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

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'.
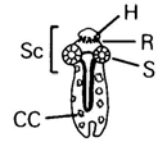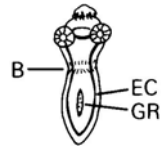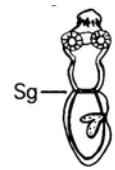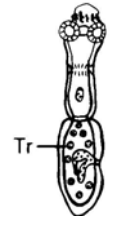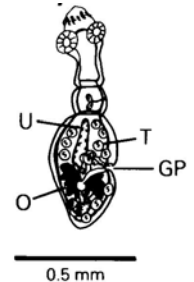
Days and stages		Description of development
Day 1		Protoscolex has evaginated and elongated; contains numerous calcareous corpuscles
	Sc : scolex CC : calcareous corpuscles H : hooks R : rostellum S : sucker	
Days 11-14		Calcareous corpuscles have disappeared; lateral excretory canals are conspicious; genital rudiment present denoting formation of first proglottid; constriction and clear area below the neck ('banding') marks the site of the first segment
	B : band EC : excretory canal GR : genital rudiment	
Days 14-17		Genital rudiment has divided into two and extends unilaterally; first segment fully formed
	Sg : segment	
Days 17-20		Rudimentary testes appear in the first proglottid; initial stages in formation of second proglottid
	Tr : rudimentary testes	
Days 20-28		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
	U : uterus O : ovarium T : testes GP : genital pore	

0.5 mm

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*
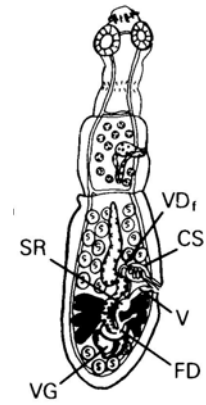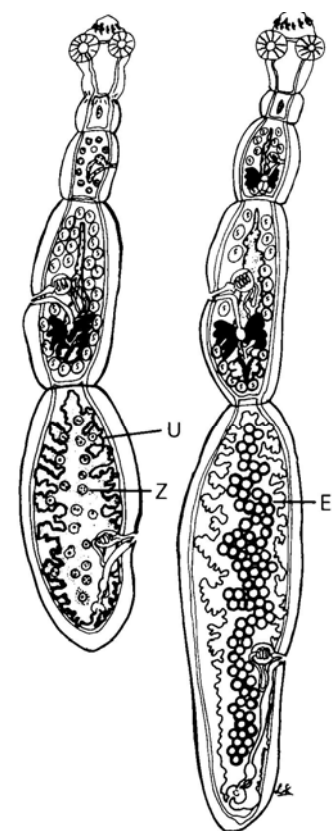
Days and stages		Description of development
Days 28-33	SR : seminal receptacle VG : vitelline gland VD$_f$: vas deferens CS : cirrus sack V : vagina FD : female reproductive ducts	Male and female genitalia in terminal proglottid fully mature; uterus still dilating; penultimate proglottid has developing genitalia; either a band or third segment appear
Days 33-37 **Days 37-45** 0.5 mm	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)
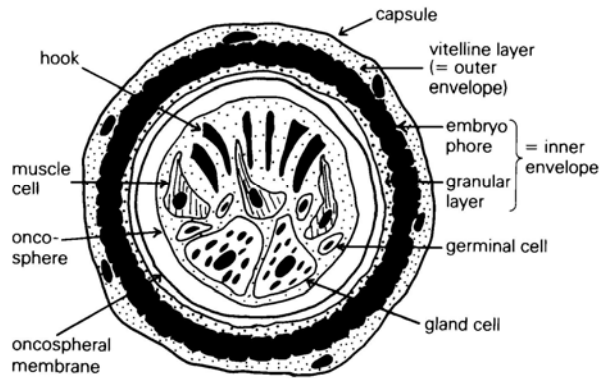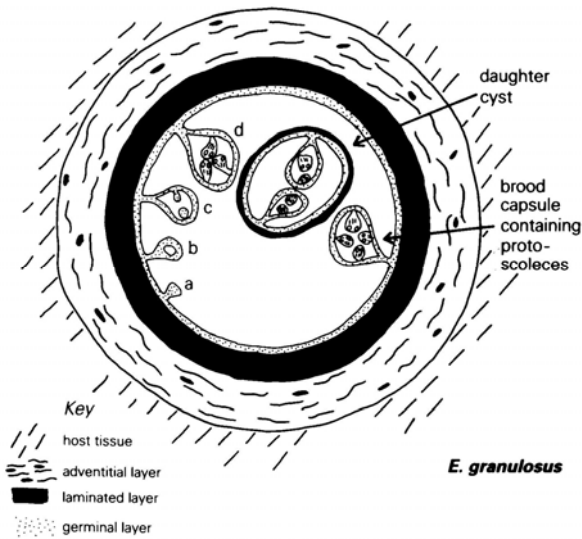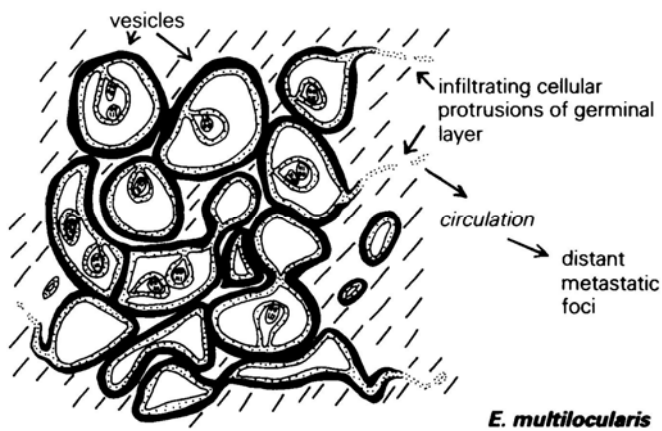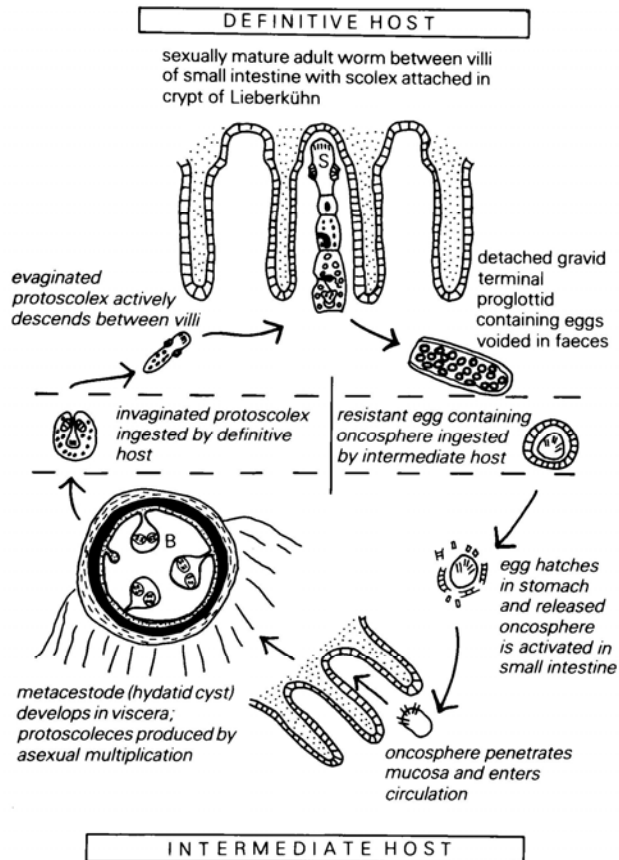Reproduced from (24) with permission from CABI *Publishing*

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                  ┌─────────────────────────────┐
                  │    D E F I N I T I V E   H O S T    │
                  └─────────────────────────────┘
          sexually mature adult worm between villi
          of small intestine with scolex attached in
          crypt of Lieberkühn
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evaginated protoscolex actively descends between villi

detached gravid terminal proglottid containing eggs voided in faeces

invaginated protoscolex ingested by definitive host

resistant egg containing oncosphere ingested by intermediate host

egg hatches in stomach and released oncosphere is activated in small intestine

metacestode (hydatid cyst) develops in viscera; protoscoleces produced by asexual multiplication

oncosphere penetrates mucosa and enters circulation

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                  ┌─────────────────────────────┐
                  │  I N T E R M E D I A T E   H O S T  │
                  └─────────────────────────────┘
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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).
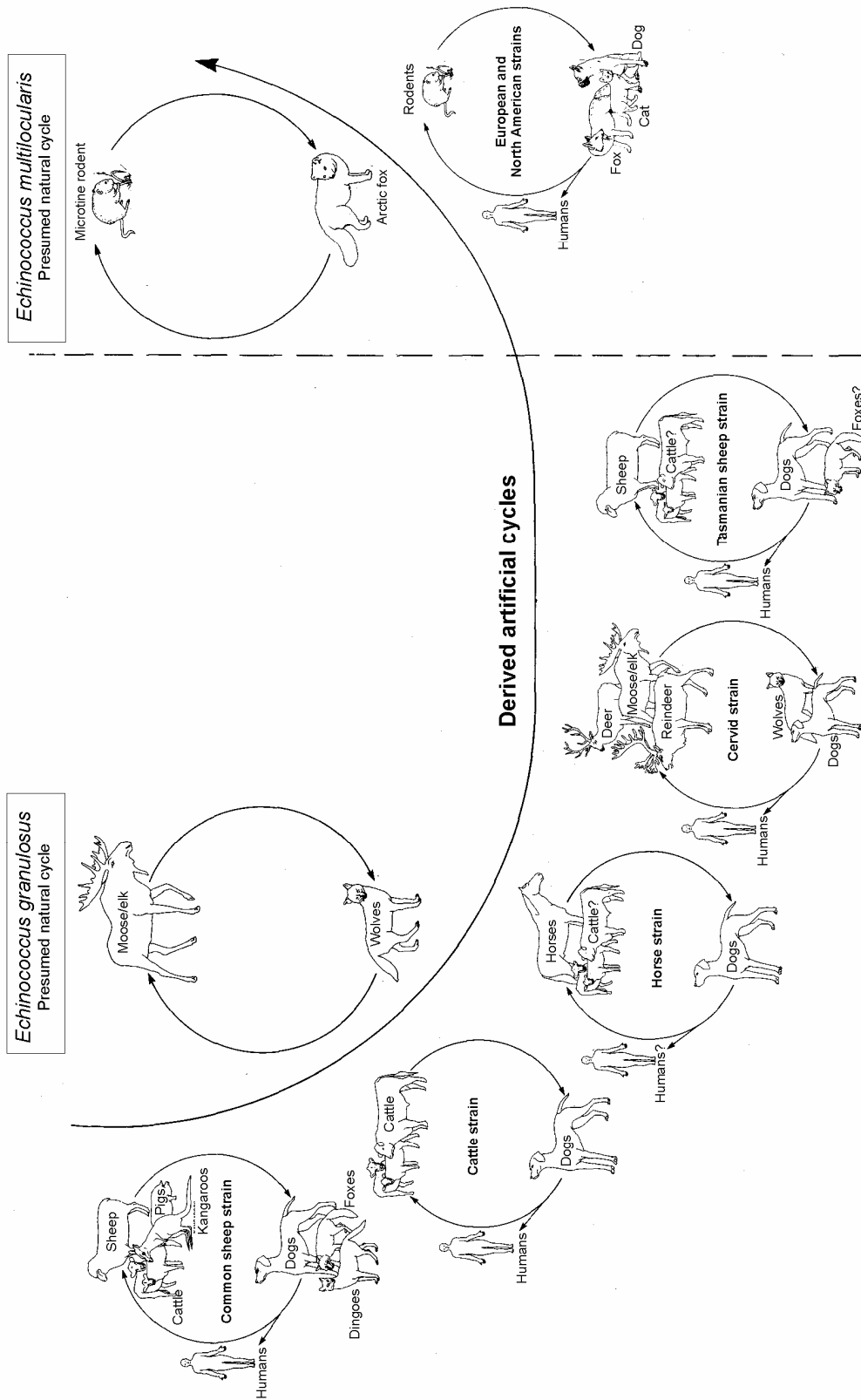
Fig. 1.5.
Life-cycle patterns of *Echinococcus granulosus* and *Echinococcus multilocularis* showing presumed natural (sylvatic) cycles and some derived artificial cycles
Source: R.C.A. Thompson and A.J. Lymbery (28)
Reproduced with permission from Elsevier Science

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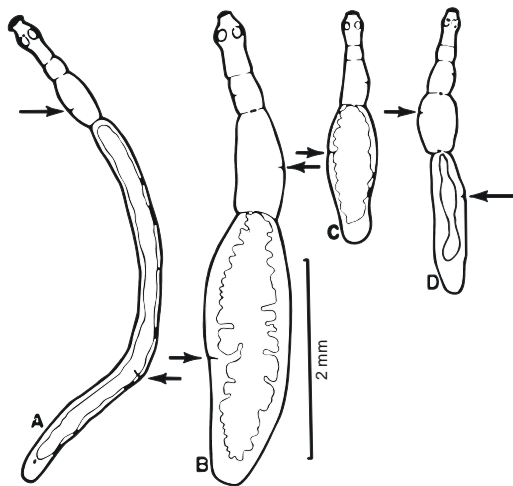
| A : *Echinococcus vogeli* | C : *Echinococcus oligarthrus* | → genital pore |
| B : *Echinococcus granulosus* | D : *Echinococcus multilocularis* | ← genital pore |

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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