies during 1993-1997 revealed that the parasite was present in 82 (20%) of the total number of villages in the GCA in either dogs or food animals or both. In 1994, the following prevalences of *E. granulosus* cysts were found in slaughter animals: cattle: 0.14% (N: 14,747), sheep: 0.03% (N: 156,152), and goats: 0.01% (N: 142,735). Strong evidence was obtained that some transmission was autochthonous as 51 cattle originating from 28 farms in 17 villages

harboured *E. granulosus* cysts, and these animals had been born and retained on these farms during their lifetime (4). In 1993, an arecoline testing programme was carried out in 48 villages, and among 2,391 dogs, 16 (0.67%) were found to be infected with *E. granulosus.* The infected dogs were found in 6 villages of the Nicosia district, situated rather close to the endemic non-government controlled area in the north, and in 2 villages of the Paphos District in the south far away from that area. The general arecoline testing re-introduced in March 1994 revealed 6 out of 7,440 dogs with *E. granulosus* (0.08%) in 5 villages, 3 of which were situated in the south of the island (Limassol, Paphos and Larnaca) (4).

Between 1980 and 1994, a total of 122 human patients were operated for CE in hospitals of the GCA, but between 1990 and 1993, there were no cases in persons under the age of 20. This implies that transmission of the infection from dogs to humans had ceased shortly after implementation of control in the GCA. This is not the case in the non-government controlled area, where CE among the Turkish Cypriots is very common and has reached alarming proportions (4) with an annual incidence of surgical cases of approximately 25 per 100,000 population, including patients of all ages, even children under 10 years (*source:* local Turkish newspapers).

Re-introduction of control for 'permanent consolidation'

In 'continental' situations with borders to other countries where *E. granulosus* is present, eradication of echinococcosis may not be possible if continuous infiltration from endemic to parasite-free areas occurs. As a continental-like situation exists in Cyprus due to the division of the country, control measures for 'permanent consolidation' have been implemented in 1994 by the Department of Veterinary Services for the GCA (4).

Measures for 'permanent consolidation' in the government controlled area of Cyprus, implemented since 1994:

- control of dogs (responsible ownership, registration, movement control and collection of stray dogs with euthanasia if dogs are not claimed)
- treatment of imported dogs with praziquantel
- prevention of smuggling of food animals
- safe destruction or deep burial of carcasses or offal of food animals
- safe slaughtering
- continuing education
- special measures in infected villages (see below).

The major measure is surveillance of all food animals in slaughtering establishments with trace-back of infected animals to the site of origin. A village is designated as an 'infected area' when a dog or food animal on any of the premises is found to be infected with either *E. granulosus* or *T. hydatigena* (4).

The infection of food animals with the metacestode stage (Cysticercus tenuicollis) of *T. hydatigena* is used as an 'early warning system' for the detection of dogs fed on raw offal. Lesions caused by C. tenuicollis in the liver of lambs or kids can be found already 3 to 4 weeks after infection with *T. hydatigena* eggs while the development of *E. granulosus* cysts may require several months to become macroscopically visible. In case of doubt, histological examination of cysts should be made for differential diagnosis. In infected villages, special control measures are applied (4).

Special control measures in infected villages:

- treatment of all dogs with praziquantel
- control of stray dogs
- movement control of dogs and food animals
- prosecution of illegal slaughtering.

In this situation the use of arecoline purging or coproantigen testing (Chapter 3) to identify individual dogs infected with *E. granulosus* appears to be of limited value as treatment of all dogs in an infected village is an essential part of the control strategy. On the other hand, coproantigen testing can be of great value in large surveillance studies in order to detect new foci of the infection which were not identified by surveillance of slaughter animals (Chapter 3).

General remarks and conclusions

Experiences from Cyprus have shown that the 'consolidation' phase may be more difficult than the attack phase. In a continental-like situation, the consolidation phase may last 30 to 50 years or longer if disease transmission cannot be prevented across borders. Therefore, a 'permanent consolidation phase' may be necessary.

Argentina

Implementation of control

In Argentina, control of *E. granulosus* has a long history since 1906. Control programmes were implemented in the Provinces of Neuquén (1970), Tierra del Fuego (1975), Rio Negro (1979), Chubut (1980) and Buenos Aires (1990) (10, 11). As an example the control programme in the Rio Negro Province is described.

The control programme was based on several measures which are summarised below.

Main control measures in the Rio Negro Province:

- registration of dogs by means of dog identity cards in which features of the individual dog and treatments against *E. granulosus* were recorded
- regular dog dosing with praziquantel (5 mg/kg bw) at intervals of 45 days in rural and of 180 days in urban areas. Drug distribution was the responsibility of the sanitary agent of the hospital in each area, dog owners were responsible for dog treatment (10, 15)
- identification of infection sources by examination of dogs for *E. granulosus* by arecoline testing (Chapter 3) and supervision of control methods by professional staff of the Veterinary Public Health Teams (13, 14)
- in 1994, a law (No. 2580) for hydatid control was approved, making dog registration in rural establishments compulsory and dog owners responsible to keep their dogs free of *E. granulosus*
- collection of information on the prevalence of *E. granulosus* cysts in sheep in slaughterhouses under provincial control
- health education (Chapter 6.1.3.).

A special computer software (DIRSAM) was developed by the Pan American Institute of Food Protection and Zoonoses, Buenos Aires in co-operation with PAHO/WHO with the aim of supporting control activities and improve administration.

The control programme was linked to medical activities, including recording of new cases of human CE, and surveys in certain population groups using serological methods and ultrasound examination (3, 6, 7, 8, 12, 14).

Evolution of control

The evolution of control in the Rio Negro Province is shown in Table 6.1.1.1. From 1979 until 1997, there was a substantial decline in prevalences of *E. granulosus* both in dog and sheep populations. Also the annual number of human cases decreased significantly from 79 to 22 per 100,000. Although progress in control has been achieved, it is not satisfactory as the incidence of CE still high. This is further substantiated by ultrasound examination in rural areas of Ñorquinco/Pilcaniyeu, where quite a high percentage of people had signs of CE (1984: 5.5%, 1986: 4.1%, and 1996: 2.1%).

Table 6.1.1.1.
Evolution of control programmes in the Rio Negro Province, Argentina

Year	*E. granulosus* in dogs: prevalence percentage[a]	*E. granulosus* in sheep: prevalence percentage[a]	CE in humans: new cases per 100,000 per year
1979[b]	41.5	61.0	79.0
1988	5.3	7.0	39.5
1991	4.2	12.7	32.6
1997	2.9	5.5	22.2

Source: E.J. Larrieu (11)
a) percentage prevalences are based on very large numbers of animals (11)
b) year of implementation of control programme

General remarks and conclusions

Control of *E. granulosus* in continental countries is very difficult, particularly in vast areas where large distances limit the implementation and effective supervision of control measures. As echinococcosis persists to be a considerable public health problem in Argentina, continuation and enforcement of control is necessary.

Uruguay

Base-line data and implementation of control

Uruguay is one of the countries in the world most affected by CE. This is reflected by a surgical prevalence of 20.2 cases per 100,000 inhabitants in 1992 (1). After several attempts of control with limited success, in 1990 the National Commission for Control of Hydatidosis promoted the passing of a law, which formed the basis for new control strategies (1, 16). In 1991, an arecoline survey for the diagnosis of the *E. granulosus* infection in dogs was performed, and a dog dosing programme was launched in the same year. Dogs were treated 12 times a year with praziquantel by staff members of the programme. In the 1991-1994 period, the coverage of treatment was gradually extended, so that in 1995, it covered more than 90% of the rural dog population (1). Surveillance of CE in sheep at slaughter plants and reporting of human cases in hospitals was also initiated. Health education was included among the field activities.

Evolution of control

Evolution of control is indicated by some selected data on the decrease of prevalence of *E. granulosus* in dogs from 10.7% in 1991 to 0.7% in 1997, and in adult sheep from 41.1% in 1992 to 14.1% in 1995 (data for lambs: 2.3%-0.17%) (1) (Table 6.1.1.2).

Table 6.1.1.2.
Evolution of control programmes in Uruguay

Year	*E. granulosus* in dogs: prevalence percentage	*E. granulosus* in sheep: prevalence percentage		Infected properties percentage	CE in humans: new cases per 100,000 per year
		Sheep	Lambs		
1991	10.7			13.2	
1992		41.1	2.3		
1993					11.3
1995		14.1	0.17		9.0
1997	0.74			1.5	6.5

Source: Anon. (1)

Strategies for continuation of control

Considering that drug administration at a national level covered all the municipalities in 1995, that some of the categories of sheep remain as reservoirs up to the age of 7 or 8 years, and *E. granulosus* eggs may survive in the environment up to one year, it is necessary to maintain the attack phase by monthly praziquantel treatment of dogs in a guided manner until 2003-2004. Some municipalities may enter the consolidation phase before that date. This phase may be reached after achieving a low re-infection potential in adult sheep (under 1%).

General remarks and conclusions

In this situation a system must be available to permit the characterisation and identification of 'infected' properties or areas (1). The activities of dog treatment should be maintained only in these areas or properties. Methods used in Cyprus (see above) for the identification and control of low-level infection may be also of value in the Uruguayan situation. One of the most important measure to reach and maintain the consolidation phase is the implementation of permanent inspection of slaughter animals for *E. granulosus* cysts in slaughter houses with significant slaughtering volumes (1). Furthermore, education and other measures will play an important role.

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6.1.2. Formulating effective and cost-effective policies in the planning phase for permanent control of *Echinococcus granulosus*

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Summary

In the planning phase, effective policies for control of Echinococcus granulosus *have to be formulated. This phase is concerned with quantifying the epidemiological and socio-economical factors that define the magnitude of parasite transmission and the benefits from and costs of control. It should also include decisions on the type of approach to control, either a horizontal approach with emphasis on education, meat inspection and upgrading of slaughterhouses, etc., or a vertical approach to include positive veterinary intervention with active surveillance programmes. These decisions have to be based on surveys, which are of fundamental importance to establish the importance of CE* vis-à-vis *other endemic diseases, to obtain base-line data and an insight into the processes of transmission, and to provide base-line information for formulating effective and cost-effective control policies for some or all the affected zones. The general methodology for surveys and surveillance is described in this sub-chapter.*

Based on experiences gained from field trials and control programmes (Chapter 6.1.1.), it seems that policies for permanent control of *E. granulosus* must be developed that can be realistically sustained within government budgets that are never enough to meet all the needs of the community. It follows that business methods will more and more have to be applied to discriminate between the claims for the limited discretionary funds available from the health and agricultural authorities for the surveillance, prevention and control of this zoonosis. In addition, it is also now known that because of the long-term nature of a control programme, a critical factor is that of obtaining sufficient funding from the legislature to complete it. Loss of confidence by the legislature, resulting from inadequate data providing evidence of success, may well result in premature withdrawal of funding. It has also now been clearly demonstrated that the collection of effective base-line data and that from subsequent surveillance are essential if the support of the legislature is to be maintained and control policies changed so that the programme remains cost-effective throughout its duration (19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 44, 45, 46, 47, 48, 49, 51).

The preparatory or planning phase is concerned with quantifying the epidemiological and socio-economic factors that define:

- the magnitude of parasite transmission
- the benefits from and costs of control.

The investigations and the sequence in which they should be carried out are also known, and the research centres required to formulate the plan, which may take several years to complete, can be recruited. Even when complete, the control of CE may be found to have a limited priority *vis-à-vis* other human health problems; or resources needed for control are simply not available to cover the whole or part of the endemic area.

Thus, decisions based on investigations in the planning phase may include:

- take no action
- apply an horizontal approach with emphasis on education, meat inspection and
- upgrading of slaughterhouses, etc., or
- introduce a vertical approach to include positive veterinary intervention with active surveillance programmes.

In the case of planning a vertical approach, several options have to be considered as recorded in Chapter 6.1.1. The current sub-chapter identifies some of the information needed from the base-line surveys for subsequent benefit-cost analyses in the 'planning' phase and the methods to be used in surveillance to monitor the programme. It is concerned with planning a permanent control programme and identifying the medical and veterinary resources needed as well as funding required from the legislature to carry out the plan and should be read in conjunction with Chapter 6.1.1.

6.1.2.1. Overall considerations during the planning phase

The 'attack' phase is costly and the various options that have been applied in this phase are described in Chapter 6.1.1.

Owing to delays in the system, reduction in prevalence of echinococcosis in animals takes time after the hyperendemic or endemic state has been transformed to extinction status. Long before this, transmission to humans will have almost ceased.

A policy decision has to be made during the planning phase, as to whether or not to:

- remove control altogether, or
- retain control permanently at a much reduced level of activity in the consolidation phase, or
- attempt eradication.

It is emphasised here that the last-named should not be considered an option in the planning phase (19, 21, 22, 25, 26, 27, 28).

Policies for upgrading education in an horizontal approach

In several highly endemic situations, particularly where effective structures or funds are not available for control, a vertical approach policy may not be possible. In this case, emphasis may be placed in the planning phase on developing an horizontal approach (option 2; Chapter 6.1.1.) by defining losses from echinococcosis to human health, and the costs of the primary health care methods that might be applied in a long-term educational approach to control, particularly directed at schools. Similarly, base-line surveys of the prevalence of the parasite in animals and the methods, by which hygiene at abattoirs may be upgraded and the costs of so doing should form part of this approach. These educational and meat hygiene components may not modify transmission, but will contribute generally towards improving standards of living and an understanding of the health problem. In addition, this horizontal approach may well form an early part of a vertical approach, in which health education in the form of 'community participation' is applied prior to the introduction of a policy decision to introduce an attack phase.

Planning policies for the attack phase and its duration

The objective of the plan is to develop long-term control policies with potentially high benefit-cost ratios that fall within the resources of the primary health care and veterinary services. If the attack phase is terminated too early before transmission between animals has ceased, the parasite may revert from extinction to endemic status. Thus, removal of all controls when transmission reaches a low level is not considered to be a valid option. Removal, however, of some of the most costly controls and retention of others of lower costs, should not lead to its recrudescence when the risk of transmission has become an unlikely event.

It seems that an important requirement in the planning phase, if permanent control is to be adopted as policy for *E. granulosus* control, is to ensure that the attack phase rapidly reduces the level of transmission to the

point where CE becomes an unlikely event. At that level, such practices as non-discriminatory dog-dosing become unnecessary and can be removed altogether. Permanent control can then be best achieved most economically by maintaining surveillance of the intermediate hosts and taking local action wherever breakdowns have been identified. An important objective in terms of minimising costs and maximising benefits of permanent control is to transfer from the attack to the consolidation phase as soon as feasible with its specific reliance on targeting of infected premises through surveillance of animal intermediate hosts during meat inspection (19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 48).

6.1.2.2. General methodology for surveys and surveillance

It is now well documented that climate plays a significant role in determining the duration of infectivity of the egg and thus the seasonal infection pressure and that this may vary widely within endemic zones. There is a need, therefore, to ensure that in any survey, the data collected belong to the region where they have been collected.

Surveys are of fundamental importance to:

- establish the importance of CE *vis-à-vis* other endemic diseases
- obtain base-line data and an insight into the processes of transmission
- provide base-line information for formulating effective and cost-effective control policies for some or all the affected zones
- provide base-line information against which to monitor the progress of control.

The information that should be obtained in the planning phase includes age-specific prevalence and geographic distribution of human, domestic livestock and wild animal echinococcosis in each zone. It is necessary to identify the main agencies involved in assembling data on CE from hospital registers, serological laboratories, health institutes, as well as those responsible for collecting data from the livestock sectors and to document the methods used in their collection and processing. In most cases, this information may have to be acquired by specific surveys and undertaken by a planning staff. Assessments must include *inter alia* estimations on the economic losses caused by echinococcosis in terms of hospital costs, man-hours lost, total and partial handicap for work, condemnation of viscera from slaughterhouses and meat packing plants, as well as on ownership and movement of dogs and occupation of owner.

6.1.2.2.1. Human cystic echinococcosis

The annual rate of hospital cases, when properly compiled, provide useful data on the significance of CE and, when measured continuously over many years, for the detection of regional changes in infection incidence. The base-line information which will contribute to defining realistic infection levels includes a five-year retrospective survey of all proven cases in all hospitals undertaking surgery within the region.

Surgical incidence surveys

The data can be expressed as the annual rate of new hospital cases (surgical cases only) or total patients per 100,000 rural and/or total/rural population. For epidemiological purposes, the rates should be broken down by age, sex, ethnic group, residence and occupation, etc. Such data can also be of great value for monitoring the effect of a control programme.

Systematic ultrasonographic and serological surveys

Ultrasonography (US) has emerged as the screening technique for CE with greatest sensitivity, specificity, and clinical correlation (48). Ultrasonography has a number of characteristics that make it an excellent screening tool:

a) high acceptability to the population

b) can explore the abdominal sites which are most commonly infected

c) provides immediate results

d) can be used under field conditions (portable ultrasonograph run on electricity or on power from a portable generator)

e) can be performed by staff after a relatively short special training

f) low cost.

Ultrasonography screening for CE has already been successfully used in Africa, South America and the People's Republic of China (48). Compared with serological screening, US detected a higher prevalence of CE and gave higher positive predictive values (48).

Immunodiagnostic tests have been improved during the last years, but they still have limitations for screening of CE:

a) a variable proportion of persons with CE do not have detectable antibodies

b) the presence of antibodies does not provide information on the location, size and other parameters of the cyst

c) many test are not highly specific so that cross-reactivity occurs with other helminthic infections (15, 48, 49).

One practical approach to serological surveys for CE is to screen serum samples with a rapid and highly sensitive test, such as ELISA, and then confirm specificity by further, more specific test (e.g. immunoblot test and arc 5 determination) (48). In cases in which cystic structures cannot be diagnosed as *Echinococcus* cysts by US, immunodiagnostic tests can help to clarify the aetiology. Systematic serological, radiological and ultrasonographic screening at a population level suggests that many cysts remain asymptomatic and provide a complementary and more direct insight into the natural history of CE, especially in highly endemic areas (2, 9, 10, 19, 31, 36, 38, 39, 41, 42, 50).

It seems, however, at the present stage of knowledge, that the basic index for evaluating the efficacy of control should be surgical incidence rates rather than serological and echotomographic prevalence levels that include cysts that will come to surgery as well as those that will always remain asymptomatic (6, 7, 8, 31).

Sensitivity, specificity and predictive value

Sensitivity, specificity and predictive value are statistics that define the performance of test procedures. These values must be defined in order to determine the performance of immunodiagnostic tests used for estimating infection levels of echinococcosis in human and animal populations. Sensitivity describes the likelihood of a positive test occurring in infected persons and animals. It can be considered as the proportion of infected persons or animals that have a positive test. Specificity is the likelihood of a negative test result in uninfected (with organisms being tested) humans or animals, or the proportion of uninfected persons or animals that have a negative test. Sensitivity (for, say, PCR) and specificity (for a variety of tests) are not constant between different groups. As an example, the specificity of *Toxacara* ELISA assay is good in northern Europe, where persons are unlikely to be infected with anything else. The same test has much lower specificity with many more false positives in South America, where patients may have multiple infections. These false positives occur particularly at lower dilutions, but by raising the cut-off point, sensitivity may be decreased. Sensitivity and specificity must be examined and adjusted to the populations under investigation. These values can be determined using a serum bank (for diagnosis) from persons within that population whose spectrum of parasitic infections are known.

The predictive value of a test is the probability its result will indicate the true state of the infection. The probability or likelihood of infection in humans and in animals with a positive test result is the positive predictive value. The negative predictive value of a test is the likelihood that humans and animals are in fact free from that infection. Infection prevalence is the important determinant of the predictive value, but does not affect predetermined sensitivity or specificity. A decline in infection prevalence reduces the positive predictive value and increases the negative predictive value. In other words, the positive predictive value in an immunodiagnostic test for echinococcosis may be much higher if it is used on persons sent to hospital with suspected CE. If, however, the same test is used on a randomly selected group where there is a low level of infection, then some of those testing positive may well be false positives. This is also true of echinococcosis in

animals. However, in this case, other cestodes may well provide a high enough level of false positives to reduce the value to a herd or flock test.

Where prevalence of infection is 50%, a diagnostic test with a 90% sensitivity and 95% specificity will have a positive predictive value of 94% and a negative predictive value of 90.05%. Both values are relatively acceptable. If the prevalence of infection is 1% in the population, the same test would have a positive predictive value of 15.2% and a negative predictive value of 99.9%. Such a test has a value only in identifying uninfected individuals.

6.1.2.2.2. Echinococcosis in food animals

Surveys of prevalence of echinococcosis in livestock are important for comparing transmission levels quantitatively within and between regions, and for determining the significance of each species of animal in the transmission dynamics. Examination of the livers and lungs at autopsy remains the only practical way of obtaining these data. Good design and sampling procedures are important and the samples should be large enough to ensure the appropriate comparisons can be made.

The information required includes:

- geographic distribution
- age-intensity prevalence
- liver/lung cyst ratios, and
- fertility of cysts.

Where there is doubt concerning the origin of the animals, the data should be excluded from the analysis of geographic distribution.

Where transmission levels have been quantified, namely, New Zealand, the People's Republic of China and Uruguay, the equilibrium steady state has been defined as endemic with R_0 only slightly above unity. This is a key measurement and the information obtained clearly showed that a dog-dosing programme at a relatively low level of intensity is feasible (11, 24, 25, 26, 27, 37, 44, 45, 46, 47).

Errors in meat inspection

The undifferentiated inclusion of parasites, other than *E. granulosus*, during meat inspection in hydatid surveys almost invariably leads to gross errors in understanding the local transmission dynamics and resources needed for control. Beside the ubiquitous *T. hydatigena*, lesions caused by other parasites such as *Toxacara* spp., *Ascaris suum*, *Parascaris equorum*, *Fasciola* spp. and *Fascioloides magna* are relatively common in the livers of sheep. Lesions that must be excluded in the lung include those caused by *Dictyocaulus filaria*, *Mullerius capillaris* and other Protostrongylidae, as well as some bacterial pneumoniasis and caseous lymphadenitis (CLA).

Monitoring progress in control

In several cases, surveillance of echinococcosis in food animals during control have been limited or even omitted. This has seriously reduced the value of the control programme or prevented success. The methods to be applied in the attack phase must be defined quantitatively and qualitatively in the planning phase. In the first, prevalence of *E. granulosus* in livers (and sometimes lungs) are compared from one year to the next in both young and old animals. This has the disadvantage that limited progress will be shown until most of those sheep born before control was initiated are no longer alive. This is usually a period of one sheep generation of say, 5-7 years, but may be longer.

A more sensitive method, particularly for field trials, includes the use of sentinel lambs. This has the advantage that changes can be determined almost as soon as a field control trial is initiated. In one study, four sentinel lambs were purchased from each of 60 farms within and outside the area (35). They were examined at slaughter for larval *E. granulosus* when either 6, 10 or 15 months of age. The livers and lungs were cut into 2-mm slices and examined histologically for parasites. Any suspicious lesions (for example, granuloma) were

removed for histological examination. Large fixed slices were cut in half and small lesions were embedded whole. Sections were stained with haematoxylin and eosin and *E. granulosus* positively identified. The PAS stain is also valuable for demonstrating the laminated membrane and thus, differentiating *E. granulosus* from other liver cysts. A similar method may be applied to define age-intensity prevalence in young animals and thus, the equilibrium steady state of *E. granulosus* during the planning phase (11).

6.1.2.2.3. Canine echinococcosis

The most accurate indicator of the prevalence and variation in the infective pattern of adult *E. granulosus* in dogs involves necropsy of the small intestine. However, it is rarely possible to obtain sufficient animals of each class (e.g. working, hunting, stray or pet dogs, etc.) to evaluate completely the epidemiological factors involved in causal relationships for infection in humans. The method has most application as a survey tool where feral and unwanted dogs are available.

Base-line surveys and field trials

As part of the key information required to complete the plan, there is a need to define the following:

- the prevalence of cestodes in dogs (by surveys)
- rates of reinfection of dogs (by field trials)
- the most cost-effective dog treatment schedule that will drive *E. granulosus* from endemic to extinction status and if possible without modifying the hyperendemic status of such tapeworms as *T. hydatigena* and *T. ovis*.

The value and limitations of treating dogs with arecoline hydrobromide in surveys of canine echinococcosis have been well documented (18) (FAO/UNEP/WHO Guidelines, 1981). The most important continuing need for arecoline testing is that for the base-line surveys and those field trials undertaken to define the reinfection levels of the canine taeniids and thus, the most economic treatment schedules for dogs in the attack phase (12, 22, 25, 29).

For the base-line surveys and data from field trials defining cost-effective dog-treatment schedules to be comparable, they should be accompanied by the following information:

- dose rate applied
- thoroughness with which doubtful samples have been excluded from the analysis
- methods used to separate worms from faeces
- visual aids used to examine the samples.

There is now ample evidence confirming that diagnosis in the laboratory is likely to give a higher infection level than diagnosis in the field, especially where worms less than 5 weeks old or fewer than 5 worms per sample are present (18).

Surveillance of canine echinococcosis

Now that praziquantel is used routinely to treat dogs in control programmes, arecoline has a less important role to play in education or surveillance during the attack phase. An alternative approach to diagnosis is based on faecal antigen detecting antibody sandwich technique. This has been developed recently and has shown promise because antigen can be detected shortly after infection, and the level declines rapidly following expulsion of the worms (1, 15, 16, 17) (Chapter 3). Further work may show that the coproantigen test may well find a useful role to identify dogs that become reinfected in the attack and consolidation phase, when qualitative rather than quantitative infection data may be required.

6.1.2.2.4. Echinococcosis in wild animals

Effective evaluations of wildlife echinococcosis may require examining relatively large numbers of animals in the planning phase. Methods of capture and sampling of the population are described in appropriate journals.

An important reason for adequate surveys in the planning phase includes a determination as to whether transmission is dependent on or independent of domestic animals. In the first situation, effective control in domestic animals should also modify the parasite population in wild animals. Evidence of wildlife involvement provides very good reasons for undertaking extensive surveys and transmission studies in the planning phase before considering the feasibility of introducing a control programme. In the event of its introduction involving 'spill-over' situations, the 'spill-over hosts' should play no essential part in the attack phase. This is because they neither need treatment nor do they serve as indices of progress in control. For this reason, surveys on feral and wild intermediate hosts should be made during the planning phase and towards the end of the attack phase.

6.1.2.3. Quantifying the economics of applying control

Mathematical models provide a logical framework within which the dynamics of parasite life-cycles are described. These models provide, via the threshold theorem (32), a criterion for deciding if a control programme can succeed in eliminating the parasite. In order to use this theorem, it is necessary to have an estimate of the basic reproduction ratio (R_0). This can only be obtained if reliable epidemiological data are available in the planning phase and before the attack phase is started. These models can be used as the basis for predicting the outcome of the various control options (Chapter 5) (24, 25, 44, 45, 46, 47).

Estimating the benefits and costs of control

An analysis requires a base point, which is usually the pre-control situation. Several indicators of economic performance are available, such as cost-benefit ratio, the net present value and the internal rate of return. The most readily understood analysis is the benefit-cost ratio. Cost-benefit analyses require the determination of the costs and losses in the uncontrolled situation. The determination of the costs and losses due to infection in humans and livestock during control programmes, requires the prediction of the effects of the selected policy on the prevalence of the parasite in its hosts (5, 14, 30, 33, 34, 40, 44). Cost-benefit analysis is not the only option for evaluating control programmes. In some cases, it might be better to conduct a cost-effectiveness analysis (J.M. Nonnemaker, personal communication, 1998).

The response to an applied control measure on the prevalence of the parasite in its hosts during the course of the programme can be approximated using the equation:

$$h = h_\infty + (h_o - h_\infty) \exp(- pt)$$

where h is the parasite infection pressure, and the effect of the measure is to change the steady-state value of h from h_o to h_∞.

The exponent p is calculated from:

$$p = \frac{h_o \lambda_\infty (1/R_{o(before)} - 1/R_{o(after)})}{h_\infty - h_o}$$

where h_∞ is the rate of parasite transmission through the system when control pressure is applied.

Before an analysis can be undertaken, information is required on all losses caused by the parasite and the effects of the different strategies on its prevalence with time. In this regard, it is important to estimate the effects of undertaking targeted or non-targeted programmes and to determine whether or not the programme will eliminate the parasite and, if so, how long it will take.

The benefit/cost ratio is defined as:

$$\frac{\text{Total discounted benefit}}{\text{Total discounted cost}}$$

A benefit/cost ratio of 1 indicates that the costs and the benefits are equal and hence the project in question should 'break even'. A ratio greater than 1 indicates that the overall benefits of the policy outweigh its costs.

Although it is recognised that control of a human disease imparts much more than just financial benefits, in practice the inclusion of non-monetary aspects into benefit/cost analysis is largely unsatisfactory.

Quantifying economic losses to human health and animal production from cystic echinococcosis (Chapter 6.1.4.)

Most studies on the losses due to CE have also confined themselves to calculating lost production due to disability and death. They are consequently gross underestimations of the true cost of disease. Nevertheless, once these limitations are recognised, benefit-cost analysis remains an extremely useful tool for assessing the comparative merits of both different public health projects and different control strategies, especially if used in conjunction with other quantitative and qualitative methods. In New Zealand, for example, two control options were studied for *E. granulosus* and 10 for *T. ovis*, giving a total of 20 control options. They were ranked according to their low and high benefit/cost ratios and net damages evaluated over a 30-year period. Such information can be most useful for decision-making by the legislature of the appropriate way to proceed with control and its cost (26, 33, 34, 40, 44).

Human health

In patients with CE, the quantifiable items include preoperative diagnosis, surgical treatment, hospitalisation, post-surgical examination, medicaments and transfers. There are variations between cases so that those complicated by infection, rupture or difficult access call for much longer periods in hospital. Quantification becomes more difficult when, in addition to these sequelae new cases occur in dramatic localisations (such as bones, eyes, brain or heart). This may result in an irreversible sequel, such as loss of an organ or death.

In this brief analysis, the convalescent period should also be considered as an economic loss in view of the well-documented fact that a great proportion of cases occur in actively working persons as the contribution of their labour to the economy is lost (5, 8, 9). To this must be added the amount of social security benefits or costs incurred. A realistic contribution to estimates of these losses from this parasitism can only be made if reliable and consistent registers are kept of case histories at all hospitals undertaking surgery.

Animal production and economic losses

To determine the economic impact of echinococcosis in domestic food animals would appear to be a simple task. It would suffice to calculate the number of kilograms of offal condemned in local abattoirs and to multiply them by their value. These calculations showed that in Chile between 1983 and 1988, offal (livers and kidneys alone) were condemned to a value of US$6,364,563 (13). However, it must be borne in mind that in some endemic areas, offal have a residual value, because they are used for making industrial pet food for animals (dogs, cats, fish, etc.), so that the real loss is that represented by the difference in price of the offal under the different sets of conditions. With respect to the losses from a reduction in the production of milk, meat and wool, very few quantitative studies have been made. Studies in Sardinia suggested that these may amount to a loss of 20,075 billion lire per annum (3, 4). Preliminary studies carried out in Region XII, Chile, indicated that carcasses of sheep born and slaughtered after implementation of control weighed on average 1 kg more than those born and slaughtered prior to control (13).

With respect to economic analyses of control programmes, a prospective analysis was performed of a 10-year project involving Sardinia. Assuming a decline in disease prevalence in sheep from 30% to 10%, the net present value of the gained milk production was evaluated at 32.5 billion liras/1982 and the internal rate of return equal to 53.6%. These studies emphasise that there is a need to obtain reliable data to convince funding authorities to support control. This identifies a research area in the planning phase, the results from which are essential if control is to be funded by the legislature for the time needed to achieve control (40).

6.1.2.4. Costs of applying control

Funding may be obtained in part or in full for the costs of control from fees attached to a dog licence or wholly through national or provincial government sources including Ministries of Health, Agriculture and Internal Affairs.

Costs of control will depend on the methods applied but will include *inter alia*:

a) training and continuous education of personnel
b) education of the public
c) dog control and treatment
d) surveillance of echinococcosis in humans
e) surveillance of intermediate host populations
f) evaluation of results
g) vehicle running and administration.

6.1.2.5. Note on benefit-cost analyses for *Echinococcus multilocularis* control

Too little is known of transmission dynamics of *E. multilocularis* to define a benefit-cost approach to control in sylvatic echinococcosis (44). However, evidence has been shown that on St Lawrence Island, Alaska, a 4-weekly dog-dosing programme reduced the infection pressure from *E. multilocularis* markedly between domestic dogs and rodents (43). This should reduce risks of transmission to humans. Further research may show that the benefits from treating this small group of dogs permanently far outweigh the costs of surgical intervention (Chapter 6.2.).

6.1.2.6. Conclusions

Sufficient experience has now been obtained to conclude that it is possible for endemic countries within their own scientific resources to:

a) identify the health problem caused by CE and its distribution within the country and quantify transmission dynamics in animals
b) compare the control options available and where appropriate predict the outcome and estimate the time needed to reach the consolidation phase
c) implement the research needed to identify the benefits and costs for control.

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6.1.3. Public health education and training in control programmes

P. Parodi, A. Mantovani and A. Seimenis

Summary

Health education (HE) is a basic component of any programme for control of Echinococcus granulosus *and cystic echinococcosis (CE) and should be closely linked to and co-ordinated with all phases of the campaign. Health education requires the motivation and participation of various population groups that are described. The educational material should address local problems in order to be effective and have the needed impact on governmental officials, managers, farmers, health professionals, etc. Health education has to take into consideration the beliefs, perceptions, behaviours, expectations and needs of the people; it should not be a passive, but a dynamic procedure, adjusted to the changing demands and progress of the control campaign. Educational materials include audio-visual aids (video films, television programmes), posters, pamphlets, text books, and others. Educational programmes at schools and personal visits of dog owners, farmers and other involved groups are of special significance. Continuing evaluation of the impact and the limitations of HE should be undertaken and modifications should be made as and when indicated.*

6.1.3.1. General aspects

Definition, goals and types of activities

Heath education (HE) is a basic tool in veterinary public health, and in particular in the prevention and control of zoonoses, such as CE. Health education has been defined as an 'educational process aimed at making the public, both as individuals and groups, responsible for the safeguarding their own and other people's health' (9). It is a frontier discipline between education and health, and relies on multi-disciplinary activities in that skills in health sciences, teaching and communication are requested. The general goals of HE are the prevention of diseases and the maintenance of health.

The main objectives of HE are to enable people:

a) to define their own problems and needs

b) to understand what they can do about these problems with their own resources combined with outside support

c) to decide on the most appropriate actions to be taken in order to promote healthy living and community well-being.

Accordingly, HE embraces all activities related to information, education and training (general and professional training).

The term HE includes at least three types of activities that are in no sense mutually exclusive, but tend to overlap and be dependent on one another:

a) Information, i.e. the transfer of knowledge from the 'expert' to the target group. This activity is generally used to call attention to given items before initiation of control programmes, implying that the community would actively participate.

b) Health education *sensu strictu* involves all those target groups not professionally concerned with the subject (e.g. public at large and school-children). The ultimate goal is a conscious and stable modification of behaviour when facing health problems.

c) Occupational training intended for persons who should apply health-oriented rules to their activities (e.g. farmers and butchers).

Participation and methodology

In order to achieve the goal of control and prevention of a disease, the participation of the community is required not only as a support to health services, but especially for assessing health priorities and for distributing available resources.

Participation may be required at different levels, such as:

a) voluntary participation in programmes for disease control and prevention

b) expression of the population's own interests and definition of priorities

c) representation of the population's interests in health policies.

The participation of various local groups of the population is important, such as:

a) medical and veterinary services

b) health committees and community health workers

c) religious bodies

d) school and adult education groups

e) police or military units.

Health education is a relevant component of any control programme of CE and should not only be aimed at specific measures but also at improving self-responsibility regarding individual and community health. It must be included into programmes by previous assessment of major objectives, target categories, restraints and resources, evaluation systems, etc.

Schematically, HE methodology consists of four steps:

a) cognition (analysis of problems and solutions)

b) planning (design of appropriate solutions)
c) operation (action and adoption of suitable behaviour)
d) evaluation (assessment of the impact and of the results of the intervention).

6.1.3.2. The general impact of health education in control of cystic echinococcosis

As mentioned above, HE is a basic component in control of CE. Its significance has already been stressed in the WHO 'Guidelines for surveillance, prevention and control of echinococcosis/hydatidosis' published in 1984 (17) and in many other publications (1, 14). Health education is essential because effective control relies on the active co-operation of population categories, such as veterinary and medical health personnel, dog owners, farmers, pastoralists, butchers, abattoir workers, and persons responsible for disposal of animal carcass. Health education should also include temporary workers, who are rarely aware of local health problems.

A control programme for CE at national or local levels calls for political decision and commitment. The actual epidemiological and socio-economic impact of the disease has to be brought out clearly to alert the community for the need of control.

Several options for control of CE have been outlined in Chapter 6.1. Health education is an integral part of all options of control programmes. In the 'horizontal approach' of control HE has to play a dominant role. This approach may be applied in two particular circumstances:

a) If control of CE relies on changes that occur in farming and slaughtering procedures and/or in the social situation in such a way that they interfere with the life-cycle of *E. granulosus* in endemic areas.
b) When the activities in a CE control programme are based on individual components (e.g. control of canine population and dog feeding, inspection and destruction of infected offal, etc.) and there is a need for co-ordinating the individual activities.

6.1.3.3. The role of health education in various phases of a control programme

In the planning phase and in early stages of a programme, HE should aim at gaining public support, in order to convince decision makers of the magnitude of the problem.

During the attack phase, it is essential that HE continues to support various measures taken for control. For example, with regard to the prevention of dogs having access to raw offal, HE should change the attitude and behaviour of people so that proper disposal of offal and safe feeding of dogs occurs. This objective is fundamental, but requires enormous commitment. When reducing dog population and launching mass treatment programmes, educational efforts must aim at maintaining the active co-operation of dog owners, e.g. in periodic diagnostic examinations of their animals and anthelmintic treatment of infected dogs. In supporting control programmes, cultural and religious traditions, habits and customs, as well as attitudes such as those induced by poverty and protein hunger should be taken into account. During the consolidation and maintenance phases of the programme, it may be necessary to introduce strict regulations or laws to deal with residual infection and habitual defaulters. In this case, educational programmes will greatly assist in the enforcement of the legislation.

6.1.3.4. Examples of the role of health education in control programmes

Italy

Veterinary services in Italy belong to the Health Administration. Health education has been an institutional task since 1978, when it was included in the reform of National Health Services. Since then, considerable experience has accumulated, including the pilot programme of CE control in Abruzzo in the early 1980s (8).

Another important programme for CE control was initiated in Sardinia in collaboration with the Istituto Zooprofilattico Sperimentale of Sardinia (2, 3, 12). This programme was based on experiences gained from other countries and adapted to the particular conditions of the island. It involved the following approaches:

a) health education
b) dog population control
c) slaughtering surveillance.

Health education was considered as a support to other activities, and effective participation of the population was sought in order to change improper behaviours related to man-dog relationships and home slaughtering practices. Mass media were intensively used with easy-to-understand messages. Radio, television, newspapers and leaflets were employed to convey information to the general public and to specific population categories such farmers, butchers, hunters, etc. Workers were instructed through the continuous presence and advice of veterinarians at the work places and at meetings. Great efforts were undertaken to stimulate farmers' responsibility for correct disposal of parasite-infected offal from home-slaughtering, for reporting of stray dogs, for support of dog population control and other measures.

Health education programmes were especially addressed to schools in order to strengthen the information platform 'school-family' and to present clear concepts in school-age groups. The purpose was to prevent children from acquiring incorrect habits from the adults (e.g. feeding dogs with infected offal). Teaching aids were adapted to individual age groups. An easy-to-remember poster was prepared for young children, in which they could see the relationships between children and the environment, with impressive suggestions for fundamental hygienic precautions. Another poster was produced for elementary and secondary schools depicting the life-cycle of *E. granulosus*, the modes of spreading of the infection and the measures of control and prevention. Furthermore, team-games were introduced to offer opportunities for play-simulated learning ('a game for understanding').

Spain

In Castilla y León, the HE campaign was based on continuing, inter-professional collaboration involving health personnel and other professional skills (1). The community co-operation was assured by requesting help from opinion leaders, parents of children at risk, people directly affected by losses due to CE, and persons operated on CE or waiting for surgery.

Health education was preferentially addressed to various populations groups, including butchers and slaughterhouse workers, pastoralists and farmers, health personnel, authorities, teachers and the general public. The activities were planned by territorial offices. Programme evaluation was based on the assessment of the level of knowledge reached by each group. Questionnaires were submitted to food-workers, farmers/pastoralists, general public and the school-age population.

Cyprus

In Cyprus, an innovative method of HE was used consisting of house-to-house visits during which issues of CE control were discussed with the families, in particular with the mothers. Information was given on the seriousness of the disease, details of the control programme, and precautions to be taken to avoid an infection. Other methods were also used such as personal visits to farmers, and teaching on echinococcosis in schools. Opportunities for information were also offered at agricultural exhibitions, school shows, and public events. Experiences of the successful control programme in Cyprus were published by Polydorou (11).

Other countries

Broad experience in HE as part of echinococcosis control has been accumulated in various other countries and regions of the world, for example Australia and New Zealand (6), the People's Republic of China (4), Argentina (5, 7), Brazil (13), Chile (16) and Uruguay (10, 15). Reports from the various regions underline the need for specific adaptation of educational approaches to regional and local conditions.

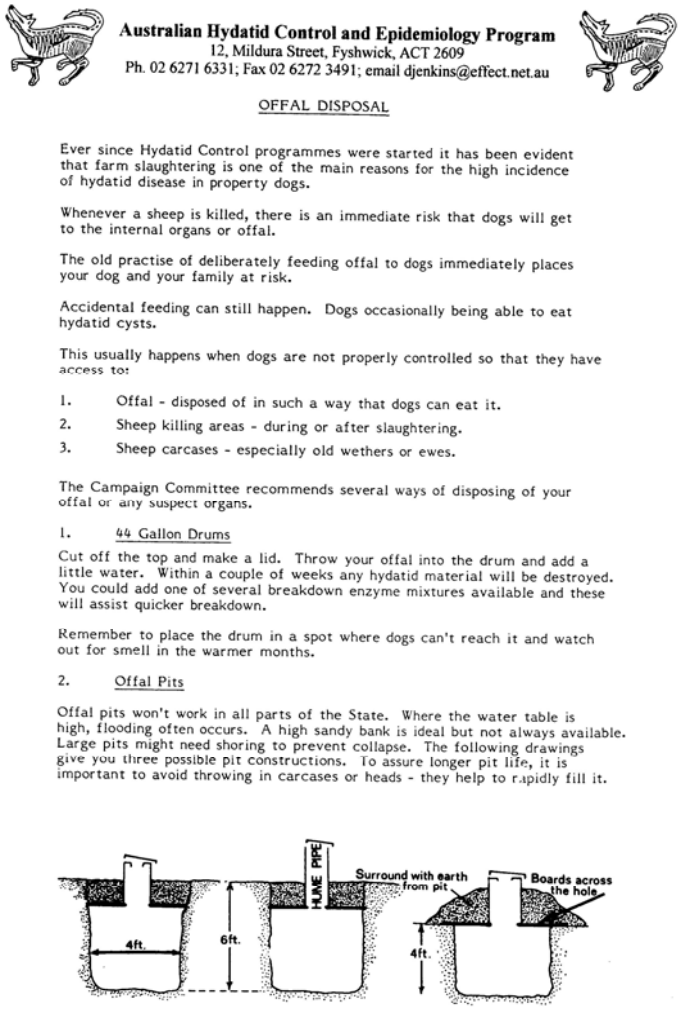
Health education materials

An excellent review on HE materials was published by Ding and Lui (4). This article is recommended for further reading.

The WHO/FAO Collaborating Centre for Research and training in Veterinary Public Health in Rome, has collected material on HE, both as a general topic and as specific problems. All this material was organised into a permanent mobile exhibition, entitled 'The instruments of information. Information, material and teaching aids in veterinary public health'. The exhibition is divided into 8 sections, one of which is devoted to *E. granulosus* and CE. The boards of the exhibition reproduce booklets, posters, folders and other informative educational material, which was used in HE programmes implemented in Cyprus, Italy, Spain and other countries. Similar activities were developed in other countries.

An example for the extend of efforts needed for HE activities in CE control campaigns was published from Brazil, where the state of Rio Grande do Sul is the most affected area (13). The following educational materials have been prepared: 250,000 flyers explaining the life-cycle of *E. granulosus* and control options, 50,000 display cards, each 30,000 technical charts and posters, 5,000 stamps, 3,000 serial albums, 2,000 copies of the pamphlet '*Programa Estadual de Controle de Hidatidose*', 230 audio tapes for radio, 150 educational video tapes, 45 sets of 75 slides each, and 2,000 charts showing how to use the educational material.

Figures 6.1.3.1. and 6.1.3.2. provide examples of educational material which is used in Australia.



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OFFAL DISPOSAL

Ever since Hydatid Control programmes were started it has been evident that farm slaughtering is one of the main reasons for the high incidence of hydatid disease in property dogs.

Whenever a sheep is killed, there is an immediate risk that dogs will get to the internal organs or offal.

The old practise of deliberately feeding offal to dogs immediately places your dog and your family at risk.

Accidental feeding can still happen. Dogs occasionally being able to eat hydatid cysts.

This usually happens when dogs are not properly controlled so that they have access to:

1. Offal - disposed of in such a way that dogs can eat it.
2. Sheep killing areas - during or after slaughtering.
3. Sheep carcases - especially old wethers or ewes.

The Campaign Committee recommends several ways of disposing of your offal or any suspect organs.

1. 44 Gallon Drums

Cut off the top and make a lid. Throw your offal into the drum and add a little water. Within a couple of weeks any hydatid material will be destroyed. You could add one of several breakdown enzyme mixtures available and these will assist quicker breakdown.

Remember to place the drum in a spot where dogs can't reach it and watch out for smell in the warmer months.

2. Offal Pits

Offal pits won't work in all parts of the State. Where the water table is high, flooding often occurs. A high sandy bank is ideal but not always available. Large pits might need shoring to prevent collapse. The following drawings give you three possible pit constructions. To assure longer pit life, it is important to avoid throwing in carcases or heads - they help to rapidly fill it.

Fig. 6.1.3.1.
Example of educational material: offal disposal
Courtesy: D.J. Jenkins, Australian Hydatid Control and Epidemiology Programme

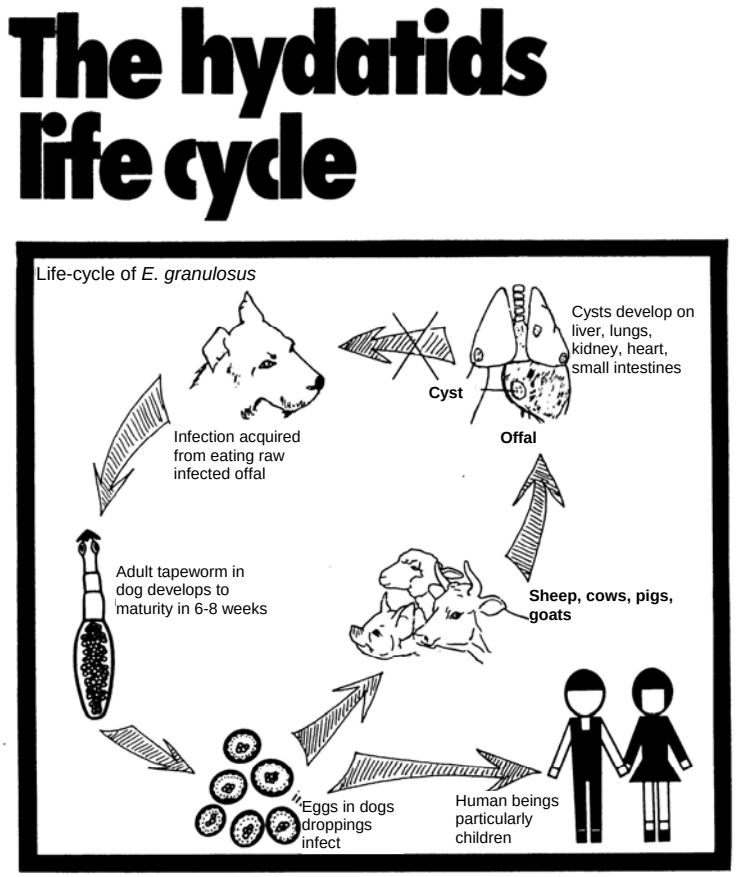

Fig. 6.1.3.2.
Example of educational material: the hydatids life-cycle
Courtesy: D.J. Jenkins, Australian Hydatid Control and Epidemiology Programme

Several videos of the life-cycle of *E. granulosus* and human cystic echinococcosis are available, for example, 'The travelling parasite' from the Australian Hydatid Control & Epidemiology Program, 1996.

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6.1.4. Socio-economic impact of the *Echinococcus granulosus* infection

G. Battelli

Summary

The socio-economic impact of cystic echinococcosis (CE), caused by Echinococcus granulosus, *in man and livestock and the costs of control programmes are reviewed. Human CE is considered both in terms of medical expenses and of social damage. In livestock the costs of CE are mainly analysed with regard to lowered production and to condemned viscera. The following costs of control are discussed: education, dog control and treatment, detection and destruction of infected viscera of livestock, diagnosis and therapy of CE in humans, and costs of programme administration and evaluation. Examples of some important costs are given. Many consequences in man and livestock are difficult to evaluate from an economic point of view, because some basic data are difficult to obtain in many countries. However, requests for funding of surveillance and control should be based on a realistic estimation of the socio-economic impact of the disease in the involved area.*

6.1.4.1. General aspects

Cystic echinococcosis (CE) in humans and livestock is an important public health and economic problem, especially in the Mediterranean Region, Latin America, Africa south of Sahara, and in other areas with high prevalence of the infection (Chapter 4). *Echinococcus multilocularis*, *E. vogeli* and *E. oligarthrus* will not be considered in this chapter. Socio-economic consequences are related to both human and livestock infections and to the costs of control programmes (3, 4, 6, 11, 15).

6.1.4.2. Socio-economic consequences in humans

In humans, CE may have various consequences, including the following:

a) cost for diagnosis of the infection
b) medical and surgical fees and costs of hospitalisation, nursing and drugs
c) loss of working days or 'production'
d) cost of travel to seek treatment for both patient and family members
e) mortality (potential years of life lost)

f) suffering and social consequences of disability

g) abandonment of farming or agricultural activities by affected or at-risk persons.

It should be noted that some of the above consequences are difficult to evaluate from an economic point of view and others, such as those under points (*f*) and (*g*), can be mainly or exclusively evaluated in social terms.

Among the costs associated with identification and treatment of CE in humans, those related to the duration of hospitalisation and convalescence represent the most important components. According to experiences from the Mediterranean region and in Latin America (5, 7, 8, 9, 14, 16), it has been calculated that the duration of hospitalisation varies from about 2 weeks to more than one month in case of surgery, and it is about 8 days for diagnosis and therapy alone. Where efficient services and modern techniques and interventions have been implemented for diagnosis, admission, surgery and treatment, the hospitalisation period has decreases by about 50% within a few years. Such an implementation leads also to a better control of the convalescent period (and to a decrease in the working days lost), which would normally last 3 to 4 weeks.

In Italy, at the main hospital of Bologna, an evaluation of hospitalisation costs was carried out using an analytical method of assessment (5). The 1995 mean specific cost of a surgical case of CE (mostly liver infection) was about US$14,000, and that of a clinical case about US$2,500. The mean number of days spent in hospital was 28 and 8 for surgical and clinical cases, respectively. The cost entities considered are shown in Table 6.1.4.1.

In Argentina, in the Rio Negro Province, the 1997 costs of surgical CE cases in two hospitals varied approximately between US$4,600 and US$6,000, and the mean costs per infected patient amounted to approximately US$4,500. The latter costs were about 31% lower than in 1980, mainly due to the introduction of chemotherapy with albendazole and of the PAIR technique (mean costs per patient approximately US$1,350 and US$2,000, respectively), which reduced the time of hospitalisation and medical care (9).

Table 6.1.4.1.
Costs of hospitalisation of human patients with cystic echinococcosis in Italy (1995 value) (5)

Cost entities	Surgical case		Clinical case	
	US$	Percentage of total costs	US$	Percentage of total costs
Days spent in hospital (net cost of stay)	10,277	73.4	1,569	61.7
Laboratory examinations	951	6.8	512	20.1
Imaging examinations	600	4.3	425	16.7
Drugs	70	0.5	25	1.0
Pharmaceutical material	9	0.1		
Anaesthesia	216	1.5		
Surgical facilities	538	3.8		
Blood and blood products	294	2.1		
Histological examinations	870	6.2		
Consultations	62	0.4	11	0.4
Surgical drapes	49	0.4		
Personnel of operating theatre	60	0.4		
Total	**13,996**	**100**	**2,542**	**100**

With regard to the costs of CE in humans, it should be remembered that in 1985, a simplified method of evaluation based on 'conventional parameters' was proposed in Spain (12, 15). This method introduces a parameter indicated as International Hydatidic Cost Rate (IHCR), defined as 'the results of what the echinococcosis/hydatidosis (= CE) takes away from the per capita income of each country by 100,000 persons'. This method is particularly useful, when it is not possible to evaluate a specific item. However, as stated by the proposers, it must be improved further and adapted to the special conditions of each country.

6.1.4.3. Economic consequences in livestock

In livestock, the following consequences of CE have to be considered:

a) reduced yield and quality of meat, milk and wool; reduced birth rate, etc.
b) delayed performance and growth
c) condemnation of organs, especially of liver and lung
d) costs for destruction of infected viscera and dead animals.

There are also other possible indirect detrimental consequences, such as ban on export of animals and their products if these are required to be free of CE.

In livestock, the importance of the above-mentioned losses will depend, to a large extent, on the characteristics of the animals or of the farming or livestock industry. For example, CE seems to cause lower economic losses in countries where sheep are primarily used for wool production, than in countries where they are primarily meat- or milk-producing. Quantification, standardised evaluation of such losses and exclusion of biasing factors in animal production are very difficult; therefore, the available data have to be interpreted with caution (Chapter 3).

Losses in sheep with CE have been reported (3, 13, 14, 16) to approximate 7%-10% of milk yield, 5%-20% of meat or total carcass weight, and 10%-40% of wool production. It has been estimated that birth weight of lambs from infected sheep may be 20%-30% less than that of lambs from healthy sheep. In Sardinia, Italy, with a population of 3 million dairy sheep, a loss in milk production was estimated to about US$13.7 million in 1982 (1, 2). This evaluation was based on a presumed decreased milk production of 7% in infected sheep and on a 80% prevalence of CE in the sheep population. This sum represented approximately 92% of the yearly losses of the whole sheep production and about 80% of the total losses in livestock productivity caused by echinococcosis. According to an evaluation in Italy (1980), the average loss (including loss of viscera) per CE-infected sheep was estimated to be 10% of the commercial value (10).

The quantification of losses caused by *Echinococcus*-infected viscera, is influenced by both the legislative rules of each country (e.g. compulsory condemnation and destruction) and the number of animals slaughtered under veterinary supervision. Depending on the utilisation of viscera and on the total or partial condemnation of infected organs, the order of magnitude of losses can vary. It should also be stressed that the costs of efficient destruction of condemned offal may be high, particularly as a starting investment to provide proper facilities (e.g. incinerators). In Extremadura, Spain, the costs of condemned viscera was estimated at approximately 2% of the total yearly costs of the *Echinococcus* infection, both in livestock and in humans (14).

6.1.4.4. Costs of control programmes

The awareness of the socio-economic impact of the disease has stimulated the implementation of control campaigns against CE in certain areas or countries. Of particular interest in this connection is a reliable cost estimation as a basis for selecting an adequate control strategy (Chapters 6.1.1. and 6.1.2.). Furthermore, it has to be determined from the beginning which costs should be paid by the public and which contributions may be obtained from private institutions or sponsors. The main costs of a control campaign are summarised in Table 6.1.4.2.

Table 6.1.4.2.
Costs entities for control programmes against *Echinococcus granulosus*

Education
Training of personnel (veterinarians, health operators, technicians, teachers, etc.)
Publications, pamphlets, posters, symposia, television and radio programmes, etc.
Dog control and treatment
Personnel
Structures (constructions, e.g. dog dosing sites, recurrent running costs)
Drugs and costs of dog dosing
Destruction of dog faeces
Disinfection
Detection and destruction of infected viscera
Provision of slaughter and incineration facilities, where not yet available
Improvement of existing structures
Personnel
Diagnosis and therapy of CE in humans
Surveillance by ultrasound examination and immunodiagnosis of the population, especially of categories at risk (Chapter 2)
Provision or improvement of diagnostic facilities
Costs of treatment (surgery, PAIR, chemotherapy) (Chapter 2)
Administration and evaluation of the programme
Personnel and equipment for administration
Expert committees (technical and administrative personnel)
Surveys in the territory involved
Publications or other means for the information (results obtained, epidemiological and economic analyses, etc.) of technicians and of the population

It should be noted that some of the expenses spent for echinococcosis control may simultaneously be beneficial to control programmes against other diseases (e.g. rabies and tapeworm infections).

According to information from Argentina, Rio Negro Province, the costs of a dog dosing programme in 1997 were US$37 per animal, including costs for dog testing with arecoline, drug distribution to dog owners and for praziquantel. Compared with 1980, a reduction of costs by US$16 per animal was achieved (9).

6.1.4.5. General recommendations

When evaluating the socio-economic consequences of the *E. granulosus* infection and the costs of a control programme, many parameters have to be considered, the majority of which are difficult to quantify in economic terms. The evaluation of the 'economic weight' of CE varies in the different countries. In man, it is related to the per capita income and social status. In animals, it is related to zootechnical economy and, in a number of cases, to the need for exportation, when the animal health regulations in the importing countries require the absence of infections with metacestodes of *E. granulosus* or *Taenia* species. Creditable efforts have been made for several years in order to assess 'losses' and 'costs', and to propose standardised evaluation methods. At present, however, no univocal methods have been accepted, also because they require data or information which are difficult to obtain in many countries or geographic areas.

Each country should define a minimal set of epidemiological, zootechnical, economic and social data to serve as a basis for the evaluation of the impact of the infection. This means that priority information should be

collected which may be converted into indicators, i.e. instruments capable of defining and measuring the epidemiological situation, the changes and their direction.

Furthermore, it should be remembered that, when submitting the requests for funding of surveillance and control to public administrators, an explication should be given as to 'why', 'where' and 'whom for' these activities must be undertaken. For this reason, an evaluation of socio-economic consequences of the disease is indispensable. Finally, it should be recognised that echinococcosis is a zoonosis whose successful control and the resulting reduction of the socio-economic impact demand continuous resources and activities in the long term, especially because it is often necessary to influence habits, customs, traditions, cultures and living and working environments, which cannot be changed in a short time.

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6.2. Control of *Echinococcus multilocularis*

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Summary

Control of Echinococcus multilocularis *is very difficult because the primary cycle is almost always sylvatic and complicated by a number of epidemiological factors. This chapter describes the presently available options for control of* E. multilocularis *in sylvatic and synanthropic cycles, and it outlines measures to reduce morbidity and mortality caused by alveolar echinococcosis (AE) in human populations.*

For the control of E. multilocularis *in sylvatic cycles treatment of foxes by praziquantel 'baits' is presently under evaluation but final results are not yet available.*

In synanthropic cycles dogs (or cats) may play a significant or a minor epidemiological role. In the first situation, mass-treatment of dogs over prolonged periods may be considered, whereas in the latter only treatment of those dogs and cats that have access to infected intermediate hosts (rodents) may be an option. The practical value of such measures remains to be evaluated.

Spread of E. multilocularis *by transfer of foxes or other definitive hosts that are reservoirs of the parasite, from endemic to non-endemic regions, should be prevented by enforcement of legal regulations and other measures, which are described.*

Measures to reduce morbidity/mortality of AE in humans by screening populations using immunodiagnostic techniques and ultrasound examination have been proven to be successful in some endemic areas. Early detection of cases significantly improves the prospects for cure of the infection.

Repeated serological screening of individuals exposed to an infection risk is recommended as a measure to prevent clinical AE. The use of highly sensitive and specific tests is necessary.

6.2.1. General aspects

In the WHO 'Guidelines for Surveillance, Prevention and Control of Echinococcosis/Hydatidosis' published in 1984 (33), it was emphasised that no attempts have been made to establish control programmes against *Echinococcus multilocularis*. It was recommended to treat all animals having access to intermediate hosts (rodents) monthly with praziquantel, to take effective measures to prevent pets from eating rodents and to practise better personal hygiene. Today, control of *E. multilocularis* is still very difficult because the primary cycle is almost always sylvatic (22), and transmission dynamics are complicated by a number of factors (Chapter 5.3.). However, some progress has been achieved in recent years. Basic principles of the presently available measures for controlling the parasite have been outlined in several reviews (5, 7, 9, 22). It has to be stressed that these measures are insufficient, and more research is required to achieve improvement.

In this chapter the following aspects of control will be discussed:

a) control of *E. multilocularis* in sylvatic cycles
b) control of *E. multilocularis* in synanthropic cycles
c) control of spreading of *E. multilocularis* by transfer of definitive hosts
d) measures in human populations to reduce morbidity and mortality caused by AE
e) education.

6.2.2. Control of *Echinococcus multilocularis* in sylvatic cycles

6.2.2.1. Elimination of final hosts

After translocation of foxes from the Kurile Islands to Rebun Island, *E. multilocularis* became endemic in Japan causing considerable morbidity and mortality in humans. Approximately 25 years later, *E. multilocularis*

was eradicated from Rebun Island by eliminating the definitive hosts of the parasite. Between 1950 and 1955, more than 2,000 foxes and 3,000 dogs were captured and killed. Without adequate numbers of definitive hosts, the parasite could not perpetuate its life-cycle (29, 36). This is the only known instance in which *E. multilocularis* has been eradicated from an area where it was previously endemic. It is important to note that the problem in Japan has been solved only on Rebun Island; in Hokkaido, the parasite persists in an endemic situation.

Elimination of definitive hosts of *E. multilocularis* cannot be carried out in larger areas; ethical and ecological reasons prohibit the large-scale application of this measure.

6.2.2.2. Anthelmintic treatment of definitive hosts

One approach to controlling *E. multilocularis* in populations of wild foxes (*Vulpes vulpes)* is the delivery of 'baits' containing praziquantel (25). This approach is under evaluation in endemic regions of southern and northern Germany (19, 26, 31).

In these campaigns, praziquantel (Droncit®) in granular form was embedded in a 'bait' matrix containing meat and fish extracts (28), and then formed into pellets; each pellet contained 50 mg of the drug. The baits were stored at –20°C until use. In southern Germany (26), baits were delivered by hand or aircraft in an area of 566 km² at a density of 15 to 20 baits per km². The baits were very well accepted (>90% had disappeared after 4 days), and the treatment was well tolerated. The prevalence of *E. multilocularis* was evaluated by necropsy and parasitological examination of 28 foxes before onset of the study and of 22 to 453 (total 1,450) foxes during the various campaigns. After 6 baiting campaigns over a period of 14 months (December 1989 to February 1991), the average prevalence of *E. multilocularis* in foxes had declined from 32% to 4% (26). Within the same endemic region, consecutive control campaigns are ongoing since 1995, covering an area of 3,400 km². Reduction of *E. multilocularis* in foxes was rapid (e.g. from 64% to 37%) after two baiting campaigns (19), but final results concerning the necessary frequency of bait distribution, the cost-effectiveness, and the development of prevalence rates after discontinuation of control are not yet available.

In control of rabies, the wide distribution of oral vaccines contained in specially prepared baits for target animals has been very successful. According to a WHO report from 1996 (35), more than 61 million vaccine baits have been distributed in 15 European countries, in Canada, and in Texas, USA, since 1978, when the control campaign first began in Switzerland. It was determined that the spread of rabies is interrupted if 50% to 80% of all foxes in the population are immunised (32).

In contrast, after baiting foxes with praziquantel and the elimination of the parasites, re-infection is likely to occur, since the previous *E. multilocularis* infection apparently does not provoke strong immunity (Chapters 5.3. and 6.2.3.). There are several open questions, for example on the optimal intervals and the necessary duration of baiting, the influence of such campaigns on disease transmission to humans, the cost-benefit-ratio, etc. Therefore, a judgement on this approach to control of *E. multilocularis* will only be possible when the final results of the studies are available.

In France, a mathematical model was used to estimate the control effort required to eradicate *E. multilocularis*. It was concluded that in areas of low prevalence (>50%), praziquantel baiting could succeed in eradicating *E. multilocularis* in fox populations (18). However, the validity of this model has not yet been demonstrated by data from field trials.

6.2.3. Control of *Echinococcus multilocularis* in synanthropic cycles

Epidemiological aspects

In endemic areas, domestic dogs and cats may become infected with *E. multilocularis* by preying on rodents harbouring the metacestode stage of the parasite (Chapter 5.3.). However, the significance of these definitive hosts to local environmental contamination with *E. multilocularis* eggs and potential human exposure may vary in different epidemiological situations.

Situation A

In a hyperendemic situation on St Lawrence Island, Alaska, domestic dogs had access to infected rodents and were regarded as the primary source of infection to humans (17). In an earlier study (1951) in one of the villages, 12% of the necropsied dogs were infected with *E. multilocularis*. More recent studies were not carried out in dogs, but in 1980-1983 high infection rates in voles (22% to 35%) trapped in villages of the island indicated that intense transmission occurred (17, 22). High prevalences of *E. multilocularis* in dogs were also reported from two provinces in the People's Republic of China, where 10% (6/58) of the animals were infected in Gansu (3) and 14% (4/28) in Sichuan (16). Recently, a 'hot-spot' was identified in the Canton of Fribourg, situated in the endemic area of Switzerland (see below), with a high prevalence of *E. multilocularis* in foxes (1993/1994: 47% [n: 73] and 56% [n: 23], respectively), in rodents (*Arvicola terrestris*) (1993: 39%; 11/28) (12, 27), and in dogs (12%, 5/41) (13).

Situation B

As far as it is known today, in endemic areas with an operating sylvatic cycle, the average prevalence rates of *E. multilocularis* in populations of dogs and cats are normally low, except in highly endemic foci (see above). It has to be stressed, however, that information on infection rates of dog and cat populations is scanty; until recently, the parasite could only reliably be diagnosed by parasitological examination at necropsy, and this was a limiting factor for larger surveys.

In a recent study carried out in an endemic area of eastern Switzerland, where approximately 33% of the foxes are infected with *E. multilocularis*, 0.30% of 660 dogs and 0.38% of 263 cats were identified as carriers of the parasite by coproantigen detection in combination with PCR or necropsy (techniques in Chapter 3) (4) (Table 6.2.1.).

Table 6.2.1.
Estimation of the epidemiological significance of various definitive hosts as carriers of *Echinococcus multilocularis* in the Canton of Zurich, Switzerland
(modified after 8, including data from 1)

Animal species	Prevalence of *E. multilocularis*		Population size (1992)	*E. multilocularis* carriers	
	Number of animals examined	Percentage infected		Total population	Percentage carriers in relation to infected foxes
Red fox	1,253	33.3	4,700[a]	1,565	–
Dog	661	0.30	48,400[b]	145	9%
Cat	263	0.38	145,200[b]	552	35%

a) *Source:* Kantonales Fischerei -und Jagdinspektorat Zurich
b) *Source:* Pet-ownership survey, 1995, Effems, Zug/CH

Considering the infection rates and population sizes of various definitive hosts, it has been shown in a model calculation for the Canton of Zurich that infected foxes are the largest group of *E. multilocularis* carriers. Infected dogs and cats represent 9% and 35%, respectively, of the group of carriers (Table 6.2.1.) (4, 8). However, in this model calculation several factors could not be included, notably the anticipated lower susceptibility of cats for *E. multilocularis,* the differences in the infection risk of dogs and cats related to their feeding habits, and the potential differences in the reproductive capacity of the parasite in various definitive hosts (Chapter 5.3.).

Control of *Echinococcus multilocularis* in dogs (and cats) in a hyperendemic situation

In a hyperendemic situation, control of *E. multilocularis* in dogs may be considered. In a village on St Lawrence Island of Alaska, all dogs were treated with praziquantel (5 mg/kg bw) at monthly intervals (17). This was effective in reducing environmental egg-contamination as evidenced by an average 83% reduction in infection prevalence in locally captured voles during the trial; the prevalence declined from an average of 29% at the beginning of the campaign to less than 5% at the end after 10 years (17). However, it was noticed that the

infection rate rebounded rapidly toward pre-campaign levels when regular treatment was discontinued (Wilson, cited in 22). Resumption of high rates of transmission was presumably related to the fact that highly susceptible dogs continued to have access to infected voles from a nearby sylvatic cycle with arctic foxes as definitive hosts (17).

Similar control measures may be considered for other hyperendemic areas or foci. For example, in a 'hot-spot' focus in Switzerland regular praziquantel treatments in intervals of 28 days of all local dogs and cats, that have access to infected rodents, has been discussed (27). It is not yet known, however, whether such a measure can reduce the infection risk for humans who continue to live in an environment contaminated by foxes with *E. multilocularis* eggs.

Education of the population about the life-cycle of the parasite, the danger of an infection, and preventive measures should form an important part of a control programme.

Control of *Echinococcus multilocularis* in dogs and cats in an endemic situation

In an endemic situation with low prevalence of *E. multilocularis* in populations of dogs and cats, control is especially difficult. Mass-treatment may not be cost-effective, and the epidemiological and preventive effects of such a strategy are uncertain and have not yet been evaluated. Furthermore, the sources of re-infection persist in the sylvatic cycle.

In these circumstances, regular treatments of only those dogs and cats that are preying on rodents with praziquantel (5 mg/kg bw) at intervals of 4 weeks may be considered. These treatments should be performed throughout the period in which activity of rodents is to be expected. Where coproantigen tests are available (Chapter 3), the treatment programme could be focused on high risk groups of animals previously identified by large-scale coproantigen testing. The potential epidemiological effects of such measures have not yet been evaluated.

6.2.4. Control of spreading of *Echinococcus multilocularis* during transfer of definitive hosts

Spreading of *Echinococcus multilocularis*

There is a real risk of spreading *E. multilocularis* by transfer of definitive hosts, that are carriers of the parasite, from endemic to non-endemic regions.

Between 1924 and 1926, 12 pairs of red foxes were imported to Rebun Island/Japan from the Kurile Islands for fur production (30). From Rebun Island, the parasite spread to Hokkaido Island, where an endemic area covered about 8% of the territory in 1965-1981. In 1991, *E. multilocularis* had expanded to 90% of Hokkaido, and apparently further south to Honshu Island (14, 29). In consequence, in a period of 25 years, approximately 250 human cases of AE were diagnosed on Hokkaido, and 60 cases on Honshu Island (17 regarded as autochthonous) (23).

In the USA, in 1989 an illegal shipment of red foxes and coyotes originating from Indiana and Ohio was confiscated in South Carolina. *Echinococcus multilocularis* was identified in 3 of 44 red foxes that were to be released into fox-hunting enclosures in the south-eastern states (22). There is no evidence that *E. multilocularis* has become endemic yet in these states but if the practice of translocation continues it almost certainly will be (22).

Recommended control measures

Legal regulations should be introduced and enforced in order to prevent the uncontrolled national and international transfer of definitive hosts of *E. multilocularis*. If transfer should be permitted, similar measures should be taken as already recommended for the prevention of rabies spreading (34). With regard to *E. multilocularis* all potential definitive hosts in national or international transit from endemic to non-endemic regions should:

a) be treated before shipment in a quarantine unit on two consecutive days with therapeutic doses of praziquantel (Chapter 3)

b) have valid certificates of the cestode-free status of the state/country of origin signed by the veterinary authorities

c) have an import license (specifying details of transport requirements) by the veterinary authorities in the state/country of destination

d) be transported in separate sealed units so that removal of the animals breaks the seals.

Although a single prazinquantel treatment is normally 100% effective, a second treatment is strongly recommended in order to minimise the risk of residual worm burdens which may be due to various factors, including incomplete drug application, drug elimination by vomiting, etc.

In view of the high risk posed by the translocation of definitive hosts these measures would be justified.

6.2.5. Measures in human populations to reduce morbidity and mortality caused by alveolar echinococcosis

If the *E. multilocularis* infection in humans is detected in an early stage, the prospects for complete cure by surgical resection of liver lesions are favourable (21). Various techniques have been used for screening of human populations with the aim of early detection of the infection and of reducing morbidity/mortality caused by AE.

Methods for screening of populations

Screening of populations for AE have been carried out in various countries, such as Alaska (15), Austria (1), the People's Republic of China (3, 10), France (2, 10), Germany (24), Japan (21, 30) and Switzerland (11).

In several of these studies, ELISAs alone or in combination with Western blot analysis for serum antibodies have been used for primary screening. In view of the low prevalence of the infection in most of the endemic areas (Chapter 4), it is essential to use only test systems which are highly sensitive and specific (Chapter 5.3.). For secondary screening, US examination of the liver is the method of choice. In suspected cases, further diagnostic examinations (for example CT and plain abdominal X-ray) might be necessary for a final diagnosis (Chapter 2). In other studies (3), US examination has been used for primary screening.

It is difficult to detect liver lesions below 10 mm in diameter either by US examination or by immunodiagnosis. In a Japanese study, 64% of liver lesions detected by US were small, ranging from 8 mm to 50 mm in diameter (30). Cases with lesions below 10 mm in diameter were always seronegative (K. Suzuki and N. Sato, personal communication, 1998).

An example for mass screening of populations in Japan

Special experience with mass-screening programmes exist in Japan (21, 30). In Hokkaido, with a population of 5.8 million people, 715,841 persons received serological primary screening during 1984 to 1993, with annual ranges between 26,356 and 96,120 persons (30). Overall 5,159 persons had a positive ELISA reaction (0.72% of the total) (30) 1,272 persons underwent secondary US screening (0.18% of the total) (K. Suzuki and N. Sato, personal communication, 1998), and finally 60 persons (0.008% of total) were detected with asymptomatic AE (30). Based on a population of 5.8 million inhabitants, this figure corresponds to an average annual incidence rate of 0.10 AE cases per 100,000 inhabitants. It has to be stressed that in the group of screened persons, the rate of complete surgical excision of liver lesions was 100%. In contrast, the resectability rate was only 20% in non-screened patients in whom with AE was detected at a later stage (29). According to a recent report, all patients, who underwent complete resection of AE lesions, did not show recurrence, and survived more than 10 years (20).

Problems of mass-screening programmes

There are several problems related to mass-screening programmes. In most countries, screening programmes have to be approved by an ethical committee, and consent of the persons to be screened has to be obtained. To achieve this, an intensive information campaign and the fulfilment of certain legal and ethical requirements are necessary (more details on ethical aspects: Chapter 2). Furthermore, many of the persons with a positive ELISA reaction will assume that they have a potentially lethal disease, and it is difficult to dispel these concerns even if in subsequent US and other examinations lesions of AE are not detected. If seropositivity persists, and nonspecific reactions can be reliably excluded by additional immunodiagnostic tests, such persons have to undergo further serological and US screening as long as a suspicion of an *E. multilocularis* infection exists. For cases with diagnosed AE, the infrastructure and financial resources for adequate treatment and follow-up have to be available (Chapter 2). Finally, in view of the low incidence of AE, it is difficult to convince health authorities and funding organisations that a prospective screening programme can be cost-effective.

Advantages of mass-screening programmes

The main advantage of mass-screening programmes is the perspective to reduce morbidity, suffering, and mortality caused by AE in a human population. The cost-benefit ratio of such a programme depends on many factors, such as prevalence of the infection, costs for primary and secondary screening, costs for treatment of AE cases, laboratory and hospital facilities and other costs.

Cost-benefit calculation

Data from Hokkaido, Japan, (30; K. Suzuki and N. Sato personal communication, 1998) are used here as an example for a cost-benefit estimate (Tables 6.2.2. and 6.2.3.). During the mass-screening programme of 1984-1993, a total of 60 human cases of AE was detected in an early curable phase of the infection. Therefore, the calculation compares the cost estimates for the mass-screening programme with the costs which would have been caused by 60 non-screened AE patients. According to these data, a mass-screening programme for the early detection of AE may well be cost-effective.

Table 6.2.2.
An example of cost estimates for a mass-screening programme in Hokkaido, Japan, 1984-1993*

Screening	Costs per patient (US$)	Number of people screened	Total costs (US$)
Blood sampling	0.80	715,841	572,673
Serological examination	4.00	715,841	2,863,364
Ultrasound examination	67.00	1,272	85,224
Treatment of AE patients in an early phase of infection	12,105.00	60	726,300
Total		–	**4,247,561**

* calculations based on 1998 prices and an exchange rate of US$1 = ¥150
Source: (30); K. Suzuki and N. Sato, personal communication, 1998

It should be noted that the costs for diagnosis and treatment vary between countries, and that they may be higher than shown in Tables 6.2.2. and 6.2.3. In a mass-screening programme in France, published in 1994 (2), the average costs for serological screening per subject were US$8.60 and per diagnosed case US$10,909. The annual costs of diagnosis, follow-up and treatment of patients with AE were US$11,148 in screened individuals and US$15,456 in patients with symptomatic AE.

Screening of individuals

Recommendations for screening of individuals exposed to an infection risk are described in Chapter 7. In principle, all persons who have been exposed to an infection risk (for example, owners of dogs or cats infected with *E. multilocularis*), or who are permanently at risk (fox hunters, laboratory personnel involved in necropsy of infected foxes, etc.), should receive serological screening as described in Chapter 7.

Table 6.2.3.
Example of cost estimates for treatment of alveolar echinococcosis in sixty non-screened patients in Hokkaido, Japan*

Treatment	Costs per patient (US$)	Number of people treated	Total costs (US$)
Large abdominal surgery	11,700.00	20	234,000
Chemotherapy and medical care			
5 years	10,000 × 5	10	500,000
10 years	10,000 × 10	50	5,000,000
Total		–	**5,734,000**

* calculations based on 1998 prices and an exchange rate of US$1 = ¥150
Source: K. Suzuki and N. Sato, personal communication, 1998

6.2.6. Education

Education is always an important part of control strategies. Similar to *E. granulosus* (Chapter 6.1.3.), education on *E. multilocularis* should include information on the parasite's life-cycle, ways of infection, infection risks, methods of prevention, etc. There are numerous ways how such information can be transmitted to the target population, e.g. through articles in the local print media, information booklets for distribution to hunters, farmers and other groups, or through information campaigns by health insurances. In some regions, a high level of awareness can be achieved by informed and responsible journalism, making costly government education programmes unnecessary. However, it has to be stressed that the risk of acquiring AE (which is low in many regions) has to be put into perspective in order to avoid unnecessary frightening or hysteria among the population.

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Chapter 7

Prevention of echinococcosis in humans and safety precautions

J. Eckert, B. Gottstein, D. Heath and F.-J. Liu

Summary

In view of the high potential pathogenicity of the Echinococcus *infection to humans safety precautions in laboratories and for field workers are of special importance. Heat remains the most reliable method for killing of* Echinococcus *eggs. They may also be inactivated by deep-freezing, but only at temperatures of –70°C to –80°C and minimum exposure times of 96 h and 48 h, respectively. The high cold resistance of the eggs of* E. multilocularis *is well documented. On the other hand, it is still unclear whether strains* E. granulosus *may differ in various regions with regard to cold resistance of their eggs. Chemical disinfection is difficult as most of the commercial disinfectants are ineffective against* Echinococcus *eggs. Of some value is sodium hypochlorite solution. Recommendations are given for disinfection of materials and objects contaminated with* Echinococcus *eggs, for decontamination of living-rooms and cars, and for inactivation of metacestode material. Furthermore, guidelines for precautions during treatment of dogs and cats infected with* E. multilocularis *and for prevention of cystic and alveolar echinococcosis in humans are presented.*

7.1. Safety precautions and disinfection

Safety precautions formed an important section in the 'Guidelines for Surveillance, Prevention and Control of Echinococcosis/Hydatidosis' published in 1984 (31) and are again emphasised in this publication.

7.1.1. Awareness of the problem

Persons at special risk and dangerous material

All personnel handling dogs, foxes and other carnivores known or suspected to be final hosts of *Echinococcus* species in endemic areas should be aware of the health risk both to themselves and to the general public (15, 31). This applies with special force to personnel involved in diagnostic work (necropsies of foxes, dogs, etc., faecal examination of carnivores) or in echinococcosis surveys and control programmes. In areas with endemic echinococcosis, they should be encouraged to regard all definitive hosts as potentially infected. Furthermore, they should always treat any faeces or other materials possibly contaminated with *Echinococcus* eggs under strict safety precautions. Safety precautions are also important in laboratory work and to some extend in clinical investigations (see below).

7.1.2. Sources and routes of infection

Primary echinococcosis in humans (Chapter 2) usually results from the ingestion of *Echinococcus* eggs. However, there is also evidence that the hatching and activation of embryos can occur in extra-intestinal sites (29). This raises the possibility that infection may result from the inhalation of eggs with subsequent development in the lungs. Experimental studies with sheep support this possibility (2). However, this has never been substantiated for natural infections. On the other hand, it may well be that eggs are inhaled, then swallowed and transported to the intestinal tract. Furthermore, secondary echinococcosis may possibly follow contamination of the conjunctiva with protoscoleces, but such cases have never been described.

Infection of humans with *Echinococcus* eggs may result from:

a) Handling infected definitive hosts, egg-containing faeces or egg-contaminated plants or soil followed by direct hand to mouth transfer. It has been shown that eggs of *Echinococcus* adhere to the coat of

dogs (25), particularly to the hairs around the anus, on the thighs, muzzles and on the paws (23). The same applies to dogs infected with *Taenia* species (5) and to foxes infected with *E. multilocularis* (unpublished findings).

b) Ingestion of vegetables, salads, uncooked fruits and other plants which have become contaminated directly with *Echinococcus* eggs. Foodstuffs or surfaces may possibly be secondarily contaminated with *Echinococcus* eggs via agents such as wind, birds, beetles and flies (see Chapter 5.1. for experimental evidence).

c) Drinking of water contaminated with *Echinococcus* eggs by faeces of infected carnivores is a potential route of infection. Recent studies in the People's Republic of China (Sichuan) have shown that people drinking water from small ditches which are accessible to animals have a higher risk to acquire CE than others consuming well-water (F.-J. Liu, personal communication, 1998) (Chapter 5.2.).

d) Inhalation of eggs in dust cannot be excluded as an infection route (15), but is apparently unimportant.

Reliable data on the actual importance of the various potential routes of infection are not available so far.

7.1.3. Resistance of *Echinococcus* eggs

Resistance to temperatures

Echinococcus eggs are highly resistant, and may remain infective for about one year in a suitable, moist environment at lower temperatures. For example, eggs of *E. multilocularis* remained viable for about 16 months at +4°C in water (30). It can be assumed that, because eggs survive at low temperatures, large numbers will accumulate during the cold season in sites where definitive hosts defecate, for example in yards where livestock live with their guard dogs. By the end of the winter, such environments must be loaded with *Echinococcus* eggs. On the other hand, desiccation and high temperatures are the two most important factors reducing the longevity of the eggs (7, 15, 16, 20, 30) (Table 7.1.).

Echinococcus eggs are killed by boiling water or dry heat. Eggs of *E. granulosus* are killed within 5 min at +60°C to +80°C and instantaneously at 100°C (Table 7.1.). *Taenia* eggs are killed by exposure to these temperatures (7), and this is also very likely for eggs of *E. multilocularis*. It has to be stressed, however, that the length of time for which contaminated materials should be heated will vary. For example, heat penetrates dog faeces slowly and such material should be boiled for at least 5 min to ensure killing of all eggs (15). Most sewage treatment processes (for example sedimentation) do not totally eliminate taeniid eggs (7). Based on experiments with *Ascaris* eggs, it can be assumed that the eggs of *Taenia* and *Echinococcus* are killed in sewage sludge and compost after exposure for at least 30 min to temperatures of +65°C or higher, generated by heating or fermentation processes (7).

On the other hand, eggs of both *E. granulosus* and *E. multilocularis* are highly resistant to freezing temperatures (Table 7.1.). Therefore, the temperatures of a household deep-freezer of −18°C to −20°C are insufficient for inactivating the eggs within a reasonable time.

However, very low temperatures of −70°C to −80°C are able to kill eggs of *E. granulosus* and *E. multilocularis* within 96 h or 48 h, respectively (Table 7.1.). The effective temperatures have to reach all parts of the contaminated material. For example, carcasses of foxes have to be frozen at −80°C for at least 4 days (routinely 7 days) in order to achieve thorough deep-freezing. The high cold resistance of the eggs of *E. multilocularis* is well documented. On the other hand, it is still unclear whether strains *E. granulosus* may differ in various regions with regard to cold resistance of their eggs.

Resistance to desiccation

The eggs of *Echinococcus* are sensitive to desiccation. At a relative humidity of 25% eggs of *E. granulosus* were killed within 4 days and at 0% within 1 day (21). Eggs of *E. multilocularis* lost infectivity to rodents after exposure at +25°C and a relative humidity (RH) of 27% for 2 days, at +43°C and 15% RH for 2 h, and at +45°C and 85%-95% RH for 3 h (30).

Table 7.1.
Resistance of *Echinococcus* eggs to heat and low temperatures

***Echinococcus* species**	**Temperature (°C)**	**Survived (+) or killed (–) after periods indicated**	**References**
E. granulosus	+45 to +55	5 min: +	Colli and Williams (3)
	+60 to +80	5 min: –	Colli and Williams (3)
	+100	1 min: –	Meymerian and Schwabe (24)
	–30	24 h: +	Colli and Williams (3)
	–50	24 h: +	Colli and Williams (3)
	–70	24 h: –	Colli and Williams (3)
E. multilocularis	–18	240 days: +	Veit *et al.* (30)
	–27	54 days: +	Schiller (28)
	–30	24 h: +	Colli and Williams (3)
	–50	24 h: +	Colli and Williams (3)
	–70	96 h: –	Blunt *et al.* (1)
	–80 to –83	48 h: –	Frank (14), Eckert *et al.* (12) Veit *et al.* (30)
	–196	20 h: –	Veit *et al.* (30)

Resistance to chemicals

Echinococcus and *Taenia* eggs are highly resistant to numerous chemicals (22). For example, eggs of *T. pisiformis* survived for 3 weeks in 10% formalin, eggs of *E. granulosus* retained viability in ethanol (50%, 70%, 95%) after 5 min to 60 min exposure (19, 24, 26), but only a few survived in glutaraldehyde (5% and 10%) (27). Most of the commercial disinfectants with activity against viruses and bacteria are ineffective against *Echinococcus* eggs (see below).

7.1.4. Ovicides and disinfection

Heat

Heat remains the most reliable and effective method for killing the eggs of *Echinococcus* and can be applied in various forms for disinfection (Table 7.2.).

Deep-freezing

Eggs of *E. multilocularis* or *E. granulosus* in carcasses or intestines of final hosts (foxes, dogs, etc.) infected with the parasite or in contaminated faecal material can be inactivated by deep-freezing at −70°C to −80°C for at least 4 or 2 days, respectively (Table 7.1.).

Irradiation

The infectivity of *E. granulosus* eggs after irradiation with doses of 10, 20 and 30 krad (= 100, 200 and 300 Gray) was diminished, but not lost (32). *Echinococcus multilocularis* eggs irradiated with a dose of 40 krad were apparently infective to rodents (as demonstrated by antibody detection), but metacestodes did not develop (30). For inactivation of taeniid eggs higher irradiation doses than 40 krad are apparently required. Indeed, after infection of rodents with eggs of *Taenia taeniaeformis* irradiated at 60 krad metacestodes did not develop (6).

Table 7.2.
Examples for disinfection of materials and objects contaminated with *Echinococcus* eggs, or of viable metacestode material

Type of material or object	Method of disinfection	Further usability of materials/ objects
Contamination with *Echinococcus* eggs		
Faecal samples	Boiling, 5 min	Examination for eggs and pro-glottids
	Steam sterilisation (autoclave)	Disposal
	Incineration	Disposal
	Deep-freezing at –80°C, 2 days	Examination for eggs and proglottids, coproantigen detection (CA), PCR
Whole carcasses or intestines of foxes, dogs, etc.	Steam sterilisation (autoclave)	Disposal
	Incineration	Disposal
	Deep-freezing at –80°C, at least 4 days	Examination for cestodes and other parasites, CA, PCR
Metal trays	Steam sterilisation (autoclave)	Re-utilisation
	NaOCl[a] solution (3.75%) for at least 1 h	Re-utilisation
Metal tables and other work surfaces	NaOCl[a] solution (3.75%) for at least 1 h	Re-utilisation
Metal instruments	Steam sterilisation (autoclave)	Re-utilisation
	NaOCl[a] solution (3.75%) for 5 min	Re-utilisation
Concrete floors[b]	Boiling water or hot water/steam mixtures	Re-utilisation
	NaOCl[a] solution (3.75% or higher) for at least 2 h-3 h	Re-utilisation
Clothing and other laundry	Steam sterilisation (autoclave)	Re-utilisation
	Washing in a washing-machine at +60°C, 1 h	Re-utilisation
Plastic sheets and disposable protective clothing	Steam sterilisation (autoclave)	Disposal
	Incineration	Disposal
Foodstuffs (vegetables, fruits, etc.) and water potentially contaminated	Heating, >60°C, at least 30 min	Consumption
***Echinococcus* protoscoleces or other viable metacestode material**		
Echinococcus metacestode material	Steam sterilisation (autoclave)	Disposal
	Incineration	Disposal
	4% formalin	Disposal or histological examination
	40% ethanol	Disposal or PCR, other examinations
	Deep-freezing, –20°C or –70°C, for at least 1-2 days	Antigen preparation, PCR, other examinations

a) sodium hypochlorite
b) alternatively the floor (or parts of it) of rooms in which potentially infective *Echinococcus* material is handled can be covered with plastic sheets which are disposed after use (see above)

Methods marked with are of variable efficacy
CA : coproantigen
PCR : polymerase chain reaction for detection of DNA

Chemical disinfection

Sodium hypochlorite solution (NaOCl) at a minimum concentration of 3.75% in water disrupts the embryophores of *Echinococcus* eggs and damages the majority of the oncospheres within a few minutes (4) (Table 7.2.). However, the effect of this disinfectant is variable and depends on the actual chlorine concentration, on temperature and the depth of penetration; it does not penetrate easily into organic materials. This may have been the reason that exposure of *E. multilocularis* eggs to a household disinfectant containing NaOCl with 'under 5% free chlorine' did not kill all eggs after 5 min (30). One should be aware that the concentration of active chlorine may decrease rapidly in a solution by evaporation. Therefore, high quality and fresh NaOCl solutions should be used. NAOCl solution is quite aggressive and has to be handled with care.

NaOCl solutions of about 1.3% to 4% are commercially available as bleaches or antifungal substances for use in households. It has been recommended (4) to use NaOCl solutions of 3.75% to wipe down work surfaces, soak instruments (3 min-5 min), plastic trays, glassware, etc. (time unlimited), both in the laboratory and in the field. In some laboratories, it is common practice to use NaOCl solutions at higher concentrations for longer exposure times for disinfecting work surfaces, floors, trays, plastic material, etc. (Table 7.2.).

In a recent study (30), the efficacy of 10 commercial disinfectants, containing phenol derivatives, aldehydes, ethanol phosphoric acid and other substances, was tested against *E. multilocularis* eggs. None of these disinfectants used in the recommended concentrations and application times killed the eggs as shown by *in vitro* activation of eggs and, in addition, peroral inoculation to rodents.

7.1.5. Decontamination of the environment

After purgation of dogs with arecoline, after drug treatment (Chapter 3) or maintenance of infected carnivores in confined areas, such as kennels, large numbers of infective *Echinococcus* eggs may contaminate the environment. Therefore, purgation or treatment should – whenever possible – be carried out in rooms or confined sites with a concrete floor, which can easily be cleaned and disinfected (Table 7.2.). Alternatively, sites of purgation/treatment may be covered with a plastic sheet, which can be incinerated. If soil has been contaminated with *Echinococcus* eggs, the surface layer (approximately 1 cm-2 cm) should be removed, and the ground thoroughly burned with a fire-lamp or a small flame-thrower. It should be considered that, although high temperatures are generated by these devices, decontamination may not be complete because of rapid decrease of temperature after contact of the flame with soil, especially moist soil (Chapter 7.1.11.).

7.1.6. Decontamination of living-rooms and cars

If dogs or cats with intestinal *Echinococcus* infection had access to living-rooms or cars, the question for an adequate method of disinfection may arise. There is no satisfactory solution, but thorough cleaning using a vacuum-cleaner, the focal application of dry heat (hair-drier, electrical heater, etc.) at sites preferably used by the animals, and heat-treatment of laundry may help to reduce the infection risk. During summer, cars can warm up to temperatures detrimental to *Echinococcus* eggs by exposing them for several hours to direct sunshine.

7.1.7. Inactivation of metacestode material

Protoscoleces of *E. granulosus* and *E. multilocularis* and germinal cells of metacestode cysts can be inactivated by heat, deep-freezing and some chemicals, such as ethanol (40% or higher concentration) or formalin (4%) (Table 7.2.). Deep-freezing (at −20°C or lower) normally kills protoscoleces of *E. granulosus* and *E. multilocularis* and also germinal cells. It should be noted, however, that cryopreservation of *E. multilocularis* tissue is possible if cryoprotectants and certain protocols for deep-freezing are used (11).

7.1.8. Precautions in laboratories

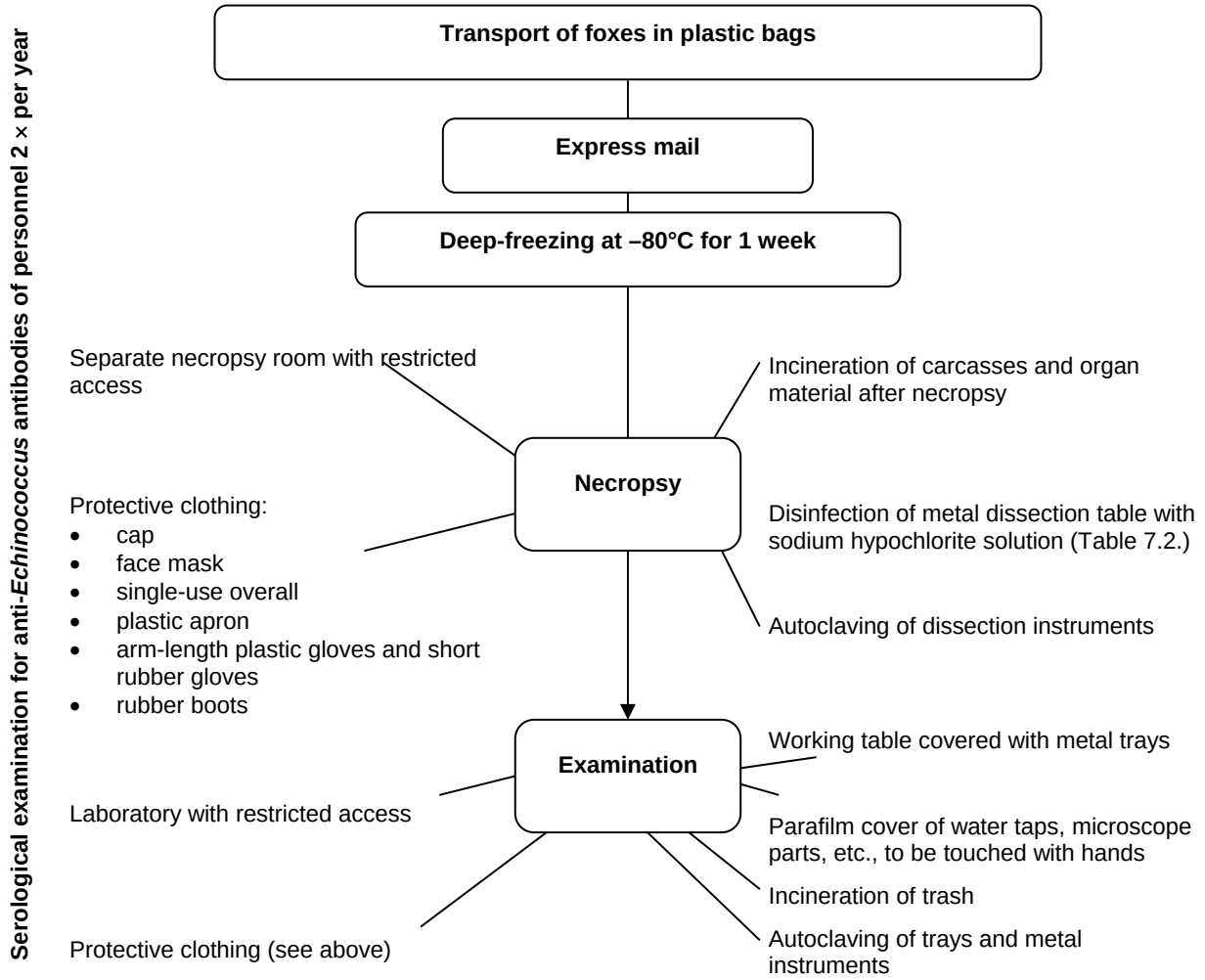
For work with *Echinococcus* infected definitive hosts, their intestines, faecal or other materials possibly containing infective *Echinococcus* eggs special laboratories or necropsy rooms should be used. In some countries, a biohazard safety level BL-3 is required. Such rooms should be marked as biohazard areas, they should be fully equipped with appropriate tables, wash-basin, containers, instruments, etc., and ideally with a sterile bench system; they should be adjacent to a changing room. Protective clothing, including overalls,

masks, caps, gloves and boots should be put on before entering the laboratory/necropsy room. Facilities should be available for decontamination of protective clothing; it should never be sent to a laundry without first being sterilised.

Infective material may be examined over sinks, in which an immersion heater can be placed to enable material to be boiled in water before it is passed into a sewerage system (15). In situations where the intestines of definitive hosts have to be examined, this should be done on metal trays or on disposable plastic foils. Following examination, the tray and all instruments should be sterilised, ideally by steam sterilisation in an autoclave. Plastic sheets, carcasses or organ material can be autoclaved or incinerated (Table 7.2.).

Faecal samples which are used for detection of coproantigen or DNA can be decontaminated prior to examination by deep-freezing at −80°C for at least 2 days.

Personnel involved in the examination of larval material from intermediate hosts should wear safety glasses. This will eliminate the possibility of protoscoleces being squirted into the eyes of the operator, with the risk of conjunctival echinococcosis. Remnants of metacestode material and infected intermediate hosts should preferably be heat sterilised or incinerated (Table 7.2.). For necropsy of foxes (and other final hosts) possibly infected with *E. multilocularis*, detailed safety precautions have been worked out (10, 12, 13) (Fig. 7.1.).



Source: Institute of Parasitology, University of Zurich/Switzerland and WHO Collaborating Centre for Parasitic Zoonoses

Fig. 7.1.
Safety precautions for parasitological examination of foxes or other definitive hosts infected with *Echinococcus multilocularis* (10)

7.1.9. Precautions in animal maintenance

Definitive hosts

If definitive hosts, experimentally infected with *Echinococcus* species, have to be maintained for research purposes special precautions are necessary. For some studies, for example drug testing, it may be sufficient to work only with prepatent infections and to finalise the experiment before excretion of infective eggs begins. For ethical reasons, maintenance of definitive hosts with patent infections should only be carried out in special isolation units under conditions in which transmission of *Echinococcus* eggs to humans is excluded. In addition, all persons working in such a unit should regularly receive screening for anti-E*chinococcus* antibodies (Chapter 7.2.).

Intermediate hosts

Maintenance of rodents infected with *Echinococcus* metacestodes by injection or surgical transplantation of metacestode material, such as protoscoleces, tissue homogenate or tissue fragments, does not require special safety precautions, but persons handling metacestode material should wear safety glasses, protective clothing and gloves. Care must be taken to inactivate metacestode material after the experiment (Chapter 7.1.7.).

After oral infection of rodents with *Echinococcus* eggs, there is a possibility of egg passage through the gastrointestinal tract and egg excretion for some days. Therefore, such animals should be maintained in the same cage for 3 to 4 days in a clean bench system preventing spreading of eggs to the environment. Thereafter, the animals should be transferred to a new cage and can be maintained under normal conditions. Isolation and handling of infective *Echinococcus* eggs requires strict biohazard safety precautions (Chapter 7.1.8.).

7.1.10. Precautions during handling of human patients with echinococcosis

Biological samples containing living protoscoleces and/or metacestode tissue of *Echinococcus* species could be infective to humans if accidentally injected to a person. Therefore, precautions are necessary, especially with regard to correct handling and disposal of needles, scalpel blades and glass ware. Spillage of such material to the face, for example during opening of a cyst, has to be avoided because of the hypothetical risk of a conjunctival infection with protoscoleces. Echinococcosis cannot be transmitted by serum samples of human patients or natural intermediate hosts.

7.1.11. Precautions for field workers

Ideally, personnel engaged in echinococcosis surveys should, at all times, wear appropriate protective clothing, i.e. impervious boots, gloves, coat or apron, and a face mask if necessary (for example during handling of faecal samples of *Echinococcus* infected carnivores). Regular screening (at least once per year) of the personnel for *Echinococcus* antibodies and the implementation of strict hygienic measures (for example thorough washing of hands after work with soap and water) are strongly recommended.

In situations in which faecal samples are being collected from potentially infected dogs following arecoline treatment, animals should be confined to a specific area. Subsequently, the ground from which faeces are collected should be thoroughly decontaminated by burning (Chapter 7.1.5.). Faeces should either be rendered safe in the field by being boiled, or by being packed in secure leak-proof containers for transport and later decontamination.

Animals necropsied in the field should be disposed according to the rules of the respective country (steam sterilisation, incineration, etc.). Intestines of potential definitive hosts should be ligated before removal from the carcass in order to prevent the dissemination of infective material. For the preservation of such material, fixative can first be injected into the litigated gut and the gut then immersed in fixative. It has to be stressed, however, that the normal fixatives (e.g. 4%-10% formalin or others) are not ovicidal (18, 25). Intestines fixed in formalin are not suitable for satisfactory recovery of *Echinococcus* species. A better method than formalin injection is deep-freezing of the intestines at −80°C for at least four days which kills *Echinococcus* eggs (see above).

7.1.12. Precautions during treatment of dogs (cats) infected with *Echinococcus multilocularis*

In view of the high pathogenicity of *E. multilocularis* to humans, special safety precautions should be observed if dogs (or cats), infected with *E. multilocularis*, have to be treated by application of an anthelmintic (8, 9) (Chapter 3).

a) Animals should only be treated under supervision of a veterinarian by informed and trained personnel.

b) Treatment should be performed under biohazard precautions in a veterinary clinic or under conditions where faecal material excreted after treatment can be collected and disinfected by heat or can be incinerated. Disinfection of kennels (for example by heat >80°C), the ground, equipment, etc. possibly contaminated with *E. multilocularis* should be feasible (Table 7.2.).

c) After treatment the animals should be shampooed and bathed in warm water in order to remove *Echinococcus* eggs adhering to the coat.

d) The result of treatment should be checked by repeated examination of faecal samples for taeniid eggs and for *Echinococcus*-specific coproantigen and/or DNA (Chapter 3).

e) Persons who had contact to a definitive host infected with *E. multilocularis*, should receive serological screening for serum antibodies using a highly specific and sensitive test (for example Em2 plus-ELISA, [17]) beginning about 4 weeks after suspected exposure and 6, 12 and 24 months later (Chapter 2).

f) These measures have to be adequately adapted to the situation of the individual case by the supervising veterinarian.

7.1.13. Precautions during purgation or treatment of dogs infected with *Echinococcus granulosus*

Several precautions have been described under Chapters 7.1.4. and 7.1.8. and in WHO Guidelines (31).

7.2. Prevention of cystic and alveolar echinococcosis in humans

- Prevention of CE

Control measures against the *E. granulosus* infection in dog populations are the basis for prevention of CE in humans. Details are described in Chapters 6.1. Some of the measures recommended in the prevention of AE (see below) are also applicable in prophylaxis against CE.

- Prevention of AE

Effective control of *E. multilocularis* in the sylvatic and the synathropic cycles is especially difficult (Chapter 6.2.). Therefore, some measures are recommended aiming at the reduction of the infection risk and of AE morbidity/mortality in humans. These measures refer to individuals or populations. For both groups education is an essential part of prevention (Chapter 6.1.3.).

Measures for individuals

The Swiss National Centre for Echinococcosis in Zurich has recommended the following measures for individuals to reduce the risk of AE (9):

a) In endemic areas where *E. multilocularis* is known to occur in foxes, wild berries, mushrooms, other plants or fruits from locations accessible to contamination with foxes' droppings should be thoroughly washed or better boiled before consumption. Deep-freezing at −18°C to −20°C does not kill eggs of *E. multilocularis* (they can only be killed at −70°C to −80°C) (Table 7.2.).

b) Foxes or other final hosts potentially infected with *E. multilocularis* should be handled with great care, always using disposable plastic gloves.

c) Special recommendations have been worked out for laboratory workers concerned with examinations of foxes for *E. multilocularis* (10, 12, 13) (Chapter 7.1.8., and Fig. 7.1.). In endemic areas similar measures may be applied to all laboratories in which necropsies of foxes are carried out, for example for rabies.

d) After agricultural or gardening work leading to contact with potentially egg-contaminated soil, hands should be thoroughly washed with soap and warm water (Chapter 7.1.11.).

e) Persons who have had single contact with infected final hosts or egg-contaminated materials (for example fox faeces), should receive serological screening for specific antibodies against *E. multilocularis* antigens at the following intervals after the suspected contact: 4 weeks, 6, 12 and 24 months. Highly sensitive and specific tests have to be employed for this purpose (Chapter 2). In unclear or doubtful cases US examination of the liver should be performed.

f) Individuals with repeated infection risk (for example fox hunters, laboratory personnel, etc.) should be serologically examined once or twice per year.

Measures for populations

In Japan and some other endemic areas, population-screening by serology and US examination of human populations has been successfully used for early detection of cases. This can reduce morbidity and mortality considerably (Chapter 6.2.5.).

7.3. Education

Education is an essential part of prevention and control of echinococcosis (Chapter 6.1.3.).

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WHO Informal Working Group on Echinococcosis (WHO-IWGE) (http://www.medicalweb.it/aumi/echinoet)

Informal Working Groups on Echinococcosis were founded in 1985 under the auspices of the World Health Organization (WHO). For 10 years, under the leadership of Professor J. Eckert (University of Zurich, Switzerland), the groups organised meetings of specialists and promoted international scientific exchange and co-operation in the field of echinococcosis research. In 1995, the WHO modified the structure of the groups and transformed them into a single group, the WHO Informal Working Group on Echinococcosis (WHO-IWGE). The aim of the IWGE is to establish international networks of co-operation on current and relevant problems on the basis of high international standards and appropriate technology. The WHO-IWGE is lead by a co-ordinator and a co-ordinating board, designated for four-year terms. Co-ordinated by Professor D.A. Vuitton (University of Besançon, France, 1985-1999) and Dr P.M. Schantz (CDC, Atlanta, USA, since 2000) the WHO-IGWE has initiated several new activities, including the following:

1. Establishment of network-groups (name of co-ordinator/s):

- Ultrasound classification of *E. granulosus* cysts (C.N.L. Macpherson)
- Natural history of small *E. granulosus* cysts (Z.S. Pawłowski)
- Staging /classification of alveolar echinococcosis (AE) in patients (P.Kern & S. Bresson-Hadni)
- Long-term follow-up of patients with cystic echinococcosis (CE) treated by PAIR (C. Filice)
- Long-term follow-up of patients with CE after surgery (Wen Hao & Menezes da Silva)
- Long-term follow-up of in-patients with CE after chemotherapy (A. Teggi & T. Todorov)
- Standardisation of immunodiagnostic tests (A. Nieto & A. Siracusano)
- Identification of *Echinococcus* in carnivores (P. Craig & P. Deplazes)
- Vaccination of intermediate hosts (M. Lightowlers)
- Health education (C. Palmas & M. Kachani)
- Socio-economic aspects (G. Battelli)
- Transmission ecology of *Echinococcus multilocularis* (P. Giraudoux & K. Takahashi)

2. EchinoNews and EchinoNet

- Preparation and distribution of a newsletter (4 issues since 1995)
- Information network established on internet in 1997. Webmaster: E. Brunetti (e-mail: slim@ipv.36.unipv.it). web site address: http://www.medicalweb.it/aumi/echinonet.

3. Publications

The WHO-IWGE was involved in the preparation of *Guidelines for treatment of cystic and alveolar echinococcosis* (*WHO Bull.*, **74**, 231-242, 1996), the *WHO/OIE Manual on echinococcosis in humans and animals* (OIE, Paris, 2001), and *PAIR, an option for treatment of cystic echinococcosis* (WHO, Geneva, 2001). Additional publications are planned.

Scientists interested in the field of echinococcosis are invited to cooperate by contacting Dr P.M. Schantz, Division of Parasitic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway NE, Atlanta, GA 30341-3724, USA (pms1@cdc.gov).
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Subject Index

Abbreviations:
AE : alveolar echinococcosis
CE : cystic echinococcosis
PE : polycystic echinococcosis
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Country/Region Index

Abbreviations:
AE : alveolar echinococcosis
CE : cystic echinococcosis

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