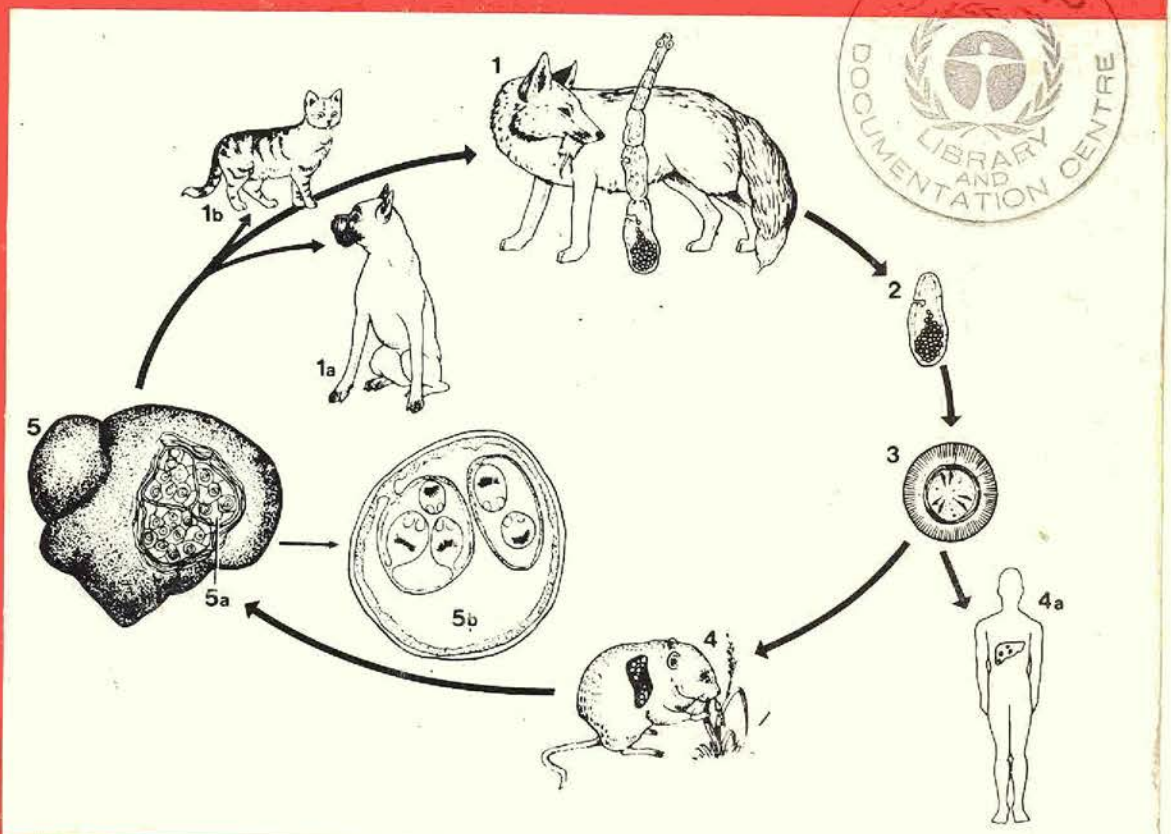
FAO ANIMAL PRODUCTION AND HEALTH PAPER 29

echinococcosis/hydatidosis surveillance, prevention and control:

fao/unep/who guidelines



FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS ROME

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echinococcosis/hydatidosis surveillance, prevention and control:

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PREFACE

General objectives

The need for the elaboration of strategies and methods for control of zoonoses and foodborne diseases was recognized by the Thirty-first World Health Assembly held in May 1978. In its resolution WHA31.48 on "Prevention and control of zoonoses and foodborne disease due to animal products", the Assembly invited Member States to formulate and implement countrywide programmes for the control of these diseases as an integral part of national health programmes and requested the Director-General to promote the extension of a network of zoonoses centres in all regions, so that the necessary support can be provided to country health programmes. It also requested further development of national, regional and global strategies, and of methods for the surveillance, prevention and control of zoonoses and foodborne diseases.

Following adoption of this resolution the WHO programme has been considerably strengthened and, at present, strategies and methods for control of selected zoonoses and foodborne diseases are being elaborated, taking into account also different epidemiological situations, such as specific animal-related human health risks in urban areas, large-scale animal production on intensive farms, areas of rapid ecological changes as well as health problems of food production, processing and distribution.

A worldwide network of WHO zoonoses centres is now being developed in order to provide essential technical cooperation to country health programmes with respect to zoonoses and related foodborne diseases. At present, services for such technical cooperation are available in the Region of the Americas through the Pan American Zoonoses Center. On 1 February 1979, the UNDP/WHO Mediterranean Zoonoses Control Programme with the participation of FAO began operations, the principal centre being located in Athens. One of the functions of the zoonoses centres will be cooperation with Member States in planning and implementation of their national programmes for control of specific diseases.

We sincerely hope that the strategies and methods as now being elaborated by WHO, in cooperation with other international organizations, and particularly FAO and UNEP, will facilitate the process of problem recognition, planning, definition of comprehensive countrywide programmes, goals, priority determination, initiation or strengthening of national projects and programmes by adaptation of principles contained in these guidelines. They aim at recognizing the important role of domestic animals and wildlife as reservoirs and transmitters of zoonotic and foodborne disease in both national and international health planning. This matter has been neglected in many countries. This is because, on the one hand, the role of animals in the spread of human diseases has not been sufficiently appreciated, and on the other hand because the administrative and legislative provisions for interprofessional collaboration are inadequate. In addition, manpower and other facilities are lacking in most of the Member States, particularly in Africa and Asia.

The guidelines will also stress that, apart from the morbidity and mortality and the human suffering they cause, zoonotic diseases and foodborne infections and intoxications are responsible for great economic losses, particularly in meat, milk and other food and products of animal origin, and that cost-effective analysis is indispensable as part of preparations for planning effective control schemes.

There is no doubt that prevention, medical treatment and control of zoonoses and foodborne diseases are an important part of primary health care. In the elaboration of the various guidelines due attention has been paid to this important tool for the attainment of the goal of "health for all by the year 2000". In addition successful zoonoses and foodborne diseases control projects will contribute to other components of primary health care, including promotion of a safe food supply and proper nutrition, safe water supply (prevention of pollution from animal sources), basic sanitation, etc. (see Article VII-3 of the Alma-Ata Declaration). The strategies and methods will also provide practical guidelines for the intersectoral coordination (Article VII-4 of the Alma-Ata Declaration).

Specific objectives

These guidelines can be regarded as a monograph embracing almost all aspects of echinococcosis/hydatidosis biology and control. Some parts will be of interest to one group of personnel and other parts will be important to other personnel employed in the development and continuation of control programmes. The monograph is set out in 13 chapters. In all sections, extensive lists of references are given in order to provide a wide range of reading matter for those who wish to become familiar with the biology, epidemiology and problems of echinococcosis control.

In the first chapter, the systematics, biology and epidemiology are discussed. The information is directed towards those senior workers responsible for training veterinarians and others involved in the more practical aspects of the control programme.

The second chapter is concerned with surveys and forms one of a group of chapters that provide the practical guidelines to control. This can be regarded as a key chapter containing the information required to plan surveys based on sound epidemiological principles. It takes account of the need to identify sociocultural and ecological factors and encourages a multidisciplinary approach involving collaboration between medical, veterinary and social science personnel.

The third, fourth, fifth and sixth chapters are concerned with defining the methods that can be applied to obtain information on prevalence in man and other animals. They embrace the specialized subject of immunodiagnosis, as well as methods used in collecting data from autopsies. A detailed account is given of the value and limitations of arecoline surveillance in canine echinococcosis.

Chapter 7 provides information on the problems of assessing the need, cost and benefit of setting up formal control programmes. This guideline is intended for those personnel establishing the case for the introduction of the programme.

The eighth chapter contains perhaps one of the most important practical aspects of the guidelines and is concerned with the establishment of the control authority and its structure. First, it outlines the sequence of events that often leads to the development of a firm case for control. Second, a detailed description is given of control structure and of the steps that should then be taken to ensure that the control authority can develop the various aspects of the programme in a logical and orderly manner. These include a definition of functions, establishment of priorities, funding, collection of base-line and continuing surveillance data, selection of staff and setting-up of training programmes, the educational component, community involvement and the selection of the appropriate control measures.

Health education is discussed in the ninth chapter. This has been found to be a most important subject, which, if properly applied, may influence the success of the programme, because it provides the key to community support. A discussion is given illustrating how some of the established programmes have benefited more than others by combining education with control measures.

Once the control authority has been established, there is an immediate need to set up a practical surveillance programme to monitor progress. This is described in chapter 10. The early introduction of surveillance embracing all potential hosts has not always been appreciated in the past. Indeed, it has been found by experience that this forms one of the more urgent tasks for the control authority. It is pointed out that, without adequate base-line and continuing data, it may become increasingly difficult to retain support for the programme.

Chapter 11 comprises four sections that are concerned with the actual control measures employed and their limitations. The first involves the prevention of infection by the introduction of safe slaughtering facilities at all levels. The second and equally important measure includes the full registration of dogs and the elimination of unwanted animals. Chemotherapy also provides an important tool in control and the efficacy of a number of

taeniacidal drugs is assessed. An account is given of the limitations of drugs that have from time to time been recommended for echinococcosis control. Chapter 12 considers drug screening programmes both for strobilate and larval phases of the parasite. In part this is concerned with research; however, it is pointed out that a need exists to define acceptable protocols for such work. The final chapter is concerned with safety precautions. Stringent measures are described, which, if followed, should provide adequate protection for personnel.

Advantage has been taken in these guidelines to describe some of the experiences gained in successful ongoing control programmes. None of them have identical problems and neither have they adopted identical approaches. It is emphasized that the measures and methods selected must take account of different social structures. Every encouragement should be given for comparative studies and exchange visits between control workers. The aims of the Veterinary Public Health unit of WHO in developing these guidelines include the provision of information to assist those countries contemplating control to select those measures and methods that most closely fit the solution to the particular control problem of that society. A summary of the sequential steps recommended for the development of an echinococcosis control programme is given below.

SUMMARY OF SEQUENTIAL STEPS RECOMMENDED FOR THE DEVELOPMENT OF AN ECHINOCOCCOSIS CONTROL PROGRAMME

1. Establish structure of control authority

This should be a recognized animal health authority. In the early stages there may be a need to achieve liaison through advisory committees with representatives of the community.

2. Define functions of control authority

Important functions of a control authority include:

(a) responsibility for the long-term funding of the programme;

(b) setting priorities;

(c) selecting personnel and organizing training activities;

(d) collecting and evaluating base-line and continuing surveillance data;

(e) defining the direction of the technical control measures and the educational components to be adopted.

3. Establish control measures

3.1 Prevent dogs gaining access to raw offal by educational and legislative means.

3.2 Direct reduction of parasite biomass by:

(a) reducing the tapeworm population; or

(b) reducing the dog population.

Both measures may be applied. Methods to reduce the tapeworm population include:

(c) "arecoline-surveillance" with or without penalties for the discovery of infection; or

(d) mass dog treatment programmes.

Methods to reduce the dog population include:

(e) banning of dogs from restricted areas;

(f) limiting the number of dogs per household;

(g) sterilization of females; or

(h) mass killing programmes.

CHAPTER 1

SYSTEMATICS, BIOLOGY AND EPIDEMIOLOGY

1.1 Introduction

Echinococcosis and hydatidosis are terms usually applied interchangeably to the zoonotic infections caused by the adult and larval stages (metacestodes) of the cestode genus Echinococcus (Family Taeniidae). In these guidelines the term hydatidosis or hydatid disease is restricted to infection with the metacestode, while echinococcosis is applied in a general way to both adult and larval infections.

1.2 General description of the parasite

Echinococcus spp. are small tapeworms, rarely more than 7 mm in length. The scolex bears four suckers, and there are two rows of hooks, one small and one large on the rostellum, the number and length of which may vary according to species. The number of proglottids of the body varies from two to six. The genital pore opens laterally and its position depends on species. The cirrus sac is horizontal or tilted anteriorly and the vitellarium is globular.

The eggs are ovoid (diameter about 30-40 μm) consisting of a hexacanth embryo (oncosphere) surrounded by several envelopes the most noticeable one being the embryophore, which gives the egg a dark striated appearance.

The metacestode basically consists of a bladder with an acellular laminated layer and an inner nucleated germinal layer, which may give rise by asexual budding to brood capsules. Protoscolices arise from the inner wall of the brood capsules or from the germinal layer.

1.3 General life cycle

Echinococcus spp. require two mammalian hosts for completion of their life cycles. Proglottids containing eggs or free eggs are passed in the faeces of the definitive host, a carnivore. The eggs are highly resistant to physical factors and can remain infective for a long period in a suitable environment (see section 1.9.2).

The intermediate host, represented by a wide range of mammals acquires infection by the ingestion of eggs. Following the action of enzymes in the stomach and small intestine, the oncosphere is released from the keratinized embryophore. Bile assists in activating the oncosphere, which penetrates the wall of the small intestine. Penetration is then aided by the hook movement 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.

Once the oncosphere has reached its final location it develops into the metacestode (hydatid larva) stage. Time of development is variable and it may take several months before protoscolices are produced (fertile metacestode). Not all metacestodes produce protoscolices (sterile metacestode). When protoscolices 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 and exposure to bile.[114] They then develop to the sexually mature tapeworms, approximately four to six weeks after infection, depending on the species and on the susceptibility of the host.

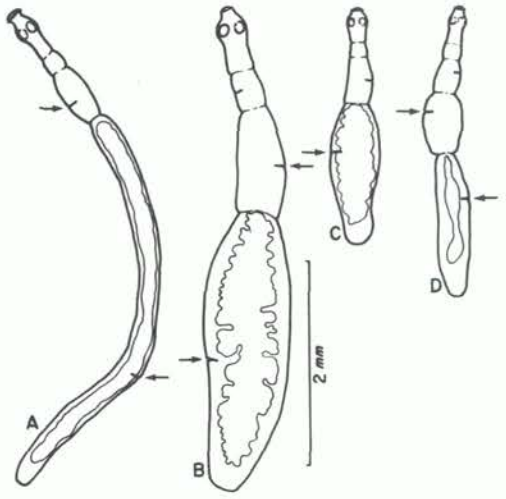
1.4 Species of the genus Echinococcus

At present four species of the genus Echinococcus are regarded as valid taxonomically. These are Echinococcus granulosus (Batsch, 1786), Echinococcus multilocularis Leuckart, 1863, Echinococcus oligarthrus (Diesing, 1863) and Echinococcus vogeli Rausch and Bernstein, 1972. 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 Fig. 1 and Table I.

FIG. 1. COMPARATIVE GENERAL MORPHOLOGY OF ADULT ECHINOCOCCUS SPECIES (ADAPTED FROM[88])

A: Echinococcus vogeli;
B: Echinococcus granulosus;
C: Echinococcus oligarthrus;
D: Echinococcus multilocularis

\+ Arrows indicate position of genital pores.



1.4.1 Echinococcus granulosus

1.4.1.1 Morphology[19,60,72,73,79,134,138,142,144,148]

The adult worm varies between 2-7 mm in length 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 characterized by well-developed lateral sacculations (Figs. 1 and 2 and Table 1). The larval stage is a fluid-filled bladder usually unilocular but communicating chambers also occur. Growth is expansive and endogenous daughter cysts may be produced (Figs. 3 and 4). Occasionally, individual bladders may reach up to 30 cm in diameter.

TABLE 1. COMPARATIVE MORPHOLOGY OF ADULT *ECHINOCOCCUS* SPECIES[a]

Taxonomic criteria	*E. granulosus*	*E. vogeli*	*E. multilocularis*	*E. oligarthrus*
Length of strobila (mm)	2-7	3.9-5.6	1.2-3.7	1.9-2.9
Rostellar hooks Length (µm): - Large hooks (average) - Small hooks (average)	 31-49 (37-42) 22-39 (29-34)	 49-57 (53) 30-47 (43)	 28-34 (31) 23-31 (27)	 43-60 (52) 28-45 (39)
Number of proglottids (range)	3 (4-6)	3 (?)	4-5 (2-6)	3 (?)
Number of testes (average)	25-80 (32-68)	50-67 (56)	16-35 (18-26)	15-46 (29)
Distribution of testes anterior/posterior genital pore	equally anterior/ posterior	majority anterior	majority posterior	majority posterior
Position of genital pore relative to middle of segment	near/ posterior	posterior	anterior	anterior
Mature proglottid	penultimate	penultimate	ante-penultimate	ante-penultimate
Form of uterus	Lateral sacculations	long, tubular	sac-like	sac-like
Ratio anterior part of strobila:gravid segment	1:0.86-1.3	1:1.9-3.0	1:0.31-0.8	1:0.96-1.1

[a] Taken from reference 88, see also references 142 and 144.

Identification of species must be based on a combination of criteria listed in the table and described in the literature.

FIG. 2. ECHINOCOCCUS GRANULOSUS, GRAVID WORM FROM A DOG.
ARROWS INDICATE POSITION OF GENITAL PORES
(PHOTO: COURTESY DEPARTMENT OF PARASITOLOGY, UNIVERSITY OF ZÜRICH)

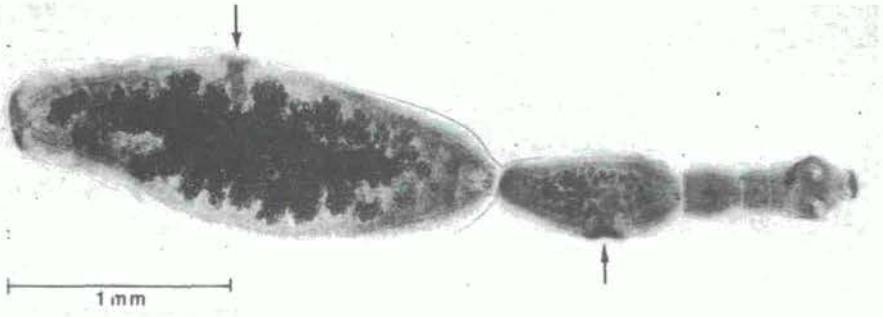


FIG. 3. HYDATID CYST OF ECHINOCOCCUS GRANULOSUS, DIAGRAMMATIC (FROM [72])

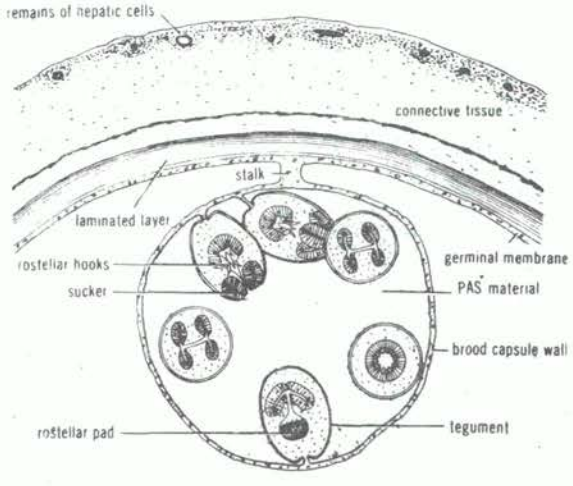


TABLE 2. SELECTED LIST OF NATURAL FINAL AND INTERMEDIATE HOSTS OF *ECHINOCOCCUS GRANULOSUS*[a]

Final hosts
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 culpaeus*) Racoon dog (*Nyctereutes procyonides*)
Hyaenidae: Spotted hyaena (*Crocuta crocuta*)
Felidae: Lion (*Panthera leo*), Leopard (*Panthera pardus*)
Intermediate hosts
Bovidae: Domestic sheep, goat, and cattle (*Ovis ammon* f. *aries*, *Capra aegagrus* f. *hircus*, *Bos primigenius* f. *taurus*), Buffalos (*Bubalus arnee*, *Syncerus caffer*), Bison (*Bison* spp.), Kongoni (*Alcelaphus buselaphus cokii*), Impala (*Aepyceros melampus*), Wildebeest (*Connochaetes taurinus*), Grant's gazelle (*Gazella granti*), Waterbuck (*Kobus* spp.), Blue duiker (*Cephalophus monticola*), Ibex (*Capra sibirica*), Mongolian gazelle (*Gazella gutturosa*), Saiga (*Saiga tatarica*)
Cervidae: Red deer, elk, wapiti (*Cervus elaphus*), American deer (*Odocoileus* spp.), Moose (*Alces alces*), Reindeer, caribou (*Rangifer tarandus*) Roe deer (*Capreolus capreolus*), Axis deer (*Cervus nippon*), fallow deer (*Dama dama*)
Camelidae: Alpaca (*Lama guanicoe* f. *pacos*), Camel (*Camelus dromedarius*) Bactrian camel (*Camelus bactianus*)
Giraffidae: Giraffe (*Giraffa camelopardalis*)
Suidae: Domestic and wild pig (*Sus scrofa*), Warthog (*Phacochoerus aethiopious*)
Equidae: Domestic horse, donkey, mule, wild ass, zebra (*Equus* spp.)
Elephantidae: Elephant (*Loxodonta africana*)
Hipoppotamidae: Hippopotamus (*Hippopotamus amphibius*)
Leporidae: European hare (*Lepus europaeus*)

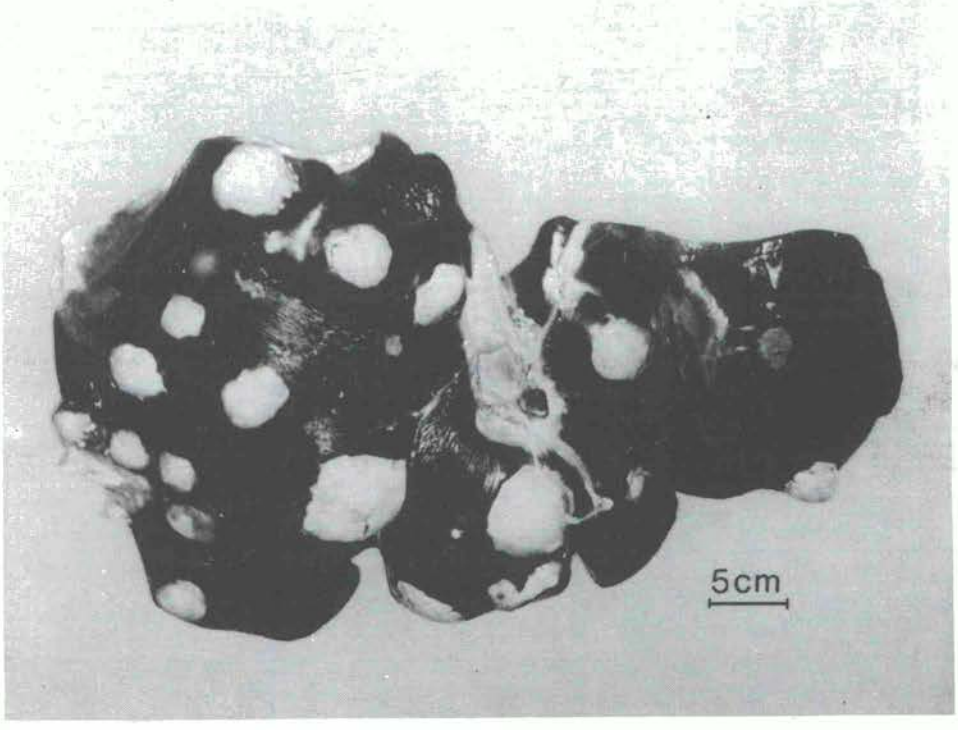
[a] Taken from references 1, 25, 51, 113, 121.

TABLE 2. SELECTED LIST OF NATURAL FINAL AND INTERMEDIATE HOSTS OF ECHINOCOCCUS GRANULOSUS[a] (continued)

Primata:
Lemur (Lemur mongoz), Macaques (Macaca spp., Cynopithecus niger), Baboon (Papio cynocephalus), Mandrill (Mandrillus spp.), Orangutan (Pongo pygmaeus) Chimpanzee (Pan troglodytes), Gorilla (Gorilla gorilla), Man (Homo sapiens)
Marsupialia:
Kangaroos and Wallabies (Thylogale sp., Wallabia spp., Macropus sp.)

[a] Taken from references 1, 25, 51, 113, 121.

FIG. 4. HYDATID CYSTS OF ECHINOCOCCUS GRANULOSUS IN HORSE LIVER (PHOTO: COURTESY DEPARTMENT OF VETERINARY PATHOLOGY, UNIVERSITY OF ZÜRICH)



1.4.1.2 Host range

Echinococcus granulosus has the least intermediate host - specificity of any of the four species, and this contributes to its cosmopolitan distribution (see section 1.8). The larval stage has been found in a wide range of mammals (Table 2). The metacestode causes "cystic" hydatid disease in man (Fig. 5).

The parasite is perpetuated primarily in a domestic cycle involving the dog (Canis familiaris) as the definitive host and domestic ungulates (e.g. sheep, cattle, pigs, goats, horses and camels) as intermediate hosts (Fig. 6). However, in some areas wild animals are involved. For example, wolves and moose in North America[82,94] and dingoes and a variety of macropods in Australia,[16,22,27,48,137] jackals and deer in Ceylon,[78] coyotes and deer in California[12,59,95] foxes and hares in Argentina[103] and jackals and wild ruminants in Kenya[25] are involved in predator-prey relationships, which support wildlife echinococcosis.

FIG 5. HYDATID CYST OF ECHINOCOCCUS GRANULOSUS IN HUMAN LIVER
(PHOTO: COURTESY DEPARTMENT OF PARASITOLOGY, UNIVERSITY OF ZÜRICH)

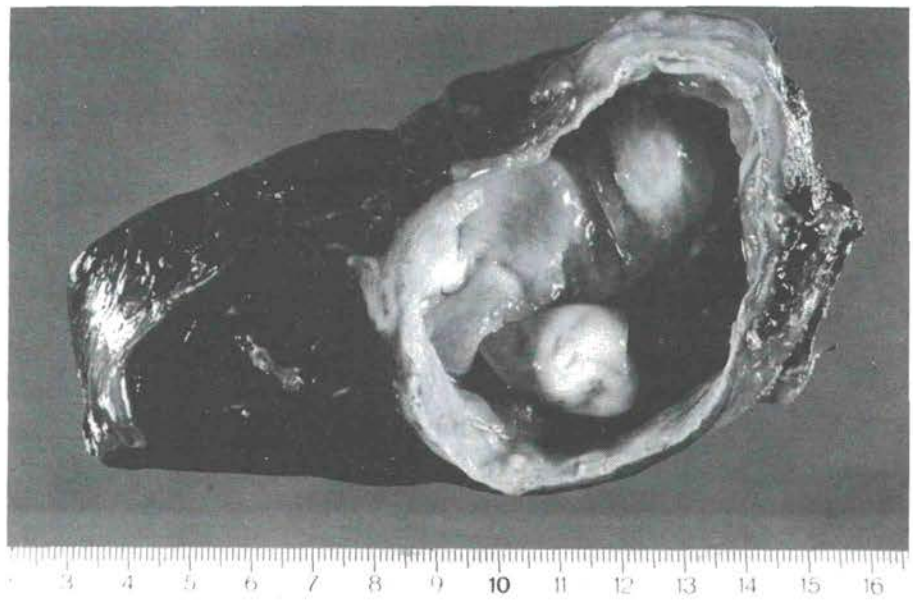
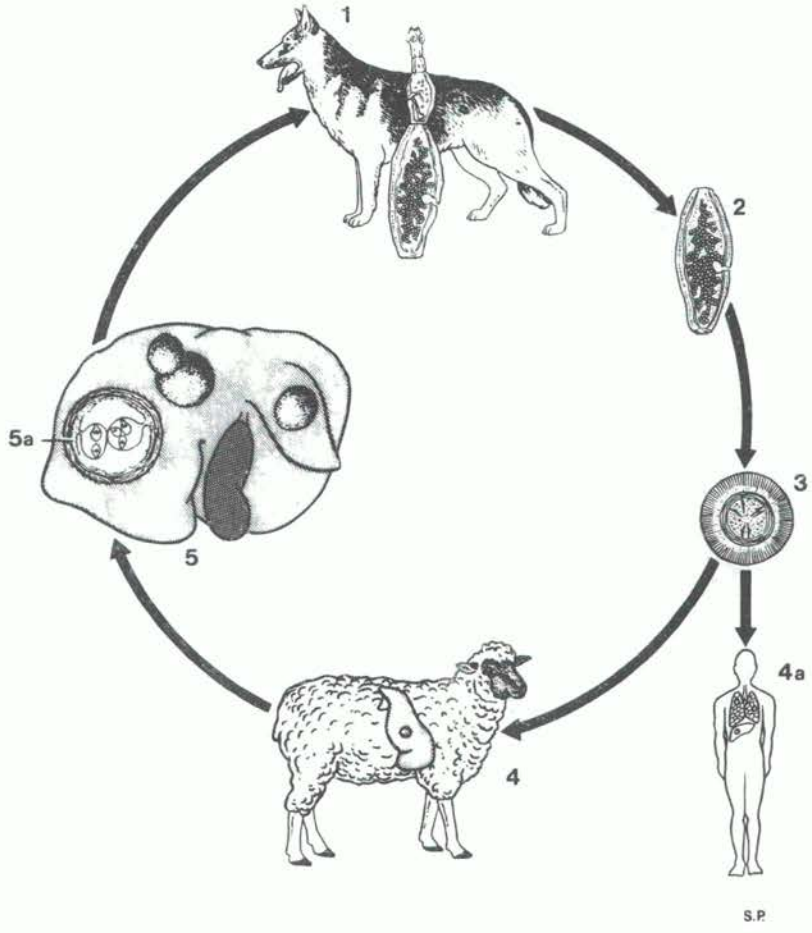


FIG. 6. LIFE CYCLE OF ECHINOCOCCUS GRANULOSUS
(MODIFIED AFTER PIEKARSKI, 1973.
COURTESY DEPARTMENT OF PARASITOLOGY, UNIVERSITY ZÜRICH)

(1) Adult worm inhabiting the small intestine of domestic dog and other Canidae, e.g. wolf, dingo, jackal. (2) Proglottid with eggs excreted in faeces. (3) Egg. (4) Ungulates, e.g. sheep, cattle, pigs, horses, camels, serve as intermediate hosts. Metacestodes (hydatid cysts) mainly develop in liver, lungs and other viscera. (4a) Accidental infection of man leads to metacestode development in liver, lungs or other organs. (5) Infected liver with metacestodes. (5a) Fertile metacestodes with protoscolices. This is the infective stage. The cycle is completed when fertile metacestodes are eaten by susceptible carnivores.

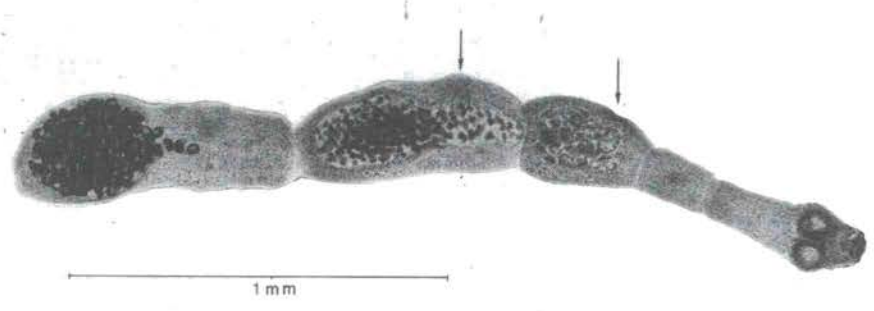


1.4.2 Echinococcus multilocularis

1.4.2.1 Morphology [19, 79, 83, 87, 88, 90, 121, 144]

The adult worm varies between 1.2-3.7 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 and Fig. 7).

FIG. 7. ECHINOCOCCUS MULTILOCULARIS, MATURE GRAVID WORM FROM RED FOX. ARROWS INDICATE POSITION OF GENITAL PORES. (PHOTO: COURTESY OF DEPARTMENT OF PARASITOLOGY, UNIVERSITY ZÜRICH)



The metacestode is a multivesicular structure consisting of conglomerates of small vesicles (diameter usually not exceeding a few millimetres). Unlike the metacestode of E. granulosus, the larval mass often contains a semi-solid matrix rather than fluid (Figs. 8 and 9). It proliferates by budding and this results in infiltration of tissues. It is commonly referred to as the alveolar hydatid.

1.4.2.2 Host range

Intermediate host specificity is relatively high and infection with the metacestode is confined to rodents, particularly members of the family Cricetidae (Arvicolidae).[26,85] Natural infections in soricid insectivores have also been reported (Table 3). The metacestode causes "alveolar" hydatid disease in man (Fig. 10).

FIG. 8. METACESTODE CONGLOMERATES OF ECHINOCOCCUS MULTILOCULARIS IN THE PERITONEAL CAVITY AND LIVER OF MERIONES UNGUICULATUS (JIRD), FIVE MONTHS AFTER ARTIFICIAL INFECTION
(PHOTO: COURTESY OF DEPARTMENT OF PARASITOLOGY, UNIVERSITY OF ZÜRICH)

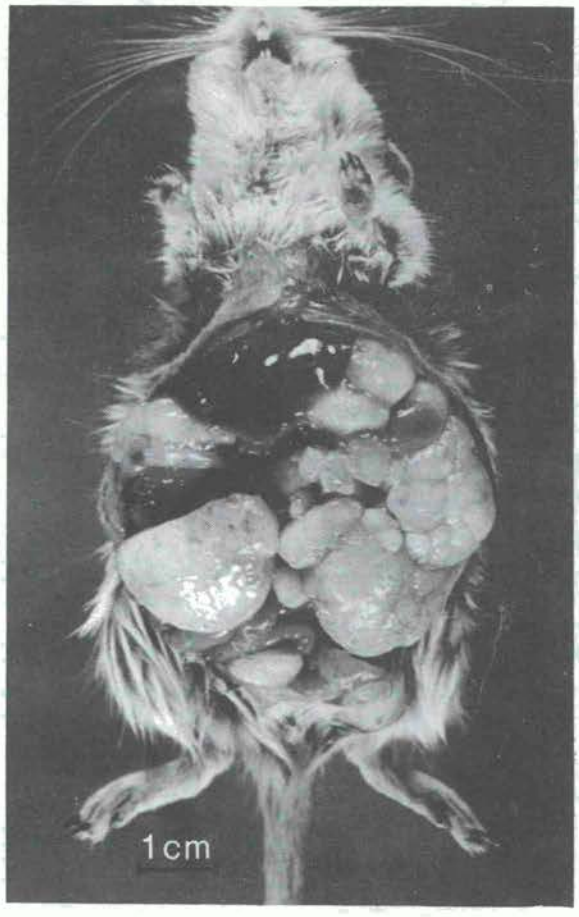


FIG. 9. HISTOLOGICAL SECTION OF METACESTODE OF ECHINOCOCCUS MULTILOCULARIS. YOUNG AND OLDER CYSTS WITHOUT PROTOSCOLEX FORMATION IN MERIONES.
(PHOTO: COURTESY DEPARTMENT OF PARASITOLOGY, UNIVERSITY OF ZÜRICH)

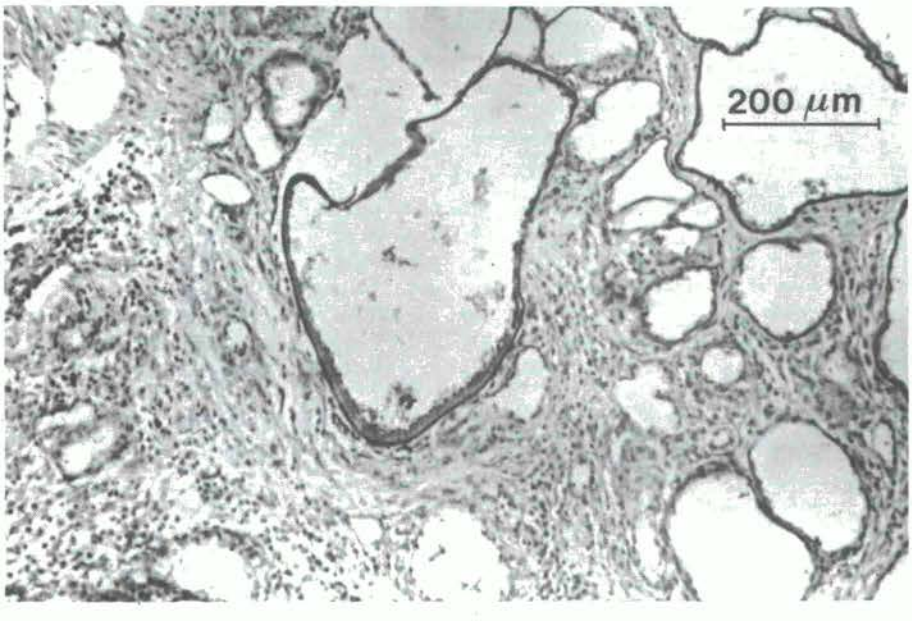
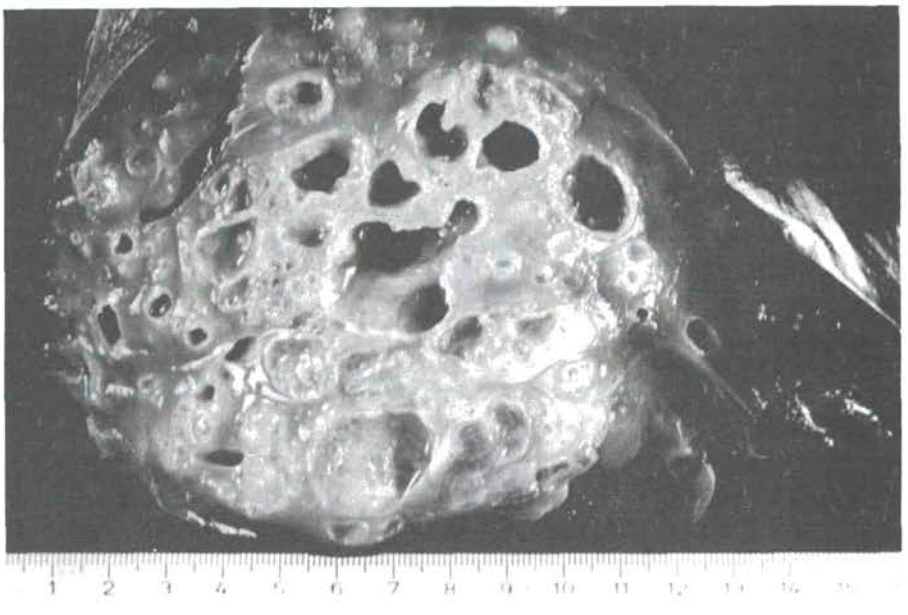


TABLE 3. SELECTED LIST OF NATURAL FINAL AND INTERMEDIATE HOSTS OF *ECHINOCOCCUS MULTILOCULARIS*[a]

Final hosts
Canidae: Red fox (*Vulpes vulpes*), Arctic fox (*Alopex lagopus*), Domestic dog (*Canis lupus* f. *familiaris*), Wolf (*Canis lupus*), Dog fox, corsac fox (*Vulpes corsac*) Racoon dog (*Nytereutes procyonides*)
Intermediate hosts
Cricetidae: Arvicola sp. Voles (*Microtus* spp., *Clethrionomys* spp.), Muskrat (*Ondatra zibethica*), Deer mouse (*Peromyscus maniculatus*), Lemmings (*Lemmus* spp.), Gerbils (*Meriones* spp., *Rhombomys opimus*), Striped hamster (*Cricetulus barabensis*), Northern mole-vole (*Ellobius talpinus*)
Muridae: House mouse (*Mus musculus*), Striped field mouse (*Apodemus agrarius*), Norway rat (*Rattus norvegicus*), Short-tailed mole rat (*Nesokia indica*)
Sciuridae: Long-tailed suslik, ground squirrel (*Citellus undulatus*), Red squirrel (*Sciurus vulgaris*)
Dipodidae: Jerboa (*Allactaga elater*)
Ochotonidae: Daurian pica (*Ochotona daurica*)

[a] Taken from references 1, 51, 61, 85 and 121.

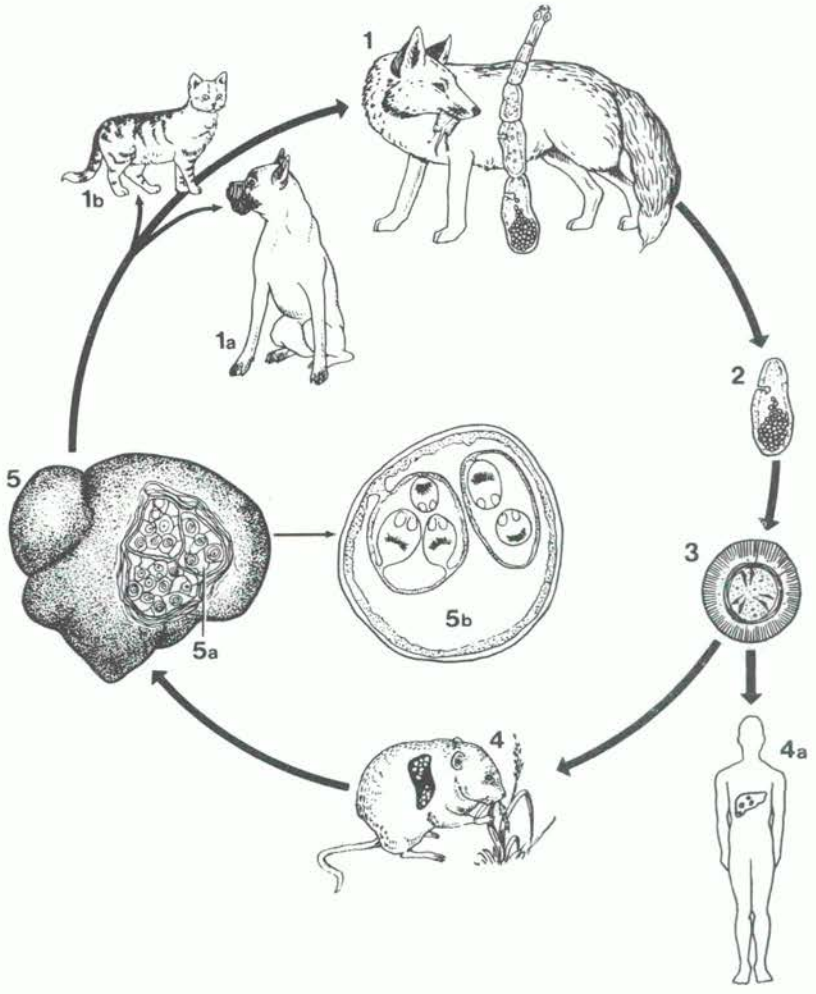
FIG. 10: METACESTODE OF *ECHINOCOCCUS MULTILOCULARIS* IN HUMAN LIVER. (PHOTO: COURTESY DEPARTMENT OF PARASITOLOGY, UNIVERSITY OF ZÜRICH)



Echinococcus multilocularis is mainly perpetuated in a sylvatic cycle involving foxes and cricetid (arvicolid) rodents. Domestic dogs and occasionally cats may also enter into this cycle when they eat infected wild rodents, or rodents commonly associated with human dwellings such as house mice[1,24,26,51,56,85,102,121,144] (Fig. 11).

FIG. 11. LIFE CYCLE OF ECHINOCOCCUS MULTILOCULARIS. (MODIFIED AFTER VOGEL, 1961. COURTESY DEPARTMENT OF PARASITOLOGY, UNIVERSITY OF ZÜRICH)

(1) E. multilocularis mainly parasitizes the small intestine of red or arctic foxes; (1a/b) domestic dogs and cats may also enter the cycle when they eat infected rodents; (2) proglottids with eggs excreted in faeces; (3) egg; (4) intermediate hosts are rodents, mainly of the family Cricetidae, natural infections in soricid insectivores have also been reported; (4a) accidental infection of man may result in metacestode development in the liver and other sites by extension or formation of metastates; (5) infected rodent liver; (5a) metacestode consisting of conglomerates of small vesicles; (5b) single vesicle with protoscoleces.



1.4.3 Echinococcus oligarthrus

1.4.3.1 Morphology[14,19,88,101,123,130]

The adult worm varies between 1.9-2.9 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 (see Table 1 and Fig. 1).

The metacestode is polycystic and fluid-filled with a tendency to become septate and multi-chambered. The single cyst may reach a diameter of about 5 cm. It has been reported that predilection sites of this metacestode are internal organs and muscles.

1.4.3.2 Host range

Echinococcus oligarthrus characteristically uses wild felids including the puma (Felis concolor), jaguar (Felis onca), jaguarundi (Felis yagouaroundi) and Geoffroy's cat (Felis geoffroyi) as definitive hosts. The agouti (Dasyprocta) and possibly other rodents serve as intermediate hosts. So far, no human infections due to E. oligarthrus have been confirmed.[19,101,122,123,130]

1.4.4 Echinococcus vogeli

1.4.4.1 Morphology[19,88,92]

The adult worm varies between 3.9-5.6 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 characterized by being relatively long and tubular in form (Table 1 and Fig. 1).

The metacestode is similar to that of E. oligarthrus. It has been reported that the two species can be distinguished by comparing differences in the dimensions and proportions of the rostellar hooks on the protoscolex.[92] A recent study has found that the protoscolex rostellar hooks of E. oligarthus vary in length between 25.9-27.9 μm (average 33.4 μm) and 22.6-29.5 μm (average 25.45 μm) for large and small hooks respectively. Those of E. vogeli vary between 39.1-43.9 μm (average 41.64 μm) and 30.4-36.5 μm (average 33.6 μm) for large and small hooks respectively.[92]

1.4.4.2 Host range

Echinococcus vogeli uses the bush dog (Speothos venaticus) and the domestic dog as definitive hosts, and pacas (Cuniculus paca) and possibly other rodents as intermediate hosts. The larval stage does develop in man causing a polycystic form of hydatid disease, predominantly in the liver.[18,19,88,92]

1.5 Variation within the genus Echinococcus

1.5.1 Reproduction in Echinococcus

The sexual phase of the life cycle may involve either cross- or self-fertilization by the adult hermaphrodite tapeworm. It has been hypothesized that if a recessive mutation occurs it may appear in both egg and sperm resulting in the formation of a double recessive. Since the metacestode reproduces asexually, a large population of genetically identical individuals can arise from a single mutant. It follows that as the parasite has a mode of reproduction which favours the expression of mutants, new variants can arise readily. If, for example, an unusual intermediate host were to ingest eggs in which a population of the oncopheres contain the mutant gene, it is possible that the mutant may be the only one suitably adapted for establishment in that host. Consequently a population of this new variant running into millions could be established rapidly from a single mutation.[117,121]

1.5.2 Inter- and intraspecific variation

Large numbers of inter- and intraspecific variants have been described, including 15 species and numerous subspecies.[86,106,113,116,137] However, only the four species, *E. granulosus*, *E. multilocularis*, *E. oligarthrus* and *E. vogeli* are currently accepted as valid taxonomically (see section 1.4). Several subspecies of *E. granulosus* have been nominated, for example, *E. g. granulosus*, and *E. g. canadensis*.[86,116] The former is infective to dogs and domestic ungulates, and was believed to have originated in Europe but was spread throughout the world by early settlers. *Echinococcus g. canadensis* is the indigenous species in the arctic region of North America and utilises wolves and wild ruminants in its life cycle. At least two subspecies of *E. multilocularis* (*E. m. multilocularis*, *E. m. sibiricensis*) have been proposed, while no subspecies of *E. oligarthrus* and *E. vogeli* have so far been described.

1.5.3 The taxonomic validity of subspecies and variants

The taxonomic validity of the numerous subspecies and variants that have been described remains controversial. It must be accepted that intraspecific variants exist.[4,5,108,117,132,136,137,138] However, to avoid further taxonomic confusion, minor morphological variants within the genus should be designated as strains until their biological status has been clarified.

1.6 Strain differences

Studies of populations of *E. granulosus* recovered from different host species and in different geographical regions have demonstrated considerable intraspecific morphologic variation[77,89,105,106,142,144] as well as biological[15,54,119,133,135,138,146,147,149] and biochemical[52,53,58,63,69,70] differences which may have important epidemiological implications. For example, one population occurring in the higher latitudes of North America has been designated the North American sylvatic strain or subspecies (*E. g. canadensis*). This form does not readily infect domestic ungulates.[15] It occurs in wolves and wild ungulates (moose and reindeer), and possibly represents the original form of the cestode as it evolved in its sylvatic animal hosts.[85] Human infection by this form is characterized by predominantly pulmonary localization, slower and more benign growth, and a less frequent occurrence of clinical complications than reported for other forms.[147] In Great Britain, a strain of *E. granulosus* recovered from the horse-dog cycle has been shown to be morphologically and biologically distinct from those recovered from the sheep-dog cycle.[53,63,138,146] Studies *in vitro* have shown different growth characteristics of the two strains, suggesting different physiological, nutritional, or metabolic requirements.[69,118,119] The apparent absence of infection in humans exposed to the British horse-dog strain suggests that this strain may not be infective to man.[138] (Table 4).

In the Soviet Union, morphological and biological differences have been reported between *E. granulosus* recovered from the sheep-dog cycle and those from the pig-dog cycle.[54,149] Cestodes recovered from the sheep-dog cycle were virtually non-infective to pigs. In contrast, the worms recovered from the pig-dog cycle were not infective to sheep. Indications that strain differences occur in *Echinococcus granulosus* have been reported from other countries - e.g. Italy, Africa and elsewhere, and there is a need for a review of the situation.

In Australia and Britain, cattle are often regarded as poor hosts of the larval stage of *E. granulosus* with cysts normally remaining sterile (e.g. without protoscolices).[37,134] However, in certain other areas (e.g. in the Republic of South Africa, Belgium and Switzerland) fertile cysts are found commonly in cattle.[23,98,143] Adult *E. granulosus* does not normally develop in felids; however, in Africa, the lion appears to be a suitable definitive host for a strain of *E. granulosus* (Table 2). In Western Australia a strain of *E. granulosus* may exist perpetuated in a cycle involving cattle but not sheep.[137] In India, there may exist another strain of *E. granulosus* perpetuated in a dog-buffalo cycle.[45] In Northern Africa and the Middle East, *E. granulosus* may be more adapted to camels than other domestic intermediate hosts. Strain characteristics may influence local patterns of transmission and health significance. It is, therefore, important for a global view of the epidemiology of echinococcosis to demonstrate the existence and characterize such strains.

TABLE 4. ECHINOCOCCUS GRANULOSUS: SUMMARY OF MORPHOLOGICAL, BIOLOGICAL AND BIOCHEMICAL DIFFERENCES BETWEEN THE HORSE AND SHEEP STRAINS[118]

	Horse-dog strain		Sheep-dog strain	
1. MORPHOLOGICAL				
Hook size: large	40.4-41.1		32.2-32.8	
small	33.1-34.2		25.0-25.5	
Cirrus sac & tube	tilted acutely		not tilted	
2. INFECTIVITY TO ANIMALS				
Intermediate hosts:				
horse	Yes		No	
sheep	rare - if at all		Yes	
cattle	rare - if at all		Yes?	
pigs	?		Yes	
Definitive hosts:				
dog	Yes		Yes	
fox	No?		Yes	
3. INFECTIVITY TO MAN	No?		Yes	
4. IN VITRO CULTURE	Almost no development		Develops to sexual maturity	
5. CHEMICAL COMPOSITION (µg/mg dry weight) (Figures rounded to whole numbers)				
Protein	550 ± 10		625 ± 12	
Polysaccharides	177 ± 1		169 ± 1	
Lipids	108 ± 4		88 ± 3	
RNA	60 ± 4		89 ± 4	
DNA	5 ± 0		5 ± 0	
6. METABOLISM (nmol/mg dry weight/3 h)	Aerobic	Anaerobic	Aerobic	Anaerobic
Glucose taken up	0	0	0	0
Glycogen utilized	46	116	102	108
O_2 consumed	84	-	120	-
Lactate	55	59	24	25
Succinate	7	61	15	42
Acetate	23	25	62	82
Propionate	2	3	0	0
Pyruvate	2	2	1	1
Malate	1	1	2	2
Ethanol	7	3	8	6
7. ENZYMES	9 isoenzyme profiles different			

1.7 Evaluation of local strain characteristics

1.7.1 Field observations

The prevalence of the parasite in all local potential hosts should be measured. Data on host range, metacestode development in the intermediate host and morphology of larval and adult parasites will provide essential base-line information.

1.7.2 Morphology

Certain morphological features may be used in supporting evidence of strain differences (Table 4), but they should not be considered as the sole criteria for the discrimination between strains.[86] This is particularly so when comparing adult worms from various definitive hosts, where morphological variations may merely reflect phenotypic adaptations of the parasite to different environments and may not reflect any genotypic differences.[115,137]

In all morphological studies, techniques for collecting, fixing, preserving and staining specimens must be standardized, and special attention should be given to comparison of characteristics of taxonomic significance.[89,138,142,144]

1.7.3 Transmission studies

The purpose of such studies includes the determination as to whether or not populations of E. granulosus recovered from one intermediate host species will infect other species. Thus, hydatid cysts collected from one intermediate host species should be fed to carnivores and the eggs produced in them should be fed to animals of the original and suspected intermediate host species.

Such studies require adequate numbers of animals and facilities for maintenance of experimental hosts under cestode-free conditions as well as isolation facilities for maintenance of infected carnivores (see also section 13.5). Attention must be given to the selection of appropriate breeds of intermediate hosts and the dose of eggs. For example, it has been shown that different strains of laboratory mice (Mus musculus) vary in susceptibility to infection with Echinococcus spp. and the same may be true for different breeds of other host species.

1.7.4 Other criteria

The experimental techniques described below have been utilized in differentiating between strains of Echinococcus. Further work is required to evaluate their potential and develop new techniques.

1.7.4.1 In vitro cultivation

Comparative studies on the in vitro growth from protoscolex to strobilate tapeworm have been used to differentiate between the horse and sheep strains of E. granulosus[118,119,120] in Great Britain.

1.7.4.2 Biochemical studies

Separation of soluble whole body proteins and enzymes by the techniques of isoelectric focusing and electrophoresis have demonstrated differences between species and strains of the genus Echinococcus.[52,58]

1.7.4.3 Developmental studies in definitive hosts

Certain strains have been shown to differ in their rates of development (growth, segmentation and maturation) in the same definitive host.[135]

1.7.4.4 Intermediate host specificity

Experimental primary and secondary infections in laboratory animals and potentially susceptible natural intermediate hosts, have been demonstrated to be valuable aids in differentiating between strains.[46,133,139,146]

1.8 Geographical distribution and prevalence

1.8.1 Echinococcus granulosus

The adaptability of E. granulosus to a wide variety of host species and the repeated introduction of domestic animals from Europe to other parts of the world has resulted in the present broad geographical distribution of the cestode from north of the Arctic Circle to as far south as Tierra del Fuego, Argentina. Within this cosmopolitan distribution, the cestode occurs in all major climates, in a wide variety of hosts at various levels of prevalence.

Several hundred articles have been published reporting the occurrence of E. granulosus infection in man and other animals throughout the world.[28,29,67,85,102,111,145]

In the higher latitudes of the Western Hemisphere, the sylvatic strain of E. granulosus occurs in wolves and wild ungulates in parts of Alaska and Canada.[85] The same or a similar strain is reported from the northernmost parts of the Scandinavian countries[96] and the Soviet Union. In these areas its confinement to sylvatic hosts limits the risk of human exposure (see section 1.6). In Alaska and Canada infection in man is largely limited to the indigenous Eskimos and Indians, especially tribes involved in hunting and trapping.[15,84] These groups feed their dogs on the lungs and other offal of reindeer and moose.

The infection occurs throughout North, Central and northern South America in cycles involving dogs and sheep (e.g. western United States of America, Mexico), and dogs and swine (e.g. south-eastern United States of America, Mexico, Guatemala, El Salvador, Venezuela, Ecuador).[102] In the Americas, highest infection prevalence is observed in the intensive-sheep-raising areas of southern South America (Argentina, Chile, Uruguay, central Peru, Bolivia and southern Brazil), where the dog-sheep cycle is most important[145] (see section 1.8.1.1).

Iceland, which a century ago had one of the highest human infection rates ever recorded (22%), is now virtually free of echinococcosis.[7]

In Europe, the highest prevalence in human and animal hosts is reported from countries adjacent to the Mediterranean Sea (e.g. Spain, Italy, Yugoslavia, Greece) where the dog-sheep cycle is dominant. In parts of western Europe, Great Britain and Ireland, the cestode is prevalent in a dog-horse cycle, but appears to account for little human morbidity.[138] A sheep-dog cycle is also present. In some countries of Europe (e.g. Belgium, Federal Republic of Germany, Switzerland) a dog-cattle cycle seems to predominate. A dog-pig cycle is prevalent in several Eastern European countries (e.g. Poland, Hungary) and in the Soviet Union (e.g. Byelorussian SSR and Ukrainian SSR). The infection is widespread throughout the remainder of the Soviet Union in the dog-sheep cycle. Control programmes have reduced the prevalence considerably in most of the Russian SSR but have had limited impact in Kazakh SSR and the Central Asian Republics.[100]

In most Eastern Mediterranean countries, infection is hyperendemic in sheep, camels, goats, and donkeys.[102] Published accounts indicate that the infection occurs in most Asian countries including the People's Republic of China, Kampuchea, Viet Nam, the Philippines, Taiwan and Indonesia,[67] with areas of high prevalences in Iran, India, Nepal and Pakistan.

In northern Africa high prevalence is reported and host-parasite relationships are similar to those in the Middle East. Infection is reported from many African countries south of the Sahara. In East Africa infection in domestic livestock is widespread, but infection in humans is mainly limited to certain groups in north-western Kenya and Uganda, where a set of poorly understood environmental and cultural factors combine to produce one of the highest human morbidity rates yet reported.[64,74,75] In this regard in Turkana, a high ratio of human infection is associated with a high rate of infection in dogs and in camels but a low rate in cattle, sheep and goats; whereas in Massailand a low ratio of human infection is associated with a high rate of infection in dogs and also in cattle, sheep and goats.[25]

Up to the 1950s infection rates in New Zealand ranked among those countries designated as hyperendemic areas.[28] However, after control efforts were intensified in 1959, the incidence of new cases dropped markedly.[13] In Australia prevalence has been considerably reduced on the island of Tasmania[68] by control measures initiated in 1965. High prevalence is still reported in parts of the States of New South Wales, Victoria and South Australia.[8,28]

1.8.1.1 Prevalence in man

There are no global prevalence data on human hydatid disease and only examples can be given. In Uruguay retrospective surveys of all medical and surgical centres on two occasions determined the number of hospital cases diagnosed annually during the 10 years 1962-1971.[80] The mean annual morbidity, whether expressed as the incidence of all hospital cases (20.7 per 100 000 population) or only new cases (17.7 per 100 000) indicated that Uruguay has one of the highest national morbidity rates for hydatid disease yet reported. Other high national figures for comparison were 12.9 cases per 100 000 in Cyprus,[65] 7.8 in Chile,[81] 7.5-8.3 in Greece,[62] 5.1-6.1 in Algeria,[76] and 3.7 in Yugoslavia[124] (see section 9.6 for recent reduction of echinococcosis in Cyprus). Infection rates expressed at the national level, however, often fail to reflect the true importance of the disease because all populations are not at equal risk. In most countries hydatid disease is most prevalent in rural areas. For example, in Uruguay the average annual incidence per 100 000 population was 123.3 for rural residents but only 10.1 for urban and suburban residents.[80] In Argentina the number of reported cases per year for the entire country varies between 1 and 2 per 100 000, but in several southern provinces the rate exceeds 150 per 100 000.[99] The same pattern is seen in many other countries in South America and elsewhere.

The use of diagnostic methods (radiology and serology) to detect asymptomatic cyst carriers indicates that symptomatic hospital cases represent only a fraction of total infections. For example, in the Province of Rio Negro, Argentina, where the annual incidence of hospital cases was 143 per 100 000, a mass miniature radiography survey of 15 000 persons demonstrated infection in 460 per 100 000, and these included only cyst infections with pulmonary localization, which represent only one-third or less of all infections.[104] The limitations of various surveillance techniques are discussed in section 2.3.

1.8.2 Echinococcus multilocularis

The geographical distribution of E. multilocularis has been reviewed recently.[61,85]

Echinococcus multilocularis is limited to the Northern Hemisphere.[85] Its known range includes an endemic region in Central Europe (southern and eastern France, Switzerland, southern Germany and western Austria) and most of northern Eurasia from Bulgaria and Turkey through the Soviet Union, extending eastward to several of the Japanese Islands (Rebun, Hokkaido, and Kuriles). In the Soviet Union the infection occurs in eastern and western Siberia, the upper and middle Volga districts, the Urals, the Caucasian Republics (Azerbaijan SSR, Armenian SSR, and Georgian SSR), Moldavian SSR, Kazakh SSR, and the Central Asian Republics (Kirghiz SSR, Uzbek SSR). Recent reports now include Greece, Czechoslovakia, northern Iran and India.[2,71,112,131] The last named represents the southernmost record of E. multilocularis.

The distribution of the cestode in North America appears to be discontinuous. It occurs at relatively high prevalence rates in the northern tundra zone, where its distribution corresponds closely to that of its most important definitive host, the arctic fox.[85] There is no present evidence that the cestode occurs in the zone of boreal forest, south of the tundra zone, but recent studies have demonstrated a large (and increasing) area of infection in south central Canada (Manitoba, Saskatchewan, and Alberta) and north central United States (North Dakota, South Dakota, Minnesota, Iowa, Nebraska, Montana and Wyoming).[57] The cestode may have been introduced to this region relatively recently by dogs brought from the endemic region in the north.[85] The availability of suitable hosts in adjacent areas presents a potential for further spread in North America.

At present E. multilocularis is not known to occur in the Southern Hemisphere. Reported cases of "alveolar hydatid disease" in Argentina and Uruguay[129,141] may have been atypical larval forms of E. granulosus or infection by E. oligarthrus or E. vogeli.

1.8.2.1 Prevalence in man

Few data exist on the incidence and prevalence of human alveolar hydatid disease. Reviews indicate that cases are seen frequently in parts of the Soviet Union,[61] northern Japan,[50] western Alaska, and Central Europe.[85] A recent report from Switzerland indicated an annual mean incidence of autochthonous cases of 0.14 per 100 000 population during 1965-1969.[21] Prevalence data are not reported from other endemic regions.

1.8.3 Echinococcus vogeli and Echinococcus oligarthrus

These species are indigenous to Central and South America. They are sympatric in several areas where ostensibly suitable hosts for both are present in humid, tropical forests as far north as the Isthmus of Tehunatepec in southern Mexico.[92] A report from Argentine Patagonia indicates that E. oligarthrus also occurs in temperate regions.[101]

1.8.3.1 Prevalence in man

The etiologic agent of a polycystic form of hydatid disease in man has been identified as E. vogeli,[19] but little is known of its prevalence. Echinococcus oligarthrus has not yet been identified as a cause of human disease.[92]

1.9 Epidemiology

The life cycle of Echinococcus species is complex involving two hosts and a free living egg stage. The dynamics of transmission of the parasite are determined by the interaction of factors associated with these two hosts and with the external environment. An understanding of these interrelationships and the way in which they influence the dynamics of the system, in particular its stability in the face of perturbation, is central for the planning and assessment of control programmes.

1.9.1 The adult worm within the definitive host

The number of worms harboured by dogs is determined primarily by the number of protoscolices ingested. Since asexual reproduction occurs within the intermediate host, extreme clustering can occur and worm burdens in the thousands are not uncommon. Dogs show a variable degree of natural resistance to infection and there is evidence that weak acquired immunity develops slowly. This immunity may affect both the numbers of worms that establish themselves in the host and their size. The biotic potential of Echinococcus spp. is low. Individual proglottids contain 200-800 eggs[6,91] and based on the growth rate of the worm it is estimated that one proglottid is shed approximately every 14 days.[30]

1.9.2 Proglottids and eggs in the external environment

Under favourable conditions, detached proglottids may remain active for a few days after being expelled in the faeces. They perform rhythmic contractions and relaxations that assist egg-expulsion[20,93] and may disperse the proglottids considerable distances from the faecal mass.[66] The majority of eggs remain free in the faeces, but evidence is accumulating that considerable dispersion of eggs occurs within a short time of deposition. For example, eggs of T. hydatigena have been shown to spread up to 80 m in only 10 days[38] and other observations suggest that small numbers are dispersed much further.[35] Since sheep generally avoid grazing areas contaminated by dog faeces, this dispersal enhances the chances of eggs being ingested. It also has important epidemiological implications because it means that a single heavily infected dog can be responsible for infecting intermediate hosts over a wide area.

Agents responsible for this dispersion have not yet been identified, although a variety of possibilities have been suggested. Wind[128] may account for some movement of eggs, but the observed radial uniformity of the dispersion[44] and the fact that the eggs are intolerant of desiccation suggests that this is not a major dispersion mechanism. An animal agent must, therefore, be suspected. The list of animals that contact faeces and have behaviour patterns that could generate the observed pattern of egg dispersal is extensive. However, birds,[110] flies,[47,97] beetles[9,10] and ants would seem to be the most likely to be involved.

Eggs deposited on the pasture are subject to the microclimate of the environment. They tolerate a wide temperature range. Heat damage does not appear to occur until temperatures reach 38°C[33] and the eggs withstand temperatures as low as -30°C[107,126] (see chapter 13). In contrast, they are very susceptible to desiccation and this factor probably dominates all others in restricting the survival of eggs of Echinococcus spp. in the natural environment.[55] As a principle applying to all taeniids so far studied, populations of eggs deposited by the definitive host appear to consist of eggs at various stages of maturity. The proportion of immature eggs would seem to differ markedly from one egg population to the next.[33] Immature eggs do not appear to activate in vitro and are probably uninfective to the intermediate host.[33] They may mature under suitable conditions.[109] Under favourable conditions mature eggs age and their life span is largely determined by the environmental temperature. This life span may vary from about three days to one year. Within certain limits the longevity of the eggs decreases as the temperature increases.[33] The aging of the eggs is characterized by a gradual loss of the ability of the oncosphere to activate in vitro and this is associated with a progressive decline in infectivity to the intermediate host.[17,49]

1.9.3 The larval parasites within the intermediate host

The density, infectivity and availability of the eggs in the environment and the feeding behaviour of the intermediate host together determine the number of infective organisms entering the host. However, the number of these that become established is strictly controlled by the host regulatory mechanisms. These consist of both a natural and acquired resistance to infection. Natural resistance causes parasite mortality at all stages during development and its extent may be related to the strain, age, sex and physiological condition of the host. Little is known of naturally acquired protective immunity in echinococcosis, but assumptions can be made from the extensive studies on other taeniid species. With these, acquired resistance develops after the ingestion of an initial immunizing dose of eggs. This response develops within two weeks[42] of the ingestion of as few as 10-50 eggs,[31,127] and prevents any further infection. The resistance is maintained throughout the life of the host by the continued ingestion of eggs, but may wane within 6-12 months in the absence of reinfection.[39] Immune mothers may transfer some degree of immunity to their offspring (e.g. via colostrum)[43,125] but animals do not appear to become fully immunologically competent to cestode infections until several weeks after birth.[41,140] The rapid development of immunity in competent animals means that the length of time that the intermediate host is susceptible to super-infection following the ingestion of the first eggs, is short (about two weeks). Thus, the events which occur during this period determine the larval worm burden for life. This means that grazing management can have a major influence on the level of infection.[32] This immunity also means that the majority of eggs deposited by the definitive host do not initiate infections. Thus, considerable regulation of parasite numbers by the host occurs at this stage.

In common with most host/parasite systems that have been observed, the hydatid organisms show an overdispersed or aggregated distribution within their hosts. The majority of hosts, therefore, contain only few parasites while a small number contain many. This distribution is generated by heterogeneity in the system and has been shown to have a vital role in determining the dynamics of transmission.

1.9.4 Parasite stability and its implications for control

The perpetuation of echinococcosis disease depends upon the common presence of the parasite, the definitive and intermediate hosts. The continued existence of host and parasite populations depends upon the fine balance of various interacting regulatory forces. The ability of the populations to survive perturbation, in the form of, for example, environmental change, without either becoming extinct or reaching plague proportions is termed stability. Theoretical work on the dynamics of host/parasite systems has indicated that such characteristics as overdispersion of parasite numbers within the host population and the development of host immunity act as important stabilizing influences.[3,11]

Control programmes are a form of man-made perturbation and it is evident that the stability of the host/parasite system determines to a major extent the effort required for their success. Evidence from the several control programmes against echinococcosis indicate that the stability of this system is quite fragile. Control programmes in Argentina, Cyprus, Iceland, New Zealand, Tasmania and the Falkland Islands have produced substantial reductions in the prevalence of echinococcosis in dogs, sheep and man, within relatively short periods of time.[34,36,40] This is in contrast to similar or concurrent programmes against other taeniid tapeworms such as Taenia ovis and Taenia hydatogena.[35] It would seem that although these parasites have similar life cycles, important differences exist that markedly influence their stability. These differences may include the low egg production of Echinococcus spp. and the occurrence of asexual reproduction within the intermediate host.

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CHAPTER 2

SURVEYS ON PREVALENCE AND GEOGRAPHICAL DISTRIBUTION OF ECHINOCOCCOSIS

2.1 Introduction

Surveys are of fundamental importance to:

(i) establish the importance of echinococcosis compared with other diseases;

(ii) obtain basic data and an insight into the processes of transmission;

(iii) provide base-line data for the subsequent establishment of control measures.

Continuing surveys (surveillance) provide the information on any changes in the prevalence brought about by the introduction of specific control measures or by such factors as changes in the standards of living or education. In this chapter, the intention is to provide information on some of the requirements for obtaining base-line data upon which the appropriate control measures can be defined.[10,23,26,38] Continuing surveillance is discussed in chapter 10.

2.2 Planning and collection of data

For the establishment of a control programme against E. granulosus, the type of minimal base-line information that should be obtained can be summarized as follows:[26]

(i) identification of the main agencies involved in assembling data on echinococcosis from hospital registers, serological laboratories, health institutions and universities as well as data from the livestock sectors;

(ii) documentation of the methods used for the collection, processing and evaluation of the data on both human and livestock and, where appropriate, wildlife echinococcosis;

(iii) assessment of the age-specific prevalence and geographical distribution of human, livestock and, where appropriate, wildlife echinococcosis;

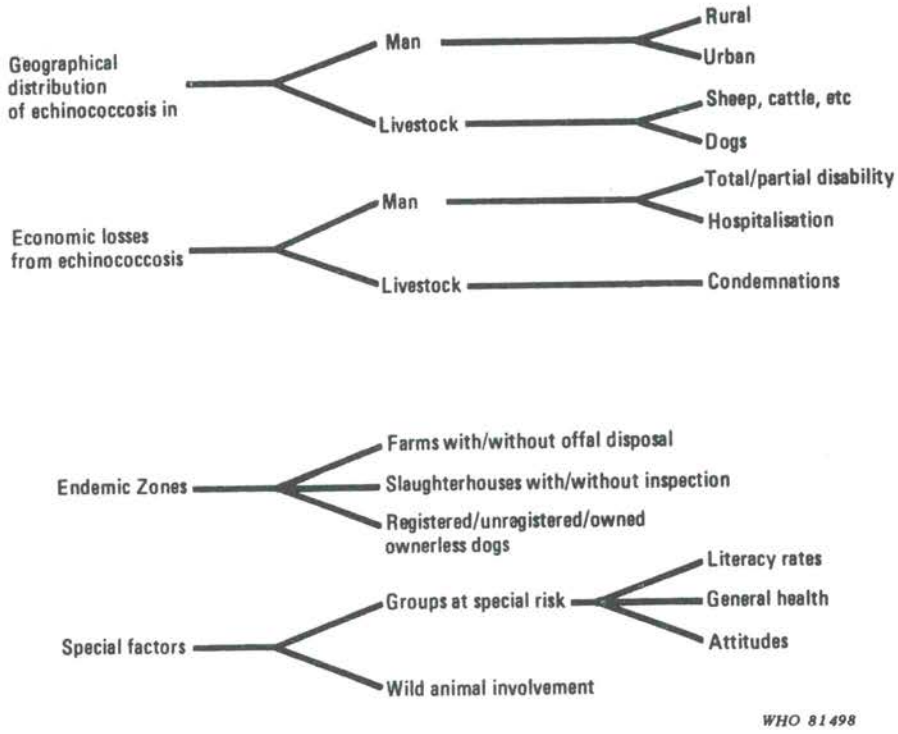
(iv) evaluation of the prevalence of E. granulosus in carnivores;

(v) correlation of data on canine echinococcosis with occupation and movement of dog and owner;

(vi) realistic 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 echinococcosis in slaughterhouses and meat packing plants.

Fig. 12 illustrates some of the factors requiring investigation in zones with fixed farming practices. It is, however, emphasized that many of the factors requiring investigation differ between the various endemic zones. For example, where nomadic farming methods are practised, special surveys are required. Studies of these type of factors in certain areas should be consulted.[4,5,16,30] A similar approach is required with E. multilocularis and other species. However, in these cases emphasis should be placed on the sylvatic aspects.

FIG. 12. BASE-LINE SURVEYS WITH INFORMATION REQUIRED TO INTRODUCE AN EFFECTIVE CONTROL PROGRAMME WITH FIXED FARMING PRACTICES (ADAPTED FROM [23])



Vital statistics on human and animal livestock populations and agricultural practices also provide valuable supplementary information for control programmes directed against E. granulosus.[26] These include:

(i) the total human population (divided into urban and rural) and individual livestock populations:

(a) of the statistical divisions of the country or of those areas in the country where echinococcosis has been regularly observed;

(b) of the proposed pilot control area;

(ii) the relative frequency of human parasitoses and zoonoses prevalent in the country or in the selected control area;

(iii) the number of:

(a) farms or premises where home slaughter is practised;

(b) slaughterhouses and/or meat packing plants with and without veterinary inspection.

Of importance is the need to select valid sampling techniques.[10] A biometrics section should be made available for designing the surveys, compiling and processing the data. This unit may either be responsible directly to the control authority or it may already exist within another recognized government agency.

2.3 Measurement of infection prevalence in man

The level of infection in human populations can be measured from hospital-diagnosed cases, mass miniature radiography, autopsies and sero-epidemiological surveys. Each has limitations.[26]

2.3.1 Hospital cases

An estimate of the public health problem of echinococcosis may be obtained by a survey of hospital cases.[6,7,17,32,35,39] Although this represents only that proportion of infections that reaches medical attention, the annual rates of hospital cases, when properly compiled, provide useful data on the significance of echinococcosis and, when measured continuously over many years, for detecting regional changes in infection incidence. The latter is surveillance.

In some countries where hydatid disease is notifiable, the hospital disease-reporting system may be sufficiently complete for the annual number of cases to be obtained from a central reporting organization; see for example, the data obtained for Cyprus,[31] New Zealand[1,17] and Tasmania.[29] These are, however, special examples where the data are used to estimate progress in control. These are discussed in section 10.4. Almost invariably, it is necessary for the investigator to obtain the data personally. A practical and systematic method is to determine retrospectively and completely the annual number of cases that have occurred over several years. The retrospective survey should include visits to all hospitals and clinics that perform thoracic and/or abdominal surgery. The decision must be made as to whether the survey report should include only surgically-confirmed new cases or whether radiologically non-operated but confirmed new cases should also be added (see section 10.4).

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 population. For epidemiological purposes, the rates should be broken down by age, sex, ethnic group, residence and occupation, etc. (An example of such data for New Zealand is given in 17.) From these data, the costs of the disease to the community in terms of total/partial disability and hospitalization can be estimated (see section 7.1). Furthermore, groups can be identified in which transmission is well above the average. Once identified, causal relationships can be investigated by statistical methods.[8,9,38] This is important because it is becoming increasingly evident that human behaviour plays a part in the epidemiology of transmission. For example, a higher risk of infection occurred among Maoris than among Europeans in New Zealand.[8,9,17] The reasons for this were found to be complex and involved land tenure, attitudes to dogs and socioeconomic factors. A high risk has also been reported among Basque Americans. This was found to be associated with their farming practices (transhumant).[5] Factors involved in other situations, however, are still not clear. For example, very high rates have been found among Turkana in north-west Kenya, but neighbouring tribes, with echinococcosis in their livestock, are rarely infected.[30] (See section 2.6 for measurement of sociocultural factors.)

2.3.2 Mass miniature radiography and ultrasound techniques

Mass radiological surveys using miniature photofluorograms have been used to determine the prevalence of echinococcosis.[32] There are considerable logistic problems that limit their application in the measurement of prevalence. Some factors that complicate the interpretation and reduce the usefulness of MMR include the difficulties in differentiating space-occupying-lesions, thereby including false-positive and false-negative information. Furthermore, their use is restricted to the area covered by the plate. The real value of MMR is that of a primary screening tool in the detection, for example, of asymptomatic lung-cyst-carriers, because these can be referred to a medical centre for further evaluation and inclusion in hospital records following identification of the parasite.

Research is in progress to adapt ultrasonic technology for field use, particularly for the identification of hydatid cysts in the abdominal cavity.

2.3.3 Autopsy series

The exact prevalence of echinococcosis within the human population can rarely be determined satisfactorily.[6,7] This is because of the large number of cases that are "silent" and do not cause clinical disease. In one study in Chile, echinococcosis was recorded over 20 years for persons dying violently. Here it was possible to demonstrate the extent of "silent" echinococcosis found incidentally at autopsy.[36] In certain circumstances it is possible to obtain good retrospective approximations of the exact prevalence from autopsy series. In Iceland, for example, autopsies have been mandatory since 1932 and it was possible to determine, retrospectively, the exact prevalence and thus the effectiveness of the control programme[12,13] (see section 10.4).

2.3.4 Sero-epidemiological surveys

Immunodiagnostic surveys are potentially useful for estimating the prevalence of echinococcosis within specific populations. It must be pointed out, however, that not all cases will be positive in serodiagnostic tests and that false-positive reactions may occur. Where hospital data are incomplete they may be the only way of measuring prevalence. Good sampling procedures are important, so that the data can be referred to the target groups selected for the survey. Methods for assessing the limitations of serodiagnosis of echinococcosis in surveys have been described.[28]

The relative value of immunodiagnostic tests for clinical diagnosis of hydatid disease is discussed in detail in chapter 3. It is increasingly suggested that the intradermal (Casoni) test using antigens from hydatid cyst fluid has very little value in epidemiological surveys on human echinococcosis.[42,45] The selection of an immunodiagnostic test as a screening tool to detect hydatid cases should be based on its simplicity, high sensitivity and specificity, and good correlation with the immunoelectrophoresis test (see chapter 3). The initial screen can be undertaken with rapid, relatively simple techniques such as latex agglutination (LA), or indirect haemagglutination (IHA), and reactive sera can then be tested in the arc 5 double diffusion (DD5) or the IEP test for diagnostic confirmation. Recent studies have shown that both the LA and IHA will detect all arc-5 positive sera; the lower nonspecificity of the LA test, its greater simplicity and its excellent correlation with the IEP test suggests that this is the screening technique of choice for use in sero-epidemiological studies of hydatid disease.[45]

The relative specificity of arc 5 (see section 3.3) is especially useful as the diagnostic criterion for surveys when results from different geographical areas are compared. It has been pointed out that since the immunoreactivity in hydatid disease cases and the intensity of cross-reaction may vary in immunological tests within and between geographical regions, it is necessary to establish titres of diagnostic significance for each region where the tests are applied.[26,33,43,44,45]

The main value of serodiagnosis, as with mass miniature radiography, is that of a primary screening procedure for detecting, for example, asymptomatic cyst carriers. These cases can be referred to medical centres for further evaluation and inclusion in the hospital records if the diagnosis is confirmed at surgery.

2.4 Measurement of infection prevalence in animal hosts

2.4.1 Livestock at killing establishments

Surveys of prevalence of echinococcosis in livestock are important for comparisons within and between regions and for determining the significance of each type of animal in the continuity of the life cycle.[26] 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 that appropriate comparisons can be made. The information required includes: (i) geographical distribution; (ii) age-specific prevalence; (iii) liver/lung cyst ratios; (iv) fertility of cysts. Where there is doubt

concerning the origin of the animals, the data should be excluded in the analysis of geographical distribution. Whole farm profile studies associated with livestock echinococcosis surveys are discussed in section 10.3.

Ideally, the surveys should be undertaken by experienced examiners and the same personnel should visit each slaughtering establishment, because this ensures uniformity and errors remain constant. This is particularly important if the surveys are to be utilized as base-line data.[19] Information on base-line and continuing surveillance are discussed in sections 5.2 and 10.3.

Care must be taken to ensure that E. granulosus is positively identified. Of particular importance is the differentiation of metacestodes of E. granulosus from those of T. hydatigena in sheep and pig livers.[19,20,40] There are six main types of parasitic lesions due to larval cestodes. These are: (i) viable cysts of E. granulosus; (ii) viable cysticerci of T. hydatigena; (iii) dead cysts of E. granulosus, which may be necrotic, calcified or caseous; (iv) dead cysts of T. hydatigena, which may be necrotic, calcified or caseous; (v) tracks, pits or scars, which may result from migrating larvae of T. hydatigena; (vi) "white spot", which may be caused by either species of helminths.

The first five parasitic lesions described above in general present few problems in identification to the experienced examiner, but "white spot" lesions, which form the majority of lesions in young animals, may cause considerable difficulties. The following classification may be found to be useful: (i) "white spot" lesions within Glisson's capsule or above the surface of the liver substance (subcapsular) or associated with a track, pit or scar, are likely to belong to T. hydatigena; (ii) "white spot" lesions unassociated with migration and within the liver tissue substance should be classified as of unknown etiology, because they may belong to either E. granulosus or T. hydatigena.

2.4.2 Dogs

Surveys on E. granulosus in dogs are fundamental in determining the factors involved in transmission and causal relationships involving infection in man.

2.4.2.1 Autopsies

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 and pet dogs, hunting, stray) to evaluate completely the epidemiological factors involved or causal relationships for infection in man. The method has most application as a survey tool where feral and unwanted dogs are available. The data provide useful information on the prevalence of E. granulosus in these classes of animal on a geographical basis. Methods of examining dogs at autopsy and identifying E. granulosus are described in section 6.1.

2.4.2.2 Arecoline surveys

The limitations and value of treating dogs with arecoline hydrobromide in surveys on echinococcosis are now well known.[20,21,26] Despite the limitations, these surveys, if combined with sociological surveys, can be very useful in determining the epidemiological and sociological factors involved in transmission and the causal relationships for varying infection rates of hydatidosis between ethnic and other social groupings within the human population. Furthermore, an estimate of the infective pattern (variability in worm counts) may reveal groups of dogs that are highly susceptible to infection, thereby increasing the infection pressure above the average.

Standardization of the technique is important if comparisons are to be made between the various factors considered to be important. For the survey data to be comparable they should be accompanied inter alia by information on the following factors: (i) methods used for treating the dogs; (ii) interval between treatments; (iii) the thoroughness with which

doubtful samples have been eliminated from the analysis; (iv) the amount and type of material examined; (v) the method used to separate worms from faeces; and (vi) the visual aids used to examine the samples.

The value and limitations of arecoline hydrobromide in base-line and continuing surveillance in control programmes are discussed in sections 6.2 and 10.2.

2.4.3 Wild animals - sylvatic cycles and "spill-over" situations

All species of Echinococcus require surveys to determine their host range. Effective evaluations on wildlife echinococcosis depend on examining relatively large numbers of animals and on transmission studies. Methods of capture and sampling wild animals are described in appropriate journals.

An important reason for adequate surveys on echinococcosis includes a determination as to whether transmission is dependent on or is independent of domestic animals. In the first situation, effective control of echinococcosis in domestic animals would also modify the parasite population in wild animals. If, however, transmission is independent, control of the domestic life cycle would not affect the sylvatic life cycle. For example, the dingo/wallaby life cycle for E. granulosus in Australia is independent of the domestic dog/sheep life cycle, although they may overlap.[11,14,18,27,41] Control directed against the dog/sheep life cycle could not be expected to affect the dingo/wallaby cycle, and cattle, the incidental host, could be expected to continue to be parasitized. In constrast, infected foxes and hares in Argentina probably represent a "spill-over" situation.[34] If so, control of the domestic life cycle could be expected to reduce the parasite population in these wild animals. A similar result would be expected if feral intermediate hosts such as sheep and goats were involved.

Evidence of wildlife involvement provides very good reasons for undertaking extensive surveys and transmission studies before embarking on a control programme. In the event of the introduction of a control programme involving "spill-over" situations, the "spill-over" hosts should play no essential part in the control programme. This is because they neither need treatment nor do they serve as indices of control progress.[26] For this reason, surveys on feral and wild intermediate hosts should be made at the commencement and towards the end of the control programme.

2.5 Measurement of environmental contamination with eggs

Almost immediately after eggs of E. granulosus have been deposited in the faeces, extensive dispersion occurs. The agent or agents responsible for this dispersion have not yet been positively identified, but birds, flies and beetles have been implicated (section 1.9). In areas containing large numbers of infected dogs, the environment may be contaminated with millions of eggs. Even a single heavily infected dog may be responsible for significant contamination over wide areas.[24]

The presence and concentration of eggs of Echinococcus in the environment can only be determined by the use of appropriate "sentinel" animals, such as sheep, pigs and goats that have been reared in a cestode-free environment and which are placed for about two weeks in the area to be investigated. They are killed after an appropriate time interval (such as six months if E. granulosus is suspected) and the cysts that have developed are counted. Although not giving the absolute numbers of eggs present, this method provides an indication of the infection pressure.[24,25]

2.6 Measurement of sociocultural factors

Echinococcosis is a parasitism of the human community and its animals. It may be manifest at both rural and urban levels. At the rural level, the community may consist of individual units such as a farmer, his family, other workers and his livestock, or it may consist of a whole village community with all the village livestock integrated into a single

unit. At the urban level, it is usually represented by a "spill-over" situation, but may also be an independent entity involving urban dogs and local livestock.

Cultural practices and social structures, land use, life styles, traditions and relationships with animals may enhance transmission of the parasite. Therefore, the survey must involve the collection of information on the several aspects of the habits and life styles of the target population, especially the attitude to dogs and to their proposed elimination.

These community profile studies should be actively encouraged as part of the base-line data because they provide information on the correct strategies for health education to ensure community participation. The forced introduction of techniques and education programmes socially unacceptable to the community may be counterproductive.

2.7 Measurement of ecological factors

An appreciation of ecological factors that affect transmission is important. Epidemic disease is thought to indicate a state of ecological imbalance and the endemic situation to denote a balanced state.[37] Echinococcosis can have either an epidemic- or endemic-type pattern. It has been recognized for a long time that there is usually a firm association between biotic factors such as the parasite, the hosts and the dispersion of the free living egg on the one hand and the abiotic factors such as geographical landscapes, weather and soil type on the other. These and other factors including the changes made by man may produce different circumstances for parasite survival. Quite frequently, the epidemiology undergoes a marked change in the habitat between two landscapes or at the interface between adjacent forms of land use (e.g. arable and pastoral).

Some final and intermediate hosts involved in the transmission of echinococcosis have well defined geographical and seasonal limits. These are determined by environment factors such as climate and soil type, which in turn influence vegetation and thus the spatial distribution of wildlife. For example, E. multilocularis in some arctic regions, such as St Lawrence Island, has seasonal high and low prevalences in both definitive and intermediate hosts depending on the abundance and predator-prey relationships of the normal host assemblages.[15] Movements of wild animals in annual migrations and domestic animals in treks to grazing areas, water holes and to market also provide opportunities for the seasonal spread of E. granulosus.

There is now convincing evidence that tapeworm eggs are widely and rapidly dispersed from the focus of deposition (see section 1.9). Extreme weather patterns and seasonal changes may affect the survival of eggs as well as the activity of the agents concerned in dispersal and their availability. Sometimes this benefits parasite survival and at other times it limits transmission.[22] For example, hot dry weather may reduce the longevity of eggs but increase the opportunities for egg dispersal, for example by invertebrates. It may also modify the spatial distribution of the animal hosts. This may prevent transmission. On the other hand, if it leads to a congregation of animals around water holes it may enhance transmission, even to the point of providing opportunities for transmission to hosts not normally involved. Moderate cold conditions, enhancing egg survival but limiting egg-transport hosts, may provide another seasonal epidemiological situation modifying transmission. The collection of meteorological data at the local level is of use for understanding factors involved in modifying transmission.

On the large scale, where migratory factors are involved, ground sampling, aerial censusing and remote sensing techniques can be used.[46] Aerial surveys have been successfully used in population studies related to echinococcosis among nomadic Turkana.[2,3]

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CHAPTER 3

IMMUNODIAGNOSIS OF HYDATID DISEASE IN MAN

3.1 Introduction

The current status of immunodiagnosis of hydatid disease has been reviewed in recent articles.[22, 35, 39, 49, 51, 56] Immunodiagnosis may be based on the detection of humoral or cellular immune responses of the host against the parasite.

3.2 Immune response in hydatid disease

Circulating IgG, IgM, IgA and IgE antibodies to Echinococcus antigens have been detected in the sera from individuals with cysts of E. granulosus in the liver or lung[14, 31, 40, 57] but it is not known how soon after infection antibodies first appear, and in some infections the antibody response may be greatly delayed or even undetectable. Levels of IgG are more frequently increased than levels of IgM and IgA. After surgical removal of cysts, IgM levels may reach normal values within four to six months in cases of pulmonary and within 12 months in hepatic hydatidosis, while elevated IgG levels can persist much longer than this.[57] Results obtained with radioallergosorbent tests (RAST) show that the percentage of patients with significantly increased specific IgE varies between 30-90%.[18, 33, 48, 70] In a few cases, high levels of specific IgE may be present without a demonstrable increase in other immunoglobulin classes.[31]

There is no distinct correlation between levels of IgG and IgM and antibody titres measured by the indirect haemagglutination and complement fixation tests.[40] It must be concluded, therefore, that only part of the immunoglobulins produced after Echinococcus infection is parasite specific. A cellular immune response in patients with hydatid disease has been demonstrated using antigen induced lymphocyte transformation.[42, 78] At present this is not applicable for routine diagnosis.

3.3 Relative merit of immunodiagnostic tests

Many immunodiagnostic techniques have been evaluated for hydatid disease.[35, 39, 49, 56] Although results of most serodiagnostic tests agree fairly well, sera from some patients are negative in one or more tests but positive in others. On the basis of recent experience it is now widely accepted that the sensitivity and the specificity of immunodiagnosis can be improved by the simultaneous application of two or more methods (partly measuring different classes of antibodies). They include, for example, the indirect haemagglutination (IHA) test and other particle agglutination tests (latex (LA) and bentonite), immunoelectrophoresis (IEP) and double diffusion (DD) tests, the indirect immunofluorescent (IIF) test, and the enzyme-linked-immunosorbent assay (ELISA).

An important consideration is that tests based on the detection of antibody to the Echinococcus antigen 5 have the highest degree of specificity. However, because antibody to antigen 5 is sometimes absent in the serum of patients with hydatid disease who have antibodies reactive with other echinococcal antigens, serum specimens should be screened initially with one or more highly sensitive procedures and then tested for antibody to antigen 5. Tests such as IHA, IIF, LA and ELISA are highly sensitive in detecting circulating antibodies in sera from patients with hydatid disease.[22, 39, 49, 51] The IHA and LA tests are recommended for initial screening because they have been shown to be highly efficient for detecting sera with antigen 5 antibodies and both are relatively simple procedures[28, 29, 36, 38, 59, 65, 66, 69, 72] (see section 2.3.4).

Antigen 5 is one of 10 or more distinct antigens of parasite origin present in hydatid fluid and somatic tissues of the metacestode. This antigen corresponds to antigen "A" and antigen "4".[8, 20, 43, 44, 46, 73] Immunoelectrophoretic studies have shown that the presence of antibody to this antigen in a patient's serum is diagnostic for hydatid disease[12] and it

has been considered to be specific for E. granulosus. The diagnostic importance of the antigen 5 has been confirmed by several workers.[13, 47, 65, 75, 76] However, antibodies against antigen 5 were demonstrated in sera of some patients infected with larval E. multilocularis, E. vogeli or Taenia solium,[64, 68, 77] and moreover, antigen 5 was found in larval E. granulosus and E. multilocularis as well as in larval Taenia hydatigena.[50, 67] In recent studies, antibodies to antigen 5 were demonstrable in sera of approximately 5-10% of human cysticercosis cases.[51, 53] Therefore, the use of antigen 5 in serology does not allow a diagnosis specific for the Echinococcus species, and cross reactions with some cysticercosis cases must be expected in areas where Taenia solium occurs. The full impact of the specificity of antigen 5 remains to be determined.

The immunoelectrophoresis (IEP) test has been most widely used to detect antigen 5. Recently, a double diffusion (DD 5) test has been described[15] in which a control antiserum is used to recognize antigen 5 positive sera (see section 3.6.6). The DD 5 test is as specific as and more sensitive than IEP and thus it may provide an effective substitute for the IEP test.

The intradermal (ID) (Casoni) test is less specific, but when properly controlled, it compares favourably in sensitivity with most serologic tests. Nevertheless, even with partially purified antigen solutions used at low nitrogen concentration, high rates of false-positive reactions have been observed in persons with other parasitic diseases, as well as in some non-parasitic pathologic conditions, especially when antigens with a high protein content are used.[35, 52, 76] Further, skin testing may stimulate the production of circulating antibodies.[31] Therefore, it has been suggested that the ID test should be replaced by serological procedures wherever possible[4] (see section 2.3.4).

3.4 Interpretation of immunodiagnostic results

In the interpretation of serological results many factors have to be considered. The performance of any serological test requires experience and careful and continuous control. Even minor technical errors may influence the results. Negative serological results do not rule out an Echinococcus infection because some of the individuals do not have detectable antibodies.

Immune responses have been associated with organ localization, the integrity of the metacestode and the size of the parasite biomass. Hepatic and peritoneal echinococcosis generally elicit stronger antibody reactions than infections of the lung, brain and eye.[1, 8, 29, 30, 34, 45, 59, 60, 61] Children between three and 15 years old were found to produce weaker serological reactions than adults.[57] Leakage or rupture of the cyst or surgical intervention leading to antigen release may be followed by an abrupt stimulation of antibodies as early as 10 days after the event.[57] Dead metacestodes may cease to stimulate the host and carriers of such parasites may become seronegative.[12, 16] The host-parasite relationships may also influence the immunodiagnostic results. Some differences in reactivity have been observed in comparative studies of patients infected with E. granulosus or E. multilocularis.[10, 29, 37] Human infection with the northern sylvatic strain of E. granulosus is characterized by more frequent pulmonary localization, slower and more benign growth than the classic pastoral strain and may be associated with diminished antigenic stimulation.[31]

Recent unpublished data on serodiagnosis of echinococcosis in Turkana indicate reduced reactivity to procedures which elsewhere would be expected to give reliable results.

3.5 Immune response after treatment

3.5.1 Immune response after surgery

Extensive studies on antibody persistence after surgical treatment of E. granulosus cases have been carried out recently.[58, 62] using IHA, LA, bentonite flocculation (BF), the complement fixation (CF) and the ID tests. Whereas the ID remains positive for many

years, serological results may provide some indications of the effectiveness of surgical intervention. A favourable prognosis can be envisaged in the following cases: (i) if the serological tests are negative before the operation and remain so up to the end of the first year after surgery; (ii) if the titres are low before operation, become negative afterwards, and remain negative for one-and-a-half years; and (iii) if the decrease in titres is small, but the downward trend continues up to the end of the second year after operation. A recurrent infection is considered likely: (i) if during one-and-a-half years after operation titres remain high or show only slight fluctuations without a marked drop, or (ii) if the titres drop after operation and then rise steadily. Prognosis should be based on at least four serological examinations, namely one, six, 12 and 24 months after surgery.[58, 62]

Experience with E. multilocularis cases is limited. In three cases with liver involvement, a more or less constant persistence of IIF and IHA antibody titres over four to five years after surgery has been observed. All patients died from the infection. In eight surviving cases, there was a considerable decline in the titres within one to three years after radical surgery. However, the significance of this observation was uncertain.[22]

3.5.2 Immune response after chemotherapy

Preliminary observations with IHA and IIF tests indicate that follow-up studies of antibody titres may not be suitable for the evaluation of drug efficacy against Echinococcus metacestodes.[2, 6, 22]

In four E. granulosus cases, it was found that after commencement of mebendazole therapy an initial increase of specific IgE levels occurred, followed by a steady decrease within several months, while total IgE concentrations remained relatively stable. The concentration of immune complexes increased during the same period.[6]

In four patients with E. multilocularis infections the IgE concentrations initially increased in three individuals and did not return to normal in any of the four persons during the six months after the commencement of treatment with mebendazole. This may have been due to persistent antigenic stimulation.[74]

In a study of 30 cases of E. granulosus infection, only 30% were found with raised levels of specific IgE antibody and immune complexes in their serum.[17] Thus, these data indicate that serological monitoring using parasite specific IgE response may be useful only in a small proportion of cases.

3.6 Description of immunodiagnostic techniques

3.6.1 Introduction

Among the numerous immunodiagnostic methods (see section 3.3), there are some that are practicable under field conditions with relatively simple laboratory equipment. These methods are the latex agglutination (LA) test, and the indirect haemagglutination (IHA) test, both recommended as primary screening tests (see section 3.3). Subsequently, the more specific arc 5 double diffusion (DD 5) test can be employed. The intradermal (ID) (Casoni) test may be used in suspected hydatid disease cases in which serum antibodies are not detectable. The relevant techniques have been reviewed[39] and more detailed information on the immunoelectrophoresis, latex agglutination, indirect haemagglutination, and arc 5 double diffusion tests can be obtained from publications[5, 63] of the Pan American Health Organization/WHO.[a]

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[a] Human Hydatidosis. Immunodiagnostic Techniques. Series of Scientific and Technical Monographs No. 7. Pan American Zoonoses Center, Buenos Aires, 1976. Human Hydatidosis. Immunodiagnosis by the Arc 5 Double Diffusion Test. Technical Note No. 22. Pan American Zoonoses Center, Buenos Aires, 1979. Address: Pan American Zoonoses Center, Casilla de Correro 23, Ramos Méjia, Buenos Aires, Argentina.

3.6.2 Blood sampling

Although the serological methods described below only require small amounts of serum, at least 1-2 ml should be obtained as examinations may have to be repeated. Moreover, it is advisable to retain a serum sample until the case is fully clarified.

Blood samples are collected under antiseptic precautions and are allowed to set at room temperature. Clotted samples are kept overnight in a refrigerator or cold room (2-4°C). After centrifugation at 700 g for 15 minutes, sera are carefully removed and stored in a refrigerator (for a few days) or deep frozen at -20°C (longer periods) until tested. When sera have to be sent to distant laboratories, precautions have to be taken to preserve them. If facilities are available, sera should be lyophilized, if not, merthiolate (Appendix 1) should be added as a preservative.

3.6.3 Antigen preparations

(a) For agglutination tests and ELISA

The antigen most commonly used is hydatid fluid of E. granulosus; it must be of high quality. Infected livers or lungs from recently slaughtered animals (sheep, cattle, pigs, horses, etc.) are transported without delay to the laboratory, if necessary under chilled conditions. Hydatid fluid is aspirated from healthy cysts (preferably from fertile ones) using a needle and syringe. The contents of suppurated or calcified cysts must not be used. Samples are collected in a graduated cylinder in which the fluid is left for about 30 minutes in order to allow sedimentation of protoscolices and other coarse particles. The clear fluid is then transferred to centrifuge tubes and centrifuged at 1000 g for 15 minutes. The supernatant fluid is stored at -20°C.

In some laboratories, the "raw", unprocessed antigen (R-antigen) is used in serological tests, in others it is dialysed and then lyophilized (DL-antigen). Methods for partial purification of Echinococcus antigen have been described.[8,20,21,43,44,46,73] New batches of antigen should be compared for their reactivities with antigens and sera of known reactivity by conventional serological tests, such as the IHA test and immunoelectrophoresis.[63] A limited number of serum samples with known reactivities can be obtained from certain laboratories.

(b) Preparation of antigen for indirect fluorescent antibody test[1]

Protoscolices are isolated from E. granulosus cysts which contain clear hydatid fluid. By repeated sedimentations the protoscolices are washed several times in PBS (Appendix 4). They can be used immediately or may be kept deep frozen at -20°C for at least several months.

Thin layers of muscle (about 2 x 2 cm) are obtained from the abdominal wall of rats. Single pieces of muscle are formed into small cylinders by rolling them around a glass or metal rod of about 3 mm diameter. After holding at -20°C for some hours the rods are removed and the cylinders are stored in plastic bags at -20°C until use.

For further processing, both ends of a frozen cylinder are cut off with a scalpel. One end of the cylinder is then attached with some drops of PBS to a glass slide or to a metal object holder previously kept at -20°C for at least one hour. In order to achieve firm attachment, the cylinders may be transferred to a deep freeze for an adequate time.

The cylinder is filled with a dense suspension of protoscolices using a Pasteur-pipette. It is again deep frozen and can be stored for some months in this state or cryostat sections can be prepared immediately.

Cryostat sections of 6-7 μm thickness are prepared and transferred to acid-washed multispot-slides (Appendix 6) at room temperature, by bringing the slide close to the microtome knife surface so that the section becomes attached. The slides are then stored in plastic boxes at -20°C until use. Under these conditions, the antigen remains active for at least two months.

3.6.4 Latex agglutination (LA) test

A. Principle

Latex particles, coated with antigen of Echinococcus are agglutinated upon contact with corresponding antibodies.

The test was introduced in 1960[26] for the diagnosis of hydatid disease and was later modified.[55,63,72]

B. Procedure I (after 63)

B.1 Latex particles

Polystyrene latex particles of approximately 0.22 μm in diameter are used. These particles may be obtained commercially in a 5% v/v suspension. The stock solution is prepared by diluting the commercial preparation 1:4 with glycine buffer pH 8.2 (Appendix 2).

B.2 Determination of optimal antigen concentration

24 mm of the lyophilized antigen (see section 3.6.3(a)) are dissolved in 2 ml glycine buffer pH 8.2 (Appendix 2). After centrifugation for 30 minutes at 2000 g, the supernatant is carefully transferred into a tube marked as No. 1.

Antigen dilutions are prepared according to the following scheme (Table 5) in three further tubes numbered as 2, 3 and 4.

TABLE 5. ANTIGEN DILUTION SCHEME

Tube number	1	2	3	4
Glycine buffer pH 8.2 (ml)	-	0.25	0.50	1.25
Antigen solution from tube 1 (ml)	-	0.5	0.25	0.25
Final antigen concentration: mg/ml	12	8	4	2

Thereafter sensitization of latex particles with several antigen concentrations is carried out as follows:

- Prepare four tubes marked with numbers 1-4.
- Place aliquots of 1.5 ml glycine buffer pH 8.2 in each tube.
- Add 0.5 ml of antigen solution from the corresponding tubes (Table 5).
- Add 0.3 ml of the latex particle stock solution (see B.1) to each tube.
- After shaking gently the flasks are incubated at 37°C for 30 minutes and then at 4°C for 24 hours.

The suspensions of latex particles sensitized with the various hydatid fluid antigen concentrations are used to examine 10 positive sera from hydatidosis patients, 10 sera from persons with other parasitic conditions and 10 sera from healthy donors. These sera are also examined in a parallel test with the latex reference reagent.[a] The sensitized latex suspension whose results coincide with those of the reference preparation is selected for use. The hydatid fluid concentration correspondingly selected for sensitization constitutes the optimal concentration for this lot of antigen for future use.

The lyophilized hydatid fluid pool selected above may then be used routinely at the pre-determined optimal concentration for the sensitization of latex particles.

B.3 Preparation of sensitized latex particles for routine use

1 ml of antigen solution, at the optimal concentration (see B.2) is added to 3 ml of glycine buffer pH 8.2 (Appendix 2). This is followed by the addition of 0.6 ml stock solution of latex particles (B.1). The mixture is agitated, incubated and stored as described under B.2.

B.4 Testing procedure

Sera

Each serum to be examined is diluted 1:5 with glycine buffer pH 8.2 (Appendix 2) and inactivated by heating in a water bath for 30 minutes at 56°C.

Agglutination test

One drop (0.02 ml) each of the inactivated 1:5 dilution of serum and the sensitized latex particles are placed on an agglutination plate and mixed with a toothpick. The plate may be a simple glass plate on which several fields have been ringed with a marking pencil. The plate is gently rocked for eight minutes and the reaction is then read against a dark background.

Reading

Agglutination of latex particles after eight minutes is indicative of a positive test. In negative sera, the particles remain in suspension.

Control sera

Each time the latex agglutination test is performed, it is necessary to include a known positive serum and a known negative serum as controls.

Latex control

Although the phenomenon is not frequently observed, some sera are known to agglutinate nonsensitized latex particles. It is recommended, therefore, that all sera which agglutinate sensitized latex particles be subjected to a control test for agglutination using nonsensitized latex particles.

A dilution of 0.3 ml of the stock suspension of latex particles (B.1) in 2 ml of glycine buffer pH 8.2 constitutes the reagent of nonsensitized latex particles. The testing procedure is as described under B.4.

The agglutination of sensitized latex particles is of no diagnostic significance in sera that agglutinate nonsensitized latex particles.

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[a] This reference reagent may be obtained upon request from the Director of the (PAHO/WHO Pan American Zoonoses Center, Casilla 3092, Correo Central, 1000-Buenos Aires, Argentina.

C. Procedure II (after 26, 27)

The original technique[26] has later been slightly modified.[19,27] It differs from the technique described under B mainly in that raw hydatid fluid and another latex preparation is used.

C.1 Latex particles

Bacto-Latex 0.81, a standardized suspension of latex particles having an average diameter of 0.81 µm is used (Difco Laboratories, Detroit, Michigan, United States of America[a]). This suspension is stable to the expiry date on the label when stored at 2-8°C.

C.2 Determination of optimal antigen

Unprocessed "raw" antigen (see section 3.6.3(a)) is used for the test.[26] In preliminary tests, several batches of cyst fluid are used to sensitize latex particles. Their reactivity is tested against several positive and negative control sera. The batch with adequate sensitivity and specificity is used for routine diagnosis (several concentrations may also be tested as indicated under B.2).

C.3 Sensitization of latex particles

The reagent is prepared by mixing 0.5 ml potent hydatid cyst fluid (see C.2) with 0.5 ml of the Bacto-Latex 0.81 suspension. After gentle shaking, the mixture is ready for use after about one hour at room temperature. It is stable for two to three weeks, if kept refrigerated.

C.4 Testing procedure and controls (see B.4)

Sera to be tested are inactivated in a water bath at 56°C for 30 minutes. One drop of coated latex particles and one drop of serum or serum dilution (1:4, 1:8, etc.) are mixed on a glass slide or plate and gently rotated either by hand or mechanically. A positive result is recorded when macroscopically visible agglutination is seen within five minutes. Prozone effects may be seen with strongly positive sera.

Negative and positive control sera must be included in each batch of sera tested. Also, a latex control should be carried out (see B.4).

D. Sensitivity and specificity

The overall sensitivity of the LA test in cases of hydatidosis was found to vary from 50% to 92% in older studies.[34] Recent work[60] indicated a sensitivity of 71% of 90 patients with pulmonary hydatidosis and of 89% in 124 cases of hepatic hydatidosis, the overall sensitivity being 80%. In the same study, the IHA test exhibited sensitivities of 71% (pulmonary hydatidosis), 95% (hepatic hydatidosis) and 83% (all cases), respectively. The LA test sensitivity is also comparable with the ELISA.[41] The percentage of false positive reactions in patients with other parasitic and miscellaneous diseases is generally recorded to be low, but may be as high as 9.1%[34] and 6.7%.[60] The latter value was comparable to nonspecific reactions of the IHA test.[60]

3.6.5 Indirect haemagglutination (IHA) test

A. Principle

Red blood cells are coated with hydatid antigen. The coating of erythrocytes is enhanced by treatment with tannic acid. Coated cells are agglutinated upon contact with corresponding antibodies.

This technique, introduced in 1957,[28] is now usually performed as a microtitration procedure.[63] It has proved to be reliable and sensitive.

[a] Mention of commercial firms or their products does not imply endorsement by the World Health Organization.

B. Procedure (after 63)

B.1 Sheep blood cells

Blood is withdrawn aseptically from a sheep into a sterile flask containing one volume of Alsever's solution (Appendix 3) or 1.2 volumes of 3.8% sodium citrate solution for each volume of blood. The suspension is distributed in aliquots and stored at 4-6°C for at least four days. Blood preserved in this manner may remain of use for three weeks.

B.2 Washing the blood cells

An aliquot of the blood is centrifuged at 800 g for 10 minutes and the cells are washed twice in saline and once in PBS, pH 7.2 (Appendix 4). Finally, a 5% blood cell (SBS) suspension is prepared in the same buffer.

B.3 Tanning the blood cells

The 5% SBS suspension is mixed with an equal volume of a solution containing 50 µg/ml of tannic acid in PBS. The mixture is incubated at 37°C in a water bath for 15 minutes, with periodic gentle shaking and then washed twice in PBS. After the last wash, the tanned red cells are resuspended to a 5% concentration in PBS and divided into two aliquots. One bottle is set aside for use as a source of uncoated cells for absorbing the heterophile agglutinins in the sera to be tested and for various controls. The other bottle contains the cells which are to be coated with antigen. As different batches of tannic acid vary in their suitability for use in this procedure, this must be checked each time a new batch is to be used.

Alternatively, red cells can be used which are fixed in formalin[7] or other fixatives[49] and afterwards tanned.

B.4 Sensitization of sheep blood cells

A 5% suspension of tanned cells in PBS is mixed with an equal amount of the optimal sensitizing concentration of hydatid fluid antigen. The mixture is incubated at 37°C for 30 minutes, with occasional shaking. The optimal sensitizing concentration of antigen is determined as described below.

After incubation, the coated cells are centrifuged at 750 g for five minutes and the supernatant is discarded. These sensitized cells and the nonsensitized cells already set aside (see above) are washed three times with PBS containing 1% previously inactivated and absorbed normal rabbit serum (Appendix 5), centrifuged at 750 g for five minutes on each occasion and the supernatants discarded. Finally, both batches of cells are made up to a 1% concentration in PBS which also contains 1% rabbit serum.

B.5 Determination of the optimal sensitizing concentration of hydatid fluid antigen

Solutions of "raw" or lyophilized hydatid fluid antigen (see section 3.6.3(a)) are prepared in PBS (pH 6.4) in 2.5 ml volumes at concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml.

A 2.5 ml aliquot of the 5% tanned SBC suspension is added to each dilution. A tanned SBC control is also set up without antigen. The sensitization procedure is then carried out as described above. Finally, all the coated cells and the uncoated cell controls are tested against a few selected known positive anti-Echinococcus human sera and negative sera from healthy donors by the IHA microtechnique, as described below. On the basis of the results, the system which gives the highest titres with positive sera and does not show haemagglutination with negative sera is then selected as the optimal antigen concentration for use in the test.

B.6 Testing procedure

The sera to be tested usually are inactivated for 30 minutes at 56°C. Microtitration equipment and plastic trays with U-shaped wells are used. Aliquots of the inactivated undiluted sera (0.1 ml) are absorbed by adding to each 0.9 ml of the uncoated cell suspension. The mixture is left on the bench for 15 minutes and then centrifuged at 750 g for five minutes to recover the serum, which is now at a dilution of 1:10. This procedure removes the antibodies directed against red cell determinants. Routine absorption may not be necessary in certain parts of the world. Doubling dilutions of the serum samples are then prepared from 1:20 to 1:40 000 using a calibrated micro-diluter in 25 µl volumes. To each well is added 25 µl of the 1% suspension of sensitized cells using a calibrated micropipette dropper. Controls with sensitized cells in diluent only and with nonsensitized cells with and without serum are also set up.

The plate is left at room temperature for one hour after which the reading is made.

Reading

A negative reaction consists of a discrete button of cells on the floor of the well. A smooth mat of cells wider than the control button is indicative of a positive reaction.

B.7 Criteria for positivity

Titres significantly above the negatives are considered positive. This value must be chosen on the basis of the titres obtained with negative sera from healthy donors and with sera from patients with other illnesses, in order to discriminate nonspecific reactions.

C. Sensitivity and specificity

In a recent study[60] a sensitivity of 71% and 95% in pulmonary and hepatic E. granulosus infections respectively were obtained. The overall sensitivity was 83%. In 16 cases of E. multilocularis infection, a sensitivity of 100% was obtained.[29] The percentage of nonspecific reactions is mostly under 10%,[34,60] and if the diagnostic titre in the area has been correctly determined, nonspecific reactions should be well below this level.

3.6.6 Arc 5 double diffusion (DD 5) test

A. Principle

The detection of antibodies against E. granulosus antigen 5 may be of value for the final confirmation of human hydatidosis (see sections 2.3.4 and 3.3). The DD 5 test[15] reveals the presence of these antibodies in the serum of a patient through a reaction of identity with a control antiserum against antigen 5. This antiserum is obtained by the artificial immunization of an animal and the dilution of the antiserum to a point where it reveals only antigen 5 under the conditions of the test.

B. Procedure

The technical procedure has been published in detail by the Pan American Zoonoses Center.[15]

C. Sensitivity and specificity

A sensitivity of 78% in 95 E. granulosus cases was reported for the DD 5 test as compared with 85% of the LA test and 71% of the immunoelectrophoresis test. 335 sera of patients who were shown not to have hydatidosis were negative in all tests.[15]

3.6.7 Indirect immunofluorescent (IFA) test

A. Principle

Antibodies reacting with antigen in sections of protoscolices are detected by fluorescein labelled anti-immunoglobulin labelled sera. The test was first used in 1964 for the diagnosis of hydatid disease and subsequently modified.[1]

B. Procedure (after 1)

B.1 Antigen separation has been described above (see section 3.6.3(b)).

B.2 Conjugate

Commercial fluorescein-isothiocyanate (FITC) labelled anti-human globulin can be used for routine diagnosis. This conjugate contains anti-IgG, -IgM and -IgA.

All conjugates, whether obtained commercially or prepared by the user, should be evaluated for their suitability in this system. In particular, the optimal working dilution has to be tested for each batch of conjugate (Appendix 7).

B.3 Sera to be tested

Nonactivated sera in serial dilutions of 1:10, 1:20, 1:40, etc., in PBS are used in the test system.

B.4 Optical system

The light source, illumination system and filter combinations are important factors in determining sensitivity.

As an example, the reaction may be examined in transmitted light using a microscope with a super pressure mercury lamp (HBO 200) in combination with 2 BG-12 (Schott and Genossen) blue glass exciter filters (peak transmission 400 nm). A yellow glass (Leitz K 510) secondary filter is used to cut off light below 500 nm.

B.5 Testing procedure

Remove the antigen-bearing slides from the freezer (see section 3.6.3(b)), thaw at room temperature for five to 10 minutes and then fix for 10 minutes in acetone.

Cover each spot of the air dried antigen on the slides with one drop of diluted serum. The following controls have to be included: (a) positive serum of known titre, (b) negative control serum, (c) PBS. The slide is then incubated in a moist chamber at 37°C for 30 minutes.

Remove the drops of serum from the slides by holding them in a sloping position.

Immerse the slides in a dish containing PBS pH 7.2 at room temperature and wash quickly for about half a minute. Use fresh PBS for each test.

Wash for 10 minutes in PBS pH 7.2 at 40°C.

Rinse the slides with a gentle stream of deionized or distilled water and dry them under an electric fan.

Cover each hole with one drop of conjugate/Evans blue mixture (Appendix 7).

Incubate in a moist chamber at 37°C for 30 minutes.

Repeat washings in PBS and water as above.

Blow dry under a fan.

Mount by placing one drop of glycerol/PBS (Appendix 8) on each slide spot and cover with cover slip (18 x 18 mm).

B.6 Reading and criteria for positivity

Examinations are carried out with the low dry system (ocular: 10 x, objective 10 x) on a fluorescent microscope equipped as described above.

Positive reactions are characterized by a distinct bright-green fluorescence.

Negative samples should not exhibit a fluorescence but may be slightly "hay-green".

The antibody titre is defined as the highest serum dilution with a distinct fluorescence.

The hooks of the protoscolices exhibit auto-fluorescence.

C. Sensitivity and specificity

The system described using E. granulosus antigen is suitable for the detection of antibodies against E. granulosus and E. multilocularis. Sera from cases of larval Taenia solium infection also may exhibit positive reactions.

The sensitivity of the test is relatively high, but this depends on the quality of antigen, organ localization of the parasite, the parasite species and other factors.

Cross-reactions may be pronounced in cysticercosis cases (see above) and also may occur in other helminthoses; however, the latter usually occur only at low serum dilutions.[1,22,29,51]

3.6.8 Enzyme-linked immunosorbent assay (ELISA)

A. Principle

The enzyme-linked immunosorbent assay (ELISA) for the diagnosis of human hydatidosis involves the sensitization of polystyrene surfaces (tubes or plates) by allowing hydatid fluid antigens to be adsorbed on to them. When a serum containing antibodies against these antigens is added, the antibodies bind to the adsorbed antigens, giving an immunocomplex attached to the solid phase. After incubation with an enzyme-conjugated anti-human IgG rabbit immunoglobulin, the subsequent addition of the specific substrate will result in a colour change which is proportional to the amount of antibody in the test serum.[3,23]

B. Procedure[10,11,32,71]

B.1 Antigens

The hydatid fluid antigen described above is used (section 3.6.3(a)). More purified antigens, prepared by chromatography have also been used.[10,32]

B.2 Enzyme-conjugated anti-immunoglobulin

This may be obtained commercially or prepared from anti-human IgG rabbit antiserum which may itself be obtained commercially or by immunizing rabbits with a gammaglobulin fraction of human serum in Freund's complete adjuvant. The serum fractions containing anti-human IgG antibodies are then isolated by chromatography and may be further purified by affinity chromatography using purified human IgG co-valently linked with glutaraldehyde. The anti-human IgG is conjugated with alkaline phosphatase or peroxidase.[3,10,11,71]

After coupling is complete, the material is dialysed against PBS (Appendix 4). The conjugate thus prepared is stored at 4°C after dilution to 10 ml with 5% human serum albumin in 0.05 M tris-HCl buffer, pH 8.0 (Appendix 9) containing 0.02% sodium azide.

B.3 Sensitization of polystyrene tubes or plates

Disposable polystyrene tubes (12 x 75 mm) or plates with flat-bottomed wells are used. The following technique is suitable for use with tubes, but the reagent volumes may be scaled down five times for use with plates. In either case the suitability of different batches of tubes or plates for use in this test varies and each new batch should be tested before it is routinely used.

A dilution of the antigen containing 10 µg/ml is prepared in 0.05 M carbonate buffer, pH 9.6 (Appendix 10). One millilitre of this dilution is added to each tube. The tubes are incubated at 37°C for three hours in a water-bath; after incubation they are stored in the cold, while still containing the antigen solution, until they are used.

B.4 Sera to be tested

Each serum to be examined is diluted 1:1000 with PBS containing 0.05% Tween 20 and 0.02% sodium azide. Tween 20 is used to avoid nonspecific adsorption.

Before assay the desired number of antigen-coated tubes are washed with PBS-Tween. The tubes are filled three times with the washing solution, allowed to stand for a couple of minutes each time and emptied by suction. One millilitre of a diluted serum is added to each tube. The tubes are incubated on a roller drum for six hours at room temperature. After further washing, 1 ml of the conjugate diluted 1:500 with PBS-Tween is added. The tubes are then incubated overnight. After incubation, excess conjugate is washed out and the amount of alkaline phosphatase bound to the tubes is determined by adding 1 ml of freshly prepared 0.05 M sodium carbonate buffer, pH 9.8 (Appendix 10), containing 1 mg/ml p-nitrophenylphosphate and 10^{-3} M $MgCl_2$. The enzymatic hydrolysis of the substrate is allowed to proceed in the dark and is stopped after 30 minutes by the addition of 0.1 ml 1.0 M NaOH. The yellow p-nitrophenolate liberated by the enzyme is measured in a spectrophotometer at 400 nm.

Each time ELISA is performed, it is necessary to include a known positive serum and a negative serum as controls. Unsensitized tubes are also included in the test, the spectrophotometer readings for these being subtracted from those of the sensitized tubes.

B.5 Reading and criteria for positivity

In the absence of a generally agreed criterion it is suggested that an absorbance reading of more than two standard deviations above the mean value for the negative control sera from healthy donors should be considered positive.

C. Sensitivity and specificity

The ELISA has similar or slightly higher sensitivity than IHA, especially when used with purified antigen 5.[24,25,32,41,54]

3.6.9 Intradermal (ID) (Casoni) test

Intradermal injection of Echinococcus antigen allows the detection of reogenic antibodies (immediate, type 1, reaction) and sensitized lymphocytes (delayed, type 4, reaction). The sensitivity of this test is 80-95%. However, specificity may be low and variable. Utilization of antigens with a high protein content (over 100 µg N/ml) may lead to 30-40% false-positive reactions.[35] Therefore ID antigens should be standardized to a protein content of about 20 µm N/ml. A disadvantage of this test is that the injections of antigen may sensitize an individual so that subsequent serological reactions may become positive in patients without hydatidosis (see section 3.3). Furthermore, the danger of a general allergic reaction cannot be excluded. Therefore, wherever possible, the ID should be replaced by serological procedures.[4] However, it may be useful for the detection of lung cysts in patients who exhibit poor or no responses to other tests.[60,61]

3.6.10 Appendices

Appendix 1

Merthiolate solution

Dissolve 1 g of merthiolate (= thiomersal) in 100 ml PBS (Appendix 4). Store solution in brown glass bottle. Add 0.01 ml of this solution to each millilitre of serum (final merthiolate concentration 1:10 000).

Appendix 2

Glycine buffer pH 8.2

Dissolve in 800 ml of distilled water:

Sodium chloride	9.0 g
Calcium chloride	1.0 g
Glycine	7.31 g
Merthiolate (powder)	0.1 g

Bring to pH 8.2 with 1 N NaOH. Add distilled water to a final volume of 1000 ml. Keep at +4 °C.

Appendix 3

Alsever's solution

Glucose	20.5 g
NaCl	4.2 g
Na citrate (tribasic)	8.0 g
Citric acid	0.55 g

Make up to one litre with distilled water and, if necessary, adjust to pH 6.1 with 10% citric acid and then autoclave.

Appendix 4

Phosphate buffered saline pH 7.2

Solution A: 0.15 M solution of monobasic potassium phosphate (20.4 g/litre)

Solution B: 0.15 M solution of dibasic sodium phosphate (53.7 g of $Na_2HPO_4.12\ H_2O$ in one litre)

28.5 ml A + 71.5 ml B diluted to a total of one litre with 0.145 M NaCl (8.5 g/litre).

Appendix 5

Buffered saline solution pH 7.2 containing 1% normal rabbit serum

This is prepared by mixing:

99 ml buffered saline solution (Appendix 4)
1 ml normal rabbit serum

Normal rabbit serum should be inactivated at 56 °C for 30 minutes or at 60 °C for 10 minutes. The serum should be checked to make sure that it does not contain agglutinins against sheep red blood cells. In the event that such agglutinins are present, the serum

should be absorbed prior to use in the following manner: one volume of sheep red blood cells is mixed in five volumes of the serum to be absorbed; the mixture is incubated at +37 °C for one hour and then at +4 °C for four hours and it is then centrifuged for 10 minutes at 800 g. The absorbed serum is separated from the packed red blood cells.

The absorbed serum is again tested with sheep red blood cells to confirm that the absorption has been effective. In the event that agglutinins are still present in the serum, the absorption procedure is repeated.

Appendix 6

Preparation of multispot slides (three spots)

- Place three drops of glycerol on a clean slide in order to cover spots of about 5 mm in diameter.

- Spray with Fluoro-Glide (Marshall-Howlett Ltd, Sidcup, Kent, England) or a similar preparation (for example: Fisons PTFE aerosol spray; Kleiner AG, Wohlen, Switzerland) under a hood![a]

- The sprayed material firmly dries in about 12 hours at +37 °C. Thereafter, the glycerol is washed off and the slides can be used after drying.

Multispot slides are commercially available.

Appendix 7

Conjugate/Evans blue mixture

(1) Evans blue (1:10 000)

Mix one part of 0.1% Evans blue solution (0.1 g Evans blue in 100 ml PBS) with nine parts of PBS working solution.

(2) Fluorescein-conjugated anti-human globulin

- Evaluate the optimal working dilution in a preceding test with sera of known antibody titres.

- Add for example one drop (= ~15 µl) of conjugate solution to 1 ml of Evans blue solution (1:10 000).

Appendix 8

Buffered glycerol

One part PBS working solution pH 7.2
Four parts glycerol

Appendix 9

Tris-HCl buffer pH 8.0

Solution A: 0.2 M tris (24.2 g/litre)

Solution B: 0.2 M HCl (16.6 ml concentrated HCl to one litre)

50 ml A + 26.8 ml B; make up to 200 ml with distilled water to give a final concentration of 0.05 M.

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[a] Mention of commercial firms or their products does not imply endorsement by the World Health Organization.

Appendix 10

Carbonate buffer 0.1 M pH 9.6

Solution A: 0.2 M solution of sodium carbonate (57.23 g of $Na_2CO_3.10H_2O$ in one litre)

Solution B: 0.2 M solution of sodium hydrogen carbonate (16.80 g/litre)

16 ml A + 34 ml B; make up to 100 ml with distilled water to give a final concentration of 0.1 M.

3.7 Commercially available test kits

Some of the test kits are available commercially. These include tests for indirect haemagglutination, complement fixation, immunoelectrophoresis latex agglutination and antigen for the intradermal test. Mention of these test kits does not imply that the quality of the reagents has been tested by the World Health Organization or endorsed by this Organization.

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CHAPTER 4

PARASITOLOGICAL DIAGNOSIS OF HYDATID DISEASE IN MAN

4.1 Introduction

The diagnosis of echinococcosis in man based on clinical findings gained from X-ray examination, ultrasonography, tomography, scintigraphy and other methods, can be supported by the detection of antibodies against Echinococcus antigen (chapter 3) and finally confirmed by the demonstration of the parasite. For direct parasitological diagnosis the techniques described below can be employed.

4.2 Examination of material discharged spontaneously

In cases of pulmonary infection with E. granulosus, cysts, parts of the cyst wall, protoscolices and hooks may be expectorated after spontaneous rupture of the metacestode. The cyst wall may be identified macroscopically, but it should be submitted for histological examination (see section 4.3.1). Protoscolices and hooks can be diagnosed microscopically in material obtained from cysts or in the sputum. Sputum samples can be examined in direct smear preparations. However, preferably sputum should be diluted with physiological saline then centrifuged and the sediment examined.

On rare occasions cysts of E. granulosus may be vomited after rupture of metacestodes in the liver. Perforation of E. granulosus and E. multilocularis metacestodes through the body wall to the surface has also been observed. The techniques described above can be applied to these cases.

4.3 Examination of material obtained by surgery

Cysts of E. granulosus must not be punctured in such a way that anaphylactic reactions and/or secondary echinococcosis may occur. In E. multilocularis cases liver biopsies performed through the body wall should be avoided as dissemination of metacestode tissue might follow this procedure. It follows that in E. granulosus and in E. multilocularis cases samples for examinations can only safely be obtained after removal of metacestodes by surgery or - in the case of E. multilocularis - by organ biopsy after surgery.

4.3.1 Histopathology and ultrastructure

Samples of metacestodes should routinely be immediately fixed after removal from the body and later submitted to histological examination after haematoxylin/eosin and PAS-staining. In special cases ultrastructure studies may be useful (for technical details see section 12.3.2).

Specific histological characteristics of metacestodes of Echinococcus spp. include a PAS-positive, acellular laminated (= cuticular) layer with or without an internal cellular, nucleated germinal layer.[5] The identification of dead and calcified metacestodes may be difficult and may require special processing and staining techniques.[7]

Differential diagnosis of E. granulosus and E. multilocularis is important in areas where both species occur. Some forms of the parasites, especially with unusual growth patterns - for example in the bones - may create considerable difficulties in identification. Therefore, macroscopic characteristics and histological features[2,5,6-9] (see also section 1.4) and where necessary results from animal experiments have to be considered in the diagnosis.

Other larval tapeworms which have to be taken into account in differential diagnosis mainly include cysticerci of Taenia solium and, in rarer instances, Taenia multiceps and Taenia brauni.[1] Criteria for the identification of cysticerci have been described.[8]

4.3.2 Viability tests

Following surgical removal of metacestodes of E. granulosus or E. multilocularis viability tests may be desirable, especially in chemotherapeutic trials. Applicable techniques are described in chapter 12. The material required for this purpose should be collected and further processed as follows:

whole cysts or parts of the cyst wall (as large as possible) of E. granulosus or pieces of E. multilocularis (about 3 x 3 cm) should be washed in sterile physiological saline after removal from the body;

thereafter, the material should be transferred to sterile vessels and covered with sterile physiological saline, preferably with an addition of 500 I.U. penicillin G and 500 µg streptomycin per ml;

although the material will survive in a refrigerator for several days, it should be sent to a specialized laboratory as soon as possible, refrigeration temperatures being maintained where possible;

material obtained in this way may be used for viability tests of protoscolices (see sections 12.3.2 and 12.5) and/or for viability tests in laboratory animals (see sections 12.2.2.2 and 12.5).

4.3.3 Identification of strains

If strain differentiation is required (see section 1.6) biochemical methods, such as enzyme electrophoresis and electrofocusing, provide useful tools. For electrofocusing Echinococcus material (cyst wall homogenate in physiological saline, protoscolices) can be lyophilized. Enzyme electrophoresis requires fresh or deep-frozen material.[3,4]

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CHAPTER 5

PARASITOLOGICAL DIAGNOSIS OF LARVAL ECHINOCOCCUS IN ANIMALS

Domestic animals are usually examined for echinococcosis at licensed killing establishments (see sections 2.4.1 and 10.3). The diagnosis of wildlife echinococcosis has to be undertaken by field survey methods. There are as yet no satisfactory techniques available for the immunodiagnosis of echinococcosis in domestic or wild animals. Thus, autopsies must be undertaken.

5.1 Autopsy

Conventional autopsy techniques are employed.

5.1.1 Collection of material

The specimens should either be preserved in a fixative such as 4-10% formol saline, or they can be packed in ice for transport and deep frozen for subsequent examination in the laboratory.

5.2 Differential diagnosis

Larval Echinococcus can often be visually detected in organs, but in large animals such as sheep and cattle, palpation and where necessary incision of the organs should be undertaken. Special care has to be taken with specific diagnosis of echinococcosis in pigs, sheep and goats, because these may also be infected with T. hydatigena. It may sometimes be difficult, if not impossible, to differentiate between these two parasites in the liver[3] (see section 2.4.1). Ascaris suum has also been incriminated as a case of "white spot" in sheep livers.[1] In wild animals, particularly, ruminants and rodents, several other larval cestodes may have to be taken into consideration in differential diagnosis.[2,5,6,7] Histological examination of larval material may confirm the diagnosis. 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 membrane can be regarded as a specific characteristic of metacestodes of Echinococcus. Morphological differentiation and characterization have been described.[4,8,9,10] Modern biochemical techniques such as isoenzyme electrophoresis and electrofocusing may be employed in differential diagnosis of larval cestodes (see section 1.7.4.2). Criteria for determining species and strain differences are given under sections 1.7.3 and 1.7.4.4.

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CHAPTER 6

PARASITOLOGICAL DIAGNOSIS OF ADULT TAPEWORMS IN CARNIVORES

6.1 Autopsy

The value and limitations of autopsy surveys are described in chapter 2. They are particularly important in the study of wildlife echinococcosis, where autopsy data together with transmission experiments are used to determine the role of each wild animal host.

6.1.1 Collection and preservation of material

Only the small intestine is required for the collection of Echinococcus spp. This should be removed as quickly after death as possible, tied at both ends, and placed in a numbered plastic bag or metal container. It should be transmitted as soon as possible to the laboratory. For long distances, the material can be placed on ice or the intestine, once ligated, can be immersed in fixative and fixative can be injected into the lumen. With unfixed material, the examination should be carried out as soon as possible, because digestion of the parasites may occur within 24 hours. Unpreserved material can be deep frozen until examined.[4] It is emphasized, however, that eggs are very resistant to low temperatures and are only killed at -70°C if retained at that temperature[3] for 24 hours (see section 1.9.2 for temperature effects on eggs). Fixatives such as 4-10% formalin can be used but they do not kill eggs.

The worms are fragile and are best handled with a Pasteur pipette with a rubber bulb after being placed into physiological saline solution (tap water may also be used). However, stainability is reduced if they are kept too long in water. The tapeworms should be separated from faecal or intestinal debris by washing in several changes of physiological saline solution. The tapeworms are then placed in a vessel with physiological saline solution. The latter is then drawn off by suction and replaced by fresh water. The worms should be left for about 30 minutes and until all movement has ceased, at which time they have relaxed.

For fixation, cold (approximately 5°C) 5-10% formalin or F.A.A. fixative (see below) is poured over the tapeworms after the water has been drawn off by suction. They should be retained in fixative for at least 12 hours.

For staining, the worms are washed in water for 15 minutes and then passed directly into Mayer's Paracarmine for 12-24 hours (progressive staining). In order to rinse out excess stain they should be immersed after staining for only a few seconds in 0.5-1.0% of HCl. Dehydration is accomplished by serial passage through increasing concentrations of alcohol (35, 50, 70, 85, 95 and 100%). The worms should remain at least 15 minutes in each concentration of alcohol with two changes in 100%.

Following dehydration the worms are placed in xylol (10 minutes) and then cleared immediately in methyl salicylate or creosote. Prior to mounting they should be returned to xylol for a few minutes. They can then be mounted in any suitable mounting medium such as balsam, picolyte, etc.

Solutions

F.A.A. fixative	
95% ethanol	80 ml
Formalin (37-40% formaldehyde)	10 ml
Glacial acetic acid	5 ml

Mayer's Paracarmine	
Carminic acid	1.0 g
Aluminium chloride	0.5 g
Calcium chloride	4.0 g
70% ethanol	100.0 ml

(Heat mixture until ingredients dissolve, then filter.)

6.1.2 Examination of the intestine

Several methods have been used in the examination of the intestine of carnivores for *Echinococcus* spp.[4,9,11,17,19] The intestine can be divided into several[11,17,19] sections and each can be immersed in saline at 37°C in a tray. Worms adhering to the intestine can then be 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.

Where accurate worm counts are required, the best method is to divide the unfixed intestine into four or six sections. Open each section along its length, then immerse each in saline at 37°C for 30 minutes. This releases most of the worms into the fluid. This is followed by washing the intestinal contents into a jar. The worms selected for studies on morphology can be removed. The intestinal wall is then scraped with a spatula. All the material is boiled and washed by sieving (see section 6.3.3) to eliminate most of the particulate 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. Sampling may be required if large numbers of worms are present.

6.2 Arecoline surveys

This technique has now been generally adopted for determining prevalence of *Echinococcus* spp. in dogs.[11,12,13,14,18]

6.2.1 Pharmacology

Arecoline is the chief alkaloid of the areca nut, the seed of *Areca catechu*. Synthetic arecoline was first used against tapeworms in dogs in 1921.[15]

Arecoline is a parasympatheticomimetic agent. Briefly, the action results in sweating and stimulation of salivary, lachrymal, gastric, pancreatic, and intestinal glands. The drug also causes increased tone and motility of smooth muscle, and this effect on the smooth muscle is responsible for purgation. The liver is the principal site of detoxification.[1,2,6,10] Arecoline also has an action on the worm itself, causing it to relax its hold on the intestinal wall, thus, it must be given by the oral route. The subsequent purgation carries the worms out with the faeces. Its action causes paralysis but not death of the worm.

6.2.2 Limitations in worm control

Under laboratory conditions, arecoline has been shown to eliminate large numbers of worms from some dogs, but none from others. Indeed, nine or more treatments may be required to free all dogs of *E. granulosus* on a group basis.[1,10,11,12] Two examples of this are shown in Table 6.

TABLE 6. LIMITATIONS OF ARECOLINE HYDROBROMIDE IN THE EXPULSION OF *ECHINOCOCCUS GRANULOSUS* FROM DOGS

	Experiment 1[1] in 16 dogs	Experiment 2[11] in 30 dogs
Percentage cleared after one treatment	12.5	20
Percentage cleared after two treatments	37	30
Percentage cleared after three treatments	69	43
Percentage cleared after four treatments	87	67
Percentage cleared after five treatments	87	90

Dose rates for Experiments 1 and 2 were 1.75 mg/kg and 3.5 mg/kg respectively.

A negative result at two or more treatments with or without purgation does not mean that an animal has been freed from E. granulosus.

Under field conditions, it has been shown that about 20% of dogs may fail to be purged, irrespective of whether the drug is given in solid or liquid form.[8,11] Most writers agree that the majority of dogs will be purged by a single treatment of arecoline hydrobromide at 1.75 mg/kg to 3.5 mg/kg within one hour. However, a proportion of dogs may purge much later.[7] For this reason in some programmes, samples are collected over a period of up to four hours after treatment.[13]

6.2.3 Side effects

Arecoline treatment of healthy dogs is regarded as a safe procedure. However, some untoward reactions do occur.[14] Vomiting is frequently observed following treatment and may, if occurring too soon after dosing, reduce the efficiency of the drug in worm removal. Usually, this side effect is transient. In some dogs, even minimal doses of arecoline can cause abnormally severe and prolonged stimulation of mucous secretions. This is shown by excessive salivation and discharge of watery mucus from the anus long after the dog has been purged. Prolonged fluid loss can cause severe dehydration of the body tissues, particularly in hot weather, and unless treated death may occur. The dog should have access to water at all times.[14]

If a considerable part of the dose is absorbed through the mucous membranes of the mouth or throat, as it may be if a dog has been struggling during the dosing procedure, this may lead to a fall in blood pressure, staggering, loss of consciousness, cyanosis, and laboured respiration. Treatment should include:

(i) ensuring continued respiration - by loosening the collar, and artificial respiration;

(ii) injecting atropine sulfate.

The dose of atropine sulfate is 0.3 to 4.0 mg according to the size of the dog. This drug is itself potentially dangerous and it should be stored where it cannot be obtained by unauthorized persons. It acts as an antagonist to arecoline hydrobromide. It is recommended to give one third of the dose intravenously and the rest by the intramuscular or subcutaneous routes. Pregnant bitches and animals with cardiac abnormalities should not be treated with arecoline salts.

6.2.4 Dosing equipment

There are several types of dosing gun suitable for the administration of tablet or liquid formulations. A metal-guarded adjustable 30-ml automatic hypodermic syringe with dose-regulator will serve for the liquid formulations. It should be capable of delivering a dose of 1 to 5 ml and it should be fitted with a nozzle. This should be made of stiff rubber tubing with a small aperture and slit valve at the delivery end. This will ensure accuracy in delivering small doses and will prevent the fluid dripping out between doses. An adapter for the attachment of the tube is also necessary. A suitable combination is a hypodermic syringe and an oesophageal tube plus an oesophageal tube-to-syringe adapter.

Other equipment includes:

(i) personal protective and hygiene items;

(ii) chemicals including some colour-coded liquids;

(iii) receptacles, shovel, buckets, containers for samples;

(iv) water tank and water boiler.

All liquids used on the dosing site must be kept in suitable plastic containers so that the exteriors of these can be sterilized before being returned to the vehicle (see section 10.2).

6.2.5 Dosing procedures

One of the problems of dosing dogs with arecoline hydrobromide is that the drug has a definite unpleasant taste and smell. Dogs tend to be nervous when they are in a strange place or being handled by strangers. It is, therefore, often wise to allow the owner to handle his own dog as much as possible, provided he is confident and capable of doing so and has been instructed on the procedure to be used.[18]

A person handling dogs should approach it deliberately in a confident and calm manner, without sudden movement. He should talk to the dog in a calm voice and allow it to sniff him, but without taking any unnecessary risk should it decide to attack. If a dog has been treated well it should be easy to handle, so the owner should be encouraged to treat dogs kindly. If their experience of being dosed is unpleasant, they will be even more difficult to handle on subsequent occasions. A few dogs are quite intractable. The use of leather gauntlets and a noose type of dog-catcher may be necessary to handle these, but only as a last resort.

6.2.5.1 Preparing the dog for treatment

Perhaps the most unfortunate result of treating dogs with arecoline hydrobromide is the occasional death due to the piercing of the bowel with sharp splinters of bone. Thus for at least three days before dosing, it is recommended that no bones be fed. There is no need to starve the dog, and a meat meal given 12 hours before dosing is preferable to a diet of dehydrated foods. Plenty of milk and water can be given. However, the dog should not be dosed on a full stomach because this increases the risk of vomiting and decreases that of purging.

Old dogs that are habitually constipated may require a suitable dose of liquid paraffin or similar preparation one or two days before dosing.

6.2.5.2 Dose rate and formulations

Recommendations on dose rates have varied slightly, but 1.75 mg/kg to 3.5 mg/kg is generally regarded as suitable for most dogs. Doubling or halving the dose rate does not increase efficacy, but the former may cause excessive vomiting. The use of a tablet form of arecoline administered with a tablet gun is strongly recommended. Standard tablet forms of arecoline hydrobromide should be coated with sugar to hide the taste and to avoid the development of skin sensitivity by susceptible handlers. An accepted dose rate is with tablets each containing 25 mg arecoline hydrobromide:

Minimum: 1 tablet per 14 kg body weight
Maximum: 1 tablet per 7 kg body weight
Optimum: 1 tablet per 10 kg body weight

Arecoline-acetarsol tablets may also be used and are available under a number of trade names. It has been suggested that this preparation has fewer side effects than the hydrobromide salt.

A standard liquid solution may be recommended as follows:

Arecoline hydrobromide: 15 mg/ml
Saccharine (or sugar) : 1.5 mg/ml
Dose : 1 ml/5 kg body weight

In the Tasmanian programme, the dose rate was reduced from 3.25 mg/kg to 1.62 mg/kg. The solution used is 0.82% arecoline hydrobromide given at a rate of 1.6 mg/kg up to a maximum of 32 mg. Dogs which fail to be purged within one hour may be redosed at the same dose.[14]

A divided dose treatment schedule frequently gives improved diagnostic results without increasing the side effects. This treatment schedule, under which the minimum dose rate may be given and followed 15 to 30 minutes later with up to a similar dose, is recommended provided that the total dose is not greater than the maximum recommended dose rate.

6.2.5.3 Dosing the average dog

The following procedure is recommended when administering tablets and liquids (Fig. 13):

(i) the dog-handler holds the chained-up dog with both hands, gripping the loose skin behind the mouth and below the ears on each side of the head, thumbs pointed downwards; the ears should never be held;

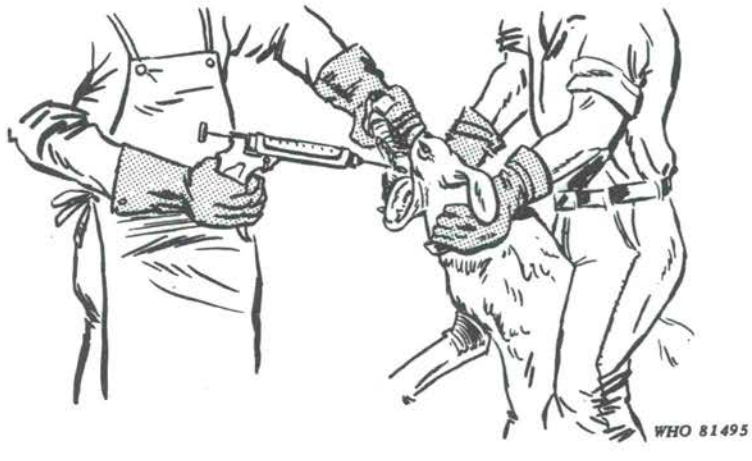
(ii) the dog-handler's legs straddle the dog, knees firmly placed against the dog's shoulders, the position is adjusted to ensure that the dog is at the full length of the chain;

(iii) the dog-doser grips the upper jaw of the dog, using the left hand (if right-handed), the fingers gently forcing the upper lips over the upper teeth, the dog's head being held in a slightly raised position;

(iv) with the right hand holding the dosing gun, the dog-doser inserts the oesophageal tube into the dog's mouth at an angle; the tip must be placed well over the back of the tongue and a steady pressure on the trigger of the dosing gun releases the required liquid dose or tablet.

On no account should forceps or other sharp instruments be used to deliver a tablet. Tablets can also be placed in a gelatine capsule and hidden in meat.

FIG. 13. ADMINISTRATION OF THE DOSE



6.2.5.4 Dosing the intractable dog

The word intractable is used here to describe all those animals that are difficult to dose. Some dogs may be well behaved under normal circumstances, but as soon as they anticipate dosing they may react violently. Other dogs may be badly behaved at all times.

The following equipment is required for use with intractable dogs:

(i) a long-handled dosing gun;

(ii) heavy gloves;

(iii) dog chains;

(iv) dog-catching stick with rope noose;

(v) piece of meat.

Often a difficult dog can be fed the drug enclosed in a capsule in a piece of meat. This is especially effective if the animal has been starved. If this fails, the animal can be constrained by pulling it close to a post. Further control can be exerted by attaching a second chain to the collar and pulling outwards on the chain. Snares can then be placed over the jaws and the capsule placed well back in the pharynx with a long-handled dosing gun. Particularly savage dogs may require tranquillizing.

6.2.5.5 Dosing the toy dog

Nervous toy dogs sometimes present a problem for the dog-doser. The dog can often be tempted if the drug is placed in a capsule and hidden in meat or other foodstuff.

6.2.5.6 The use of enemas

Some dogs resist purgation. The additional use of arecoline hydrobromide as an enema has been recommended.[5] This will increase the number of dogs purged following oral treatment, but because worms may not be reached and paralysed by the rectal route it may give rise to "false negatives" and thereby underestimate the actual prevalence.[11]

6.2.6 Hygiene and safety precautions

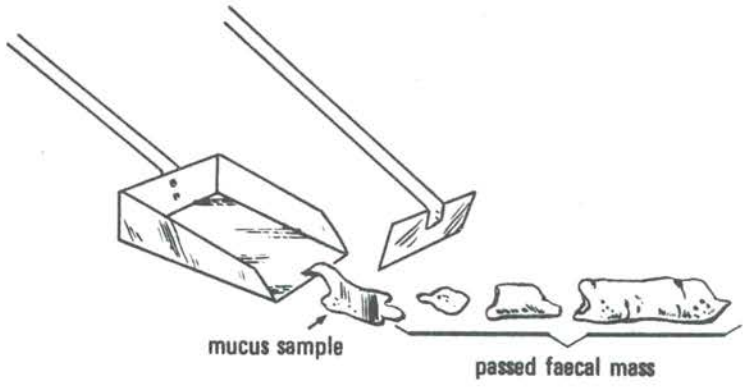
The safety precautions required for arecoline surveys are discussed in detail in chapter 13. Owners should be advised not to transport their dogs in private cars. This is because further purgation may occur long after treatment has been given.

Particular care must be taken in endemic regions of rabies to ensure that immunization has been carried out on all dogs to be examined and on persons handling the dogs.

6.2.7 Collection of the faecal material

The dog that purges successfully produces at least two motions. Typically the first consists entirely of formed faeces, which should be ignored. Even one teaspoonful (4 ml) of the mucus that follows may be productive, but formed faeces are not, and they need not be examined. The mucus is picked up with a trowel and placed in a small stainless-steel receptacle together with one of the metal discs with the identity number from the dog's chain. The jug is carried into the mobile laboratory by the gloved operator (Fig. 14).

FIG. 14. COLLECTION OF THE FAECAL SAMPLE



In the laboratory the specimen of mucus is diluted with 100 ml of tap water and then covered with a thin layer of kerosene (c. 1 ml) and boiled on a gas flame or other device for five minutes. The kerosene prevents foaming and stops most of the smell.

6.3 Examination of faeces following arecoline treatment

Echinococcus organisms are often difficult to detect by direct examination of faeces and methods have been developed to overcome this problem.[11] With all procedures, if no worms are detected by naked eye, visual aids such as a hand lens or dissecting microscope should be used. It seems that the size (age) of the worms rather than their number presents the greatest difficulty when no visual aids are employed.

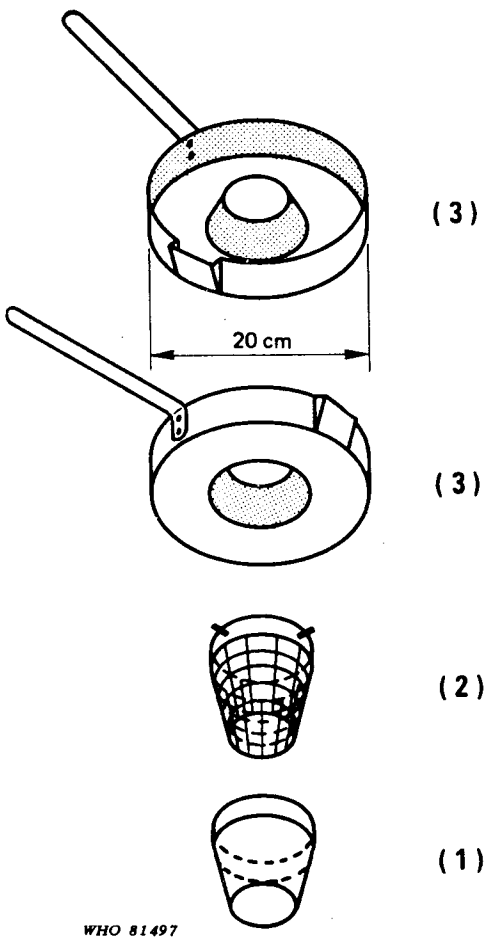
6.3.1 Direct examination

The mucus part of the faecal purge can be divided into several samples and each can be broken up in a dish containing water. Almost invariably, worms are difficult to detect because of the coloured debris. This method is not recommended unless very small samples are diluted in a large amount of water.

6.3.2 Flotation technique

This technique is independent of a piped water supply. It relies on the fact that Echinococcus will float in a medium with a specific gravity of about 1.2, whereas most other faecal debris sinks. A flotation technique using saturated salt (NaCl) has been described[11] (Fig. 15) in which a faecal sample is placed directly into a fine-mesh (80-100 meshes/linear inch) wire basket (1) and boiled. If a faecal mass contains large particles it may be placed in a coarse mesh (10 meshes/linear inch) basket (2) which fits inside the fine-mesh basket and this will retain the larger particles. After boiling, a stainless-steel collecting pan with a cone in the centre and having a 5-cm opening at the apex and a wall 15 cm deep and 10 cm wide (3) is attached by ear clips to the top of the fine-mesh basket. The basket and pan are then slowly and completely immersed in a saturated salt solution. The contents of this pan can then be poured into a black tray for examination. If no worms are detected initially, the flotation should be repeated at least twice.

FIG. 15. FLOATATION EQUIPMENT FOR FIELD USE



6.3.3 Sieving technique

Apparatus for large-scale screening of samples has been described in detail.[11] This method involves the washing of the faecal sample under pressure through a sieve, and examining the retained material for the presence of worms.

Another less elaborate method uses a constant stream of water to wash boiled mucous faeces through a sieve. The material retained on the sieve is washed off into a black tray for examination for worms. For field survey purposes, a mesh of 80-90 meshes per linear inch can be used and for anthelmintic research studies a mesh of 120 apertures per linear inch is recommended. Details on losses of worms according to sieve size and washing time are given in reference 11.

6.3.4 Sedimentation technique

When exact quantitative data are required, a sedimentation technique is warranted.[11] This involves suspending the boiled faecal sample in saline (0.85%) and allowing the worms to sink for at least 30 minutes. The supernatant is then removed and the worms resuspended in saline. This procedure should be repeated several times, until the majority of the light flocculant material has been removed in the supernatant fluid. The sediment can then be examined for worms by pouring a small amount at a time into a black tray containing saline. This is considered to give far more accurate results and is less damaging to the worms than the two methods described previously, but is considered to be too time-consuming to apply when handling large numbers of samples.[11]

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CHAPTER 7

NEED, COST AND BENEFIT OF CONTROL PROGRAMMES

Echinococcosis is a public health and economic problem of global proportions. It is a health problem, which except in its wild life elements, is entirely preventable. In parts of Latin America and Europe, for example, the prevalence of human cases requiring surgery may be in excess of 10 per 100 000 per year and in some areas 60-70% of the livestock are infected.[4] In parts of Africa and Asia, although not accurately documented, the human prevalence is even greater. In man, the prognosis is often poor, and at the present time, surgery is the only practical treatment (see section 12.4 for discussion on drug treatment).

7.1 Public health and economic importance of hydatid disease in man

The evaluation of the public health importance of any disease is difficult, and the methodology is still far from being perfected. Most studies have relied almost entirely on economic criteria to assess the importance of particular diseases. This involves estimating the cost not only of treating the disease, but also of the wage and productivity lost through illness, treatment and convalescence. It also requires evaluation of the losses incurred through illness or death of the affected individual. Problems of this economic approach include the difficulty of putting monetary values on different degrees of disability and suffering incurred and on human life itself. This applies in both developing and developed countries.

For investigation purposes, the public health and economic significance of echinococcosis can be broken down into the following components:

(i) medical and surgical fees and cost of hospitalization, nursing, drugs and convalescence;

(ii) loss of income and loss of productivity as a result of absence from work, reduced capacity for work or permanent incapacity;

(iii) losses due to mortality;

(iv) social consequences of disability and mortality.

The epidemiological survey techniques described in chapter 2 will provide much of the data required to evaluate the above.

7.2 Economic importance of echinococcosis in livestock

The economic importance of echinococcosis in livestock is due to the condemnation of livers, other organs or even whole carcasses. In severe infections the parasite may cause retarded performance and growth, reduced quality and yield of meat, milk or wool. The importance of these losses will depend to a great extent on the characteristics of the farming or livestock industry. For example, echinococcosis causes a lower loss in Australia, where the sheep are primarily wool producing, than in New Zealand, where sheep are primarily for meat production.

Most of the data collection methods required to make an evaluation of the economic importance of echinococcosis in livestock are described in chapter 2.

7.3 Cost and benefit

Evaluation of the public health and economic importance of the disease is the first step to establishing priorities. It is then necessary to examine the alternative programmes, their expected effect, cost, and the benefits that should result. Comparison

of the costs of various programmes with the benefits that should ensue by means of cost-benefit analysis should further aid decision-making on priorities. Once a decision has been made concerning the level of resources available for control and the particular objective that should be pursued (reduction in transmission or eradication, for example), cost-effectiveness analysis can be used as an aid to decide the most effective use of the resources.

7.3.1 The cost of control

Several different strategies for the control of the parasite may be possible. For example, an intensive long-term educational programme may be felt to be sufficient on its own, or it may be combined with legislation designed to prevent the definitive host from gaining access to raw offal by strict control of slaughtering and carcass and offal disposal. Alternatively, or in addition, control may be directed against the definitive host. The dog population may be severely reduced, the movements of dogs strictly controlled and infected dogs detected and either destroyed or treated (see chapters 9, 10 and 11 for options in control).

It may be necessary to assess the cost of the options separately so that the decisions can be made on the strategy that combines economy and effectiveness. Assessment of the costs of control programmes should include:

(i) the costs of surveillance of the human population by immunological techniques or mass X-ray surveys;

(ii) the costs of arecoline surveillance of dogs and post mortem surveillance in sheep and other intermediate hosts;

(iii) elimination of surplus dogs and registration, periodic examination and possibly anthelmintic treatment of others;

(iv) provision of slaughterhouses and meat inspection services, disposal of condemned offal;

(v) health education of butchers, farmers and other dog owners;

(vi) planning, evaluation and monitoring of the programme and of enacting legislative and administrative measures;

(vii) staff training including symposia, workshops and exchange visits;

(viii) provision of safe water, etc.

Detailed cost estimates of programmes against echinococcosis in Tasmania[2] and Chile[1,3] have been published. These can be taken as examples, but not as complete guides, because of the particular economic and epidemiological conditions under which the estimations were made.

7.3.2 Benefit of control

Based on the experience gained in the successful control programmes against E. granulosus in Cyprus, New Zealand and Tasmania, it can be expected that a reduction in human morbidity from echinococcosis will occur and benefit all age groups within a few years of the introduction of control measures. Similarly, a reduction will occur in the domestic food animals. Other short- and long-term benefits of a control programme should be assessed. For example, the costs of diagnosis and treatment of echinococcosis would be reduced or eliminated; surveillance of the human population may detect other treatable medical conditions; education combined with the control of animal killing facilities may contribute to raising the level of general food hygiene and the removal of echinococcosis may open up new markets for animals and their products and increase the confidence of established ones.

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CHAPTER 8

ESTABLISHMENT OF CONTROL AUTHORITY

8.1 Sequence of events and time scale leading to control

In most successful control programmes, early attempts to introduce formal measures have often been unsuccessful and the time scale to achieve success has often been extended over a long period. It is emphasized here that a control programme or its precursor may in fact involve only a small circumscribed area such as a village or a group of villages and farms. Indeed, there are instances where local action, involving universities, research institutes and/or veterinary practices, has been successful without the introduction of formal control programmes. In this section, a description is given of the sequence of events, which most frequently occur, before a formal control programme can be instituted.

Phase 1 — Event: Increased awareness by surgeons and patients of the overall effect of hydatidosis to community health and by veterinarians involved in meat inspection that echinococcosis is preventable.

Action: Discussion only.

Phase 2 — Event: Formation of discussion groups at first involving mainly medical and veterinary officials.

Action: Widening of discussion group, leading to formation of Action Committee.

Phase 3 — Event: Formation of Committee involving medical, veterinary and other group leaders representing farming, dog owning, religious and political groups.

Action: Committee enters early planning stages and discusses legislation, etc.

Phase 4 — Event: Committee seeks information on such factors as prevalence of echinococcosis and estimates of dog population.

Action: Early collection and collation of available data.

Phase 5 — Event: Committee requests official help in obtaining base-line data and encourages studies by universities and other research institutes.

Action: Government officials formally appointed to Committee to assist in providing departmental information and support.

Phase 6 — Event: Committee collates information and discusses control structure, functions, funding, scope of operations, training, priorities and options based on local knowledge and also seeks information from officials operating successful schemes elsewhere.

Action: Intensive educational programme begins with particular attention to gaining community support. At this stage Committee may decide, with assistance from universities and local veterinary practitioners, to introduce control within a circumscribed area without recourse to further official government action, but with departmental support.

Phase 7 — Event: Committee decides to request the legislature to undertake a full-scale formal control programme and draws up plan of action with options.

Action: Committee submits plan to government departments and legislature.

Phase 8 Event: Legislature discusses plan and approves funding or returns plan for further evaluation and resubmission. Here, success may be dependent on priority status of echinococcosis vis-à-vis other community health problems.

Action: Legislature approves plan and instructs the appropriate government department to proceed.

Phase 9 Event: Government department appoints Director of control programme and Director appoints Committee as advisory body.

Action: Final plans drawn up and implemented (see chapters 9, 10, 11 and 13).

8.2 Structure of the control authority

The philosophy and approach to the control of hydatidosis is comparable to that for diseases such as tuberculosis, brucellosis and rabies. Thus, the control of echinococcosis should follow established practices by utilizing government health or agricultural organizations and the enactment of appropriate legislation. There is rarely any need or justification to set up a special organization.

There are several models involving national and provincial authorities that have developed successfully to the stage where they can be evaluated.[8,9] Most of them use existing governmental organizations. They also represent island and continental situations and include Iceland,[4] Cyprus,[12-19] Tasmania[10,11] and the Province of Neuquén, Argentina.[2,5] Their structures are illustrated in Fig. 16. In some instances, these involve health and in others agricultural authorities. In all recent programmes there has been a strong veterinary component.

While hydatidosis control should be undertaken ab initio by an existing government organization, there have been instances when this has not occurred. These include Uruguay[3] and New Zealand.[1,6] Thus, special Acts of Parliament were promulgated and these provided for the erection of a national control authority to take charge of the programmes.[6]

Irrespective of whether or not control is vested in health or agricultural departments for administrative purposes, the main administrative and decision-making process should flow through the veterinary and animal health services. There are many advantages in this. These include experience of other disease control programmes, laboratory services, staff training, career structure and permanency for staff within existing divisional and regional activities. The person responsible for the control of the programme should be the director or chief of the veterinary services of the agricultural authority or the appropriate veterinary department or division of the health authority.

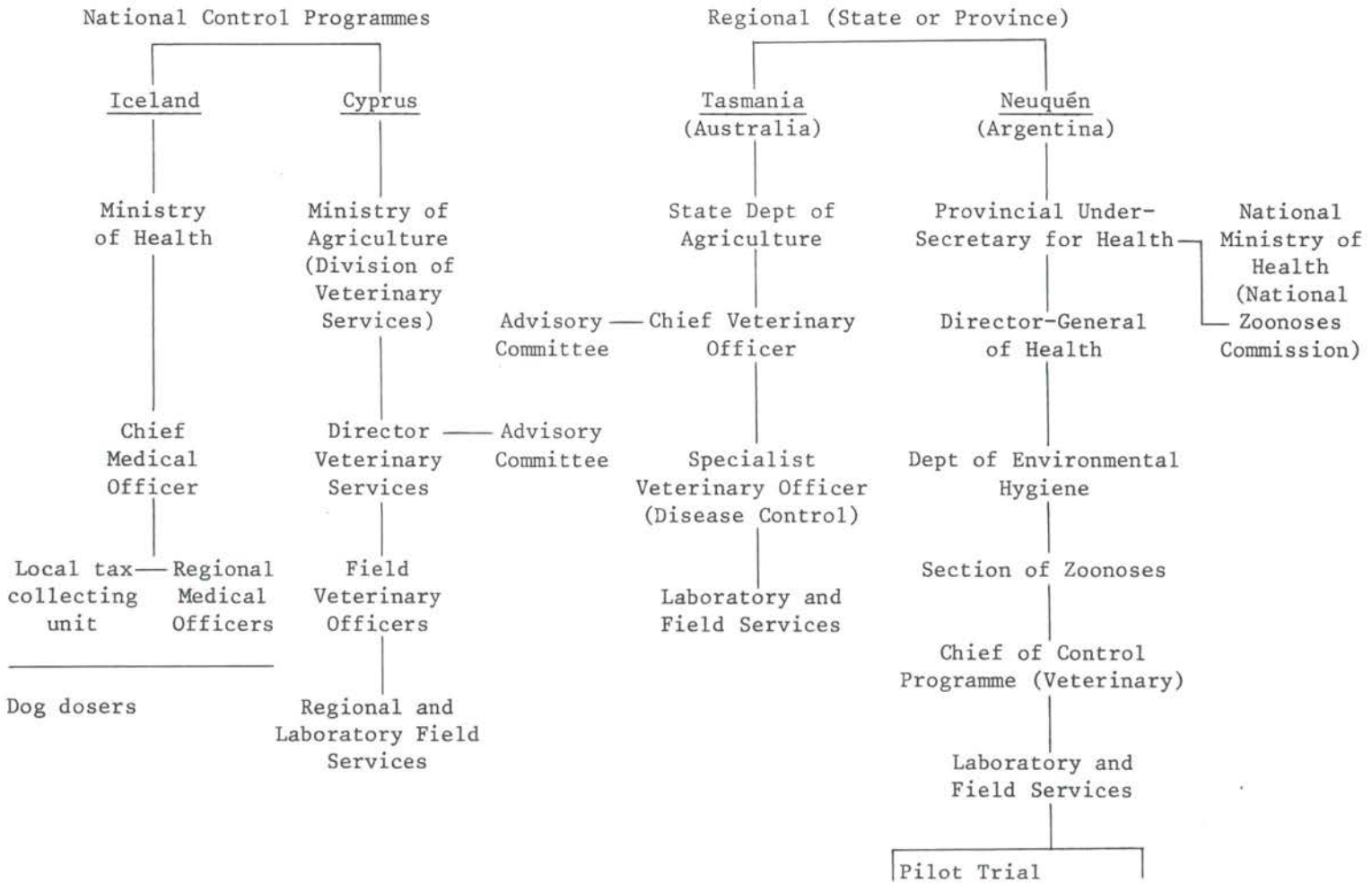
8.3 Functions

There are four important functions of any control authority be it at the national or regional level. The first is the securing of funds for the programme and its planned expansion. The second is the collection of the base-line and subsequently the continuing surveillance data. The third is the selection and training of personnel and the fourth is the direction of the educational component and the technical control measures to be adopted.

8.4 Funding the programme

It is advantageous that the control campaign should be funded by central and/or regional governments. This should be done on at least a triennial basis with guarantees of continuity until the programme has reached its declared objectives. Funds such as those from dog license fees, fines and other taxes should be regarded as important for local control measures, but they should not be regarded as substitutes for central funding.

FIG. 16. OPERATIONAL STRUCTURE FOR THE ADMINISTRATION OF AN ECHINOCOCCOSIS CONTROL PROGRAMME[8,9]



8.5 Collection of base-line and continuing surveillance data

While it is recognized that surveys may have to be undertaken before a decision can be made to implement control, the Director must assume responsibility for ensuring that the collection and evaluation of base-line data and the establishment of the continuing surveillance programme be given a major priority (see chapters 2 and 10). This has not always been appreciated in the past. It may be necessary to employ special personnel to obtain some of these data. It is emphasized that it is impossible to judge the future success of the programme without such base-line information.

8.6 Selection of staff and training programme

The Director of the programme must be responsible for the selection and training of the veterinary and paraveterinary personnel at the national, regional and district levels. He must ensure that appropriate courses are established or that the staff receive effective training in control, epidemiology, health education and meat inspection.

At the paraveterinary level, courses in animal management, meat inspection, slaughterhouse and farm hygiene, recording procedures and community health education must be developed. The paraveterinary personnel should be part of the permanent establishment and career opportunities for their professional advancement in other aspects of animal health should be encouraged.

8.7 Priorities

Competition between animal health projects almost inevitably means that finance is limited. An early decision has to be made as to whether or not a programme should begin with a pilot project in one of the most endemic zones or a full-scale nation-wide control effort should be mounted ab initio. This decision will determine the scale of staff recruitment, training programmes and the extent and intensity of the educational component.

Priorities, of course, will vary and must take account of socio-economic factors. In some countries, special emphasis may have to be placed on developing modern slaughterhouse facilities and closing unsanitary establishments. In others, full scale "arecoline surveillance programmes" may be linked to an educational programme and to the construction of effective on-farm killing facilities. In others again, a massive reduction of the dog population and the development of village-level sanitary slaughterhouses must be applied as the most urgent measure. Other priorities may become apparent following the introduction of drugs such as praziquantel both for the treatment of dogs in dosing programmes or "baiting" programmes with medicated food in regions with uncontrolled or feral dog populations.

8.8 Educational component

Almost all successful programmes rely heavily on health education to initiate and sustain interest in control. Once the sociocultural factors have been identified, the direction of the educational effort can be defined. There have been several programmes in which the educational component has provided a major contribution to control. An historic and often quoted example is that of Iceland, where in 1864, Krabbe produced a booklet in the Icelandic language. This was regarded as being unusually successful because of the natural literary disposition of Icelanders.[4] However, often it has been found elsewhere that pamphlets and other visual programmes have had little measurable effect. In both New Zealand[1,6] and Tasmania,[10,11] the special educational feature was community involvement. Emphasis was placed on the need to prevent dogs gaining access to raw offal and on encouraging the building of effective on-farm offal-disposal systems. This was reinforced with "arecoline surveillance" when the official control programmes were introduced.

A special effort to reduce the dog population was necessary in Cyprus.[12,16] This was supported by a special team of educationalists who gained community acceptance for the plan. Included in this was the direction of some of the educational effort through other central and local government agencies.[12]

8.9 Control measures

The decision has to be made on the measures to be adopted and methods by which they are to be applied.[1,3,6,7,9,12,16] There are two main control measures available, but several methods of application[7,8,9] (see chapter 11). First, most control authorities regard the prevention of dogs gaining access to raw offal at abattoirs, village slaughterhouses and on farms as vital even though it does require great effort in terms of legislation, education and manpower. The second important measure is the reduction of the parasite biomass either by reducing the dog population or by mass dog-treatment programmes. Both measures may be applied. Selection and emphasis on measures and methods depend on socio-economic factors. For control measures against E. multilocularis see section 11.3.5.

8.10 Community involvement

Several programmes have benefited by the formation of voluntary committees to assist the control authority in gaining acceptance for the plan at the community level. These committees have involved many important groups among rural and urban communities. They should be actively encouraged. However, it has been observed in some programmes that their value decreases once the programme has become accepted by the community.[9]

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CHAPTER 9

HEALTH EDUCATION

As previously stated (see section 2.6), echinococcosis is a parasitism of the human community and its animals. This community may consist of individual units, such as the farmer, his family, other workers and his livestock, or it may embrace a whole village unit, an urban area or a much wider ecosystem. The lives and lifestyles may be adapted to a wide range of environmental and socioeconomic realities.

Community health education must be regarded as a basic component of all echinococcosis control programmes. This is because effective control relies on the active cooperation of persons such as butchers, persons responsible for disposal of animal carcasses and dog owners.[3] Health education alone was largely responsible for the success of one of the earliest and effective efforts at control of the parasite in Iceland, but as has been pointed out, there were several other factors which contributed to this notable success[6] (see section 8.8). These included: (i) the small but highly literate human population; (ii) the custom of feeding dogs on cooked household scraps; (iii) restricted season for sheep slaughtering; and (iv) centralized slaughtering. Most of these favourable factors are absent in many currently endemic areas. Health education, however, must be regarded as an integral component of the control programme, and must follow closely its various phases. It cannot stand by itself as a separate campaign. Careful planning of the long-term strategy and tactics of the educational component and its integration within the control programme is essential.

9.1 Objectives

An echinococcosis control programme at the national or provincial level requires political decision and commitment. This is unlikely to be achieved only on the basis of reported morbidity in man as this is usually low with under 10 (often near five) cases treated surgically per 100 000 population. The full socioeconomic impact, which may be considerable in endemic areas, has to be brought out clearly in order to alert the community on the need for control (see chapter 7). In the planning and early phases of the programme, health education should aim at gaining public support. This has to be done in order to convince the decision-makers of the importance of the problem.[5,7]

In the operational phases, it is essential that educational programmes continue to support the various measures adopted for control.

For example, with regard to the prevention of dogs gaining access to raw offals, health education should aim at changing the attitude and behaviour of the people concerned in order to attain proper disposal of offal and safe feeding of dogs. This objective is essential, but requires enormous effort. With respect to reducing the dog population and instituting mass treatment programmes, educational efforts must be directed to maintaining the active cooperation of dog owners in, for example, periodic diagnostic examination of their animals and possible elimination or anthelmintic treatment of infected animals. In supporting the control programme, the health educator has to take into account cultural and religious traditions, traditional habits and customs, as well as attitudes such as those induced by poverty and protein hunger.

In the latter phases of the programme, it may be necessary to introduce rigid regulations or laws to deal with residual infection and with habitual defaulters. Here educational programmes will greatly assist in the enforcement of such legislation.

9.2 Target groups

In the early phases of the programme, the general public, especially communities in endemic areas have to be made aware of the danger to health as well as the economic importance of echinococcosis. As far as possible, full use should be made of the mass media. All available means of informing each community should be used, but an effective method has been found to be discussion in small groups. In such discussions, the health worker (educator) suggests some kind of concrete action, for example, formation of working committees which may be formed soon after the discussions. Such committees have proved extremely useful in the initial early phases of several control programmes. However, their value recedes once the technical aspects of the programme have been successfully introduced (see section 8.1).

In securing community support and participation, it is useful to enlist the aid of:

(i) opinion leaders who are identifiable in all communities;

(ii) parents, especially mothers of children at risk;

(iii) persons who suffer direct economic losses because of condemnation of edible viscera, reduction in weight of affected animals, etc.; and

(iv) persons who have undergone treatment or still suffer from this disease, and their relations.

An important group to be informed in the early stages is that of policy makers. Community support and involvement in the programme can often help to achieve political commitment with appropriate funding. Cost-benefit studies are helpful in this regard.

In the operational phases of the programme, health education should continue, but special attention has to be paid to the following groups:

(i) abbatoir workers;

(ii) others who slaughter animals, including shepherds;

(iii) those concerned with disposal of animal carcasses and handlers of animal by-products;

(iv) dog owners, animal attendants, shepherds and pastoralists who use dogs to tend livestock.

These groups need to be continuously reminded of their responsibilities.

In the long-term approach, important target groups for health education are school-age children and other young persons. They are not only the citizens of tomorrow, but may be particularly at risk from E. granulosus through contact with infected dogs. They can be informed through formal or informal teaching in schools or through combined home, community or school efforts. It has to be remembered that many school-age children and youths in endemic areas do not attend school. Health education should, therefore, extend beyond the school to reach educationally deprived children.

In the case of E. multilocularis echinococcosis, the respective target groups (for example, hunters, trappers) can be approached in a similar way.

9.3 Methodology

Most of the highly endemic areas for echinococcosis are situated in developing countries with relatively low literacy rates. The educational aids, such as audio-visual material, should be relevant to the content to be learned and appropriate also to the target group. Use can be made of mass media, but group discussions have been found to be much more useful

and effective in many of the successful control programmes. Written leaflets and pamphlets (see Fig. 17) may be used to supplement these discussions and lectures, but this type of material on its own is considered to have a limited impact. For school children, however, it is useful to include instruction on echinococcosis in textbooks on biology and hygiene. For younger children, line drawings of the life cycle of the parasite to be coloured have also been used with advantage in some countries.

Where social, religious or other community welfare organizations already exist, efforts should be made to secure their cooperation and support in carrying out health education. For reaching remote areas and nomad populations, mobile units equipped with appropriate educational material should be provided.

9.4 Training of health workers and school teachers

As far as possible, the health educators should be drawn from the community in which they will be working. Everyone involved directly or indirectly in a control programme against echinococcosis must carry out public health education. It is, therefore, essential that this subject should have an important place in staff training. Such training should be planned and preferably imparted by a specialist, who should also advise on the selection of appropriate educational methods and preparation of educational material suited to local conditions and to the various phases of the programme. The general training that health workers may have received in schools of public health also needs to be supplemented with briefing on the local control programme itself and the various aspects of the local situation.

It is useful to prepare and distribute a booklet or manual dealing with the technical, administrative and educational aspects of the programme. This can then be used by all persons involved in the programme, including lay members of committees or other groups set up to obtain public cooperation and support. Such a manual helps to avoid confusion caused by different answers to the same questions given by different people.

9.5 Other considerations

It is important that health education should be included in the control project from the start and should be closely linked to and coordinated with all changes in it. Continuing evaluation of the impact and limitations of health education should be undertaken and modifications should be made as and when indicated. The control programme should be technically sound, realistic and useful and should not make promises which cannot be fulfilled later on. The educational material used should take into full consideration the beliefs, perceptions, behaviour, expectations and needs of the people (felt and unfelt). This highlights the need to carry out sociocultural and socioeconomic surveys (see section 2.6) to ensure that the information imparted will be accepted by each target group.

9.6 Impact of the educational component in control programmes

So far, educational requirements have been considered in the broad sense, embracing a wide range of socioeconomic situations. There have been several programmes where the impact of the respective educational components linked to control strategy can be compared.[13,14,15] The special case of Iceland has already been considered.

A key to the control of echinococcosis in both New Zealand and Tasmania was community involvement with the formation of local committees representing interested groups at the rural and urban levels.[5,7] Once each Government's commitment to control had been made, arecoline hydrobromide was used as a diagnostic agent (see sections 6.2 and 10.2). This technology, when intelligently applied, was found to be a powerful tool in changing attitudes favourable to control.[10,11] This technology was more successful in Tasmania[1,8,9,16,17] than in New Zealand.[2] In the former programme, a mobile testing laboratory was used and the owners of infected dogs were immediately informed of the results in front of their neighbours. In addition, quarantine was applied to infected dogs with follow-up visits by control authority personnel (both veterinary and paraveterinary) to ensure that effective facilities

to prevent dogs gaining access to raw offal were introduced. In contrast, in New Zealand, the use of mobile laboratories was considered impractical because the control personnel were not employed by the central control authority. By using a central diagnostic laboratory, owners could not be immediately informed of their results. In addition, quarantine was not applied with follow-up visits and no assurance could be made that effective killing facilities were installed. The difference in the impact of these educational/control approaches was associated with a more rapid reduction in ovine echinococcosis in Tasmania than in New Zealand.

In the case of Cyprus[18-23] the control authority was part of the Department of Veterinary Services, Ministry of Agriculture and Natural Resources, being a miniature service within the Department and under the direct control of the Director. The Tasmanian control authority was similar. Here, arecoline surveillance was carried out four times each year also with mobile laboratories. Infected dogs were destroyed rather than quarantined. This programme was linked to a positive commitment to reduce the dog population (see section 11.3). This combined educational/control approach was also rapidly followed, as in Tasmania, by a reduction in the prevalence of echinococcosis in sheep.

Less successful than either of these three educational/control programmes cited above was the project in Uruguay.[4,14] This project introduced a similar control structure to that of New Zealand, and even though, as in Tasmania and Cyprus, mobile laboratories were used, the killing of infected dogs, but with no firm policy for reducing the population, tended to encourage a lucrative breeding programme. In this case, no early concurrent reduction in ovine echinococcosis was observed.

It seems that while arecoline surveillance can assist in education, it must be linked to other control policies if the maximum impact is to be achieved. In both Tasmania, with its emphasis on quarantine and follow-up visits, and Cyprus, with its emphasis on dog population control, the effective structure of the control organization together with mobile diagnostic laboratories with their educational value, may have all contributed to the strong impact of arecoline surveillance. With the unusual control structure of New Zealand, with its centralized laboratory and limited follow-up, and that of Uruguay with its mobile laboratory, but lack of a positive dog control policy, maximum impact was not achieved. Both programmes changed policy away from arecoline surveillance as an educational tool to a mass dog-treatment programme. As yet only pilot projects[12,13] have been instituted to define the educational component in echinococcosis control in the absence of arecoline surveillance. Early evidence suggest that the educational effort may have to be channelled through "traceback" of infected food animals with follow-up farm profile studies and quarantine (see section 10.3).

These valuable experiences in the educational aspects of control represent both developed and developing countries. They do not, however, take account of the problems of education faced by countries where subsistence farming still applies and primary health care is in its infancy. It is the intention of the Veterinary Public Health Unit of the World Health Organization through its zoonoses centres to ensure that experiences in health education such as those described in this section can be readily made available to all countries contemplating control of echinococcosis.

FIG. 17. MODIFIED FROM A POSTER ILLUSTRATING THE LIFE CYCLE OF ECHINOCOCCUS GRANULOSUS

(Courtesy of the Department of Agriculture, Tasmania)

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CHAPTER 10

MONITORING OF CONTROL PROGRAMMES

10.1 Introduction

Surveys (see chapter 2) and surveillance (continuing surveys) may serve distinct purposes and require different approaches. Of fundamental importance to the success of echinococcosis control is the establishment of a long-term surveillance programme to act as an indicator of progress. It is strongly recommended that this surveillance be initiated before other steps are taken to control this zoonosis. A programme has two main functions. The first is to identify problems requiring special attention. The second is to provide data on progress or lack of it. Three measurements may be required. These are changes in:

(i) prevalence of adult tapeworm infections in definitive hosts;

(ii) prevalence of larval tapeworm infections in livestock;

(iii) prevalence of larval tapeworm infections in man. Details of the methods and techniques used are presented in chapters 2, 3, 4, 5 and below

10.2 Infection rates in dogs

Several control programmes, such as those of Cyprus,[15-20] New Zealand[1] and Tasmania,[7,8,13,14] have successfully used arecoline surveillance to monitor progress in control. It must be emphasized, however, that other programmes such as those of Iceland and the Falkland Islands, have been equally successful without using this method.

Arecoline surveillance of all dogs can be undertaken every three months as in Cyprus, once each year as in New Zealand or embrace that part of the dog population considered to be the most at risk, as in Tasmania. All three programmes have used the information to deal with infected dogs. In the first-named programme euthanasia of the infected dog was mandatory, in the second, a redosing-fee-penalty was applied and in the third infected dogs were quarantined.

The value of arecoline surveillance as an indicator of progress in echinococcosis control is limited. It provides early evidence of progress before this can be assessed in the intermediate host population. However, once a low plateau has been reached, it has limitations.[11,12] The data become unreliable and do not altogether portray progress accurately. The arecoline surveillance test has a high specificity but low sensitivity (see chapter 6). Applied to a group of dogs, the test can only identify a proportion of infected individuals; therefore it does not reflect the exact prevalence of the parasite but gives an approximation of the number of infected households. Obviously, the greater the frequency of testing the more likely it will be that the majority of infected dogs will be detected. Arecoline surveillance becomes virtually useless as an indicator of progress in control where short-interval dog-dosing programmes have been implemented. However, from the educational point of view, it can still be used for identifying some breakdowns in a dog-dosing programme if applied at the same time as the taeniacide is given (see section 11.4.6).

10.2.1 Development of arecoline surveillance

To conduct surveillance programmes, it is necessary to train staff in dog handling, treatment, collection of samples, record keeping and hygiene. Dogs can be tested on special dosing strips or on individual premises. Generally the former is preferred for several reasons. These include: (i) standardization of hygiene; (ii) number of dogs that can be treated at any one time; (iii) ease of collection of samples; (iv) an educational programme can be conducted demonstrating the number of dogs with infection.

10.2.2 Testing site

The dosing strip, which should be permanent, must be isolated as far as possible from areas where people gather, for example, school playing areas. The site should be determined in consultation with local health authorities. It should be well drained and fenced and the gate should be kept locked when not in use. The area should be signposted so that it is identifiable as a dog-dosing area. When a strip is sited adjacent to a road, portable signs to warn traffic should be displayed when in use. A hard surface is to be preferred. The surface may be concrete or bitumen, or it may be grassed provided it is closely mown.

A satisfactory structure for tethering dogs consists of a permanent fence with the following features:

(i) posts at each end;

(ii) standards at 1 m intervals;

(iii) wires at the following heights above ground, bottom - 2 cm, middle - 45 cm and top - any suitable distance - say 90 cm. Suitable wire includes 4 mm mild steel galvanized and 2.5 mm - high tensile wire;

(iv) wire netting - height 1 m, attached to the three wires.

The dogs can be tethered at about 2 m intervals, and the tethering chain should not exceed 0.3 m. The aim of this spacing is to avoid confusion in the identification of faeces. An alternative mobile method includes the use of steel standards. These are driven into the ground and connected by a chain at about 45 cm above ground. The short dog chains are attached to this long chain. This is relatively easy to erect and dismantle. In Tasmania the system of tethering on testing strips consist of a series of steel pegs, about 50 cm long each with a ring welded to the top, and sunk in the ground for their whole length. Dogs are attached to these rings by chains 80 cm in length. The pegs are spaced about three metres apart, so that the areas on which purges can be deposited, a series of circles of about 150 cm radius, do not overlap.

10.2.3 Laboratory versus field testing

Both fixed laboratory and mobile field testing technology are available to carry out surveillance.[11] There is no advantage of one method over the other with regard to using the data as an indicator of progress. The real advantage of field over laboratory diagnosis lies in its educational value (see section 9.6). With the former it is possible to identify infection immediately and treat the dog with an appropriate drug. The educational value lies in the fact that the identification of the infected dog is undertaken in the presence of the owner and his neighbours.

10.2.4 Health precautions

All equipment must be heat sterilized before the day's work begins and boiling water must be made available throughout the period of activities. Each dosing team must have:

(i) personal protective and hygiene items such as rubber boots and overalls;

(ii) dog-dosing equipment including dosing gun;

(iii) receptacles;

(iv) water tank and boiler.

All liquids used on dosing strips should be kept in suitable plastic containers so that the exteriors can be sterilized by dipping in boiling water before they are removed from the strip.

The same thoroughness should be applied to personal hygiene. Absolute freedom from contamination of vehicles and related equipment is essential. All implements such as trowels, spades, buckets, chains, syringes, boots and rubber gloves must be thoroughly cleaned with water maintained at boiling point before they are replaced in the vehicle. A good source of hot water includes a gas-heated boiler. Hand washing facilities must be made available to all personnel using the strip. Used paper towels must be burned or buried. Overalls must be sterilized before being placed in the vehicle. They should never be sent unsterilized to a laundry. As far as possible, only trained staff should enter the dosing strip. Smoking should not be permitted. Where arecoline testing is conducted on a household basis the hygiene precautions detailed above must be maintained. The high standard adopted by control authorities may itself be regarded as health education.

10.3 Infection rates in food animals

Several programmes have utilized changes in the prevalence of echinococcosis in livestock as a major indicator of progress. This can be done by "trace-forward" or "trace-back" procedures identifying each consignment submitted to slaughter at licensed killing establishments. This is a powerful tool of high sensitivity and specificity, provided certain precautions are taken to exclude from the data parasites such as Taenia hydatigena and Fasciola hepatica. This surveillance technology should be given the highest priority. Ideally, all infected animals slaughtered at official killing establishments should be recorded. Where possible estimates should be made of the age-specific infection rates for each animal owner. Limitations of the system include the fact that animals may be transferred over various distances or through several ownerships during their lifetime and some may have become infected outside the control zone.

"Trace-forward" techniques are usually applied in small abattoirs where the ownership of the consignment is known to the inspector before slaughter. 'Trace-back' procedures (information of ownership determined after slaughter) can be applied in large killing establishments by using a ticketing system. These data can be readily computerized. In some control programmes,[7,8,13-20] special staff have been employed on echinococcosis surveillance at killing establishments. In others, especially where a chain system of slaughter is operative and ticketing of infected carcases can be applied, this can be done by the normal meat inspection service.

In the early years of the control programme, the data from the older animals may be of limited importance, because they reflect events that have occurred several years before the commencement of the programme. However, as the control programme proceeds, the data become important not only for measuring progress, but also for control purposes. At a certain stage, clustering effects may be observed. This is a reflection of "breakdowns" in control. By either "trace-forward" or "trace-back" procedures, ownership can be identified and the flock or herd can be purchased by the control authority for slaughter under supervision or the flock or herd can be placed under quarantine. It is being recognized more and more that the identification of those flocks or herds with echinococcosis long after most have been freed from infection provides an important control tool. By undertaking whole farm profile investigations, errors in on-farm killing procedures and dog control can be identified and changed. An example of a questionnaire for a whole farm surveillance scheme in New Zealand is given in Figure 18. In this scheme, T. ovis (T.o) and T. hydatigena (T.h) in lambs are included as indicators of recent breakdowns in the control measures.[2] Such data lend themselves to processing by modern mathematical procedures, e.g. multiple regression analysis.[22]

10.4 Infection rates in man

Authorities contemplating control measures should note that systematic recording of prevalence or incidence as an indicator of progress needs to be started as soon as possible. Ideally, retrospective data should be collected over a period of at least five years. Survey methods and their limitations are described in section 2.

FIG. 18. EXAMPLE OF QUESTIONNAIRE FOR FARM PROFILE STUDIES

OFFICE ..... DATE .....

MINISTRY OF AGRICULTURE AND FISHERIES
ANIMAL HEALTH DIVISION
JOINT ON-FARM INVESTIGATION FOLLOWING HYDATIDS INFECTION IN STOCK

Investigating SLO/LO ..... REPORT No. .....

Hydatid Control Officer ..... Highest Infection

Name and address of Property ..... T.o. Lambs .....

..... T.o. Ewes .....

..... E.g. Ewes .....

How long has owner been on property?.....Yrs.

Were any of consignment brought in? Yes/No Lambs Yes/No Ewes Yes/No

Name and address if known .....

DETAILS OF PROPERTY

Size ..... Hectares Nearest township ..... Distance .....kms

Do hunters/fishermen/picnicers/etc. come onto property? Yes/No

Type of Land: Flat/Rolling/Hill Clean/Semi/Rough

Type of Farming: Sheep/Cattle Mixed/Dairy/Crop Stores/Fat/Breeding/Stud

General state of property: Very tidy/Average/Untidy

How many Ewes? ..... Hoggets? ..... Wethers? .....Rams? .....

DOGS ON PROPERTY

Number Dogs. Working ..... Pet ..... Retired ..... No. dog owners .....

Killing/feeding facilities shared by all dog owners? Yes/No

If NO fill in separate form for each other owner and omit from this form.

Infection history. T.o ..... E.g. ..... T.h. .....

HOME KILLING

Regular killing done on property? Yes/No

If Yes, by whom. Owner/Manager/Son/Employee

How many sheep/year for house ..... for dogs .....?

Is occasional killing done by Relative/Seasonal Worker/Other? Yes/No

Where is killing done? Under tree/in Paddock/in Woolshed/in Cowshed/in Killing Shed/Other

Is killing site dog-proof? Yes/No In good repair Yes/No Clean and tidy Yes/No

Drain and sump present? Yes/No In good repair? Yes/No Dog-proof Yes/No

Was door of killing site closed at time of visit? Yes/No

Is cutting up site dog-proof? Yes/No

OFFAL DISPOSAL

How is offal disposed of? Pit/Commercial bore/Trench/Ditch/Mineshaft/Gully/Cliff/Incinerator/ Offal cooker/Other (specify)

Is site dog-proof? Yes/No Incorporated with killing site? Yes/No

If not, distance from killing site .....

Is offal container suitable? Yes/No Stored in a dog-proof site? Yes/No

Is system adequate for farm requirements? Yes/No

FIG. 18 (continued)

NAME ..... REPORT No. .....

CARCASE DISPOSAL

Estimated stock loss .....p.a. Abnormal losses (specify) .....

Carcases disposed by: Shovel/Pit/Commercial bore/Trench/Ditch/Mineshaft/Gully/Cliff/ Incinerator/Bulldozed into ground/Burnt/Other (specify)

More sites required? Yes/No Sites dog proof? Yes/No

Carcases removed promptly? Yes/No Dog access to sheep skins? Yes/No

Evidence carcases present or recently removed from property? Yes/No

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DOG FEEDING

Who usually feeds dogs? Owner/Family/Manager/Employee

What is fed?

Home killed mutton/Mutton ex works/Ex Abbattoir/ex Butcher's shop/ Household scraps. Opossum/Sheep Heads/Untreated Flap or shank/ Commercial pet meat/Dog crackers or nuts/Boiled offal/Cooked mutton/ Beef/Horse meat/Other (specify)

How often are dogs fed? 1/day 1/2 days Other (specify)

If frozen Mutton Fed Freezer size ..... Adequate for requirements? Yes/No

Below 10°C? Yes/No Rotation system? Yes/No

7 days supply Yes/No Offal/Sheepheads present? Yes/No

Does dog owner know freezing requirements? Yes/No

If offal/meat boiled - boiled for required time? Yes/No

Has there been any occasion during past year when untreated meat was fed? Yes/No

If yes, elaborate .....

.....

DOG CONTROL

Any untreated dogs? Yes/No Untreated "Pups"? Yes/No Age .....

Annual dog turnover. No. acquired .....No. transferred .....

Dog housing. Loose/Kennel/Motel/Tied in shed/in House

Dog housing in good condition? Yes/No Fresh water supplied? Yes/No

Dogs tied up when not in use? Yes/No Snap hooks on collars? Yes/No

Loose at time of visit? Yes/No Pet dogs under control? Yes/No

Stray dogs. Stray dog problem? Yes/No

If yes, elaborate .....

Does owner take action on stray dogs? Yes/No

If not, elaborate .....

Does owner ask for treatment certificates of all visiting dogs? Yes/No

If not, why not .....

In the opinion of investigating Officers:

What is dog owners knowledge of Hydatids like? Good/Average/Poor

What was owners attitude to investigation and recommendations? Good/Average/Poor

What was probable cause of infection on farm? .....

.....

Signed: Date.....

The exact prevalence of echinococcosis cannot be measured directly, because of the number of "silent" cases.[3-6] It can, however, be estimated retrospectively from serial necropsy data. Symptomatic cases can be collected by compulsory notification and by recording hospital admissions. Notifications are generally unreliable as they frequently include readmissions. Even with this loading, notifications frequently fall short of surgical incidence. For this reason, the data should be collected by a specialist designated for this purpose.

10.4.1 Necropsy studies

Serial necropsy studies such as those in Iceland have been found to be of considerable value.[9,10] These data contain a large number of "silent" cases. In the case of Iceland, autopsies were made compulsory in 1932. It was confirmed that much of the health problem from hydatid disease had been resolved before that time.

10.4.2 Surgical prevalence or incidence

Annual prevalence and surgical incidence of echinococcosis in man over a sufficiently long period of time provide the most useful indicator of the effectiveness of the control measures that have been applied. They also identify foci where control has been less successful and that may require special measures which if applied to the whole community could not be justified on account of cost.

Comparisons between different time periods and geographic regions must take account of the fact that some reports use estimates of prevalence and others of incidence. Also the rate of progress in the total population may not be comparable between zones that have substantial differences between urban and rural distribution in the population. It has been pointed out [21] that it would be more satisfactory, when multiple readmissions occur, to report the surgical incidence (i.e. the admission rate for surgically confirmed new cases). The advantage of this is that some control programmes have shown an early and substantial reduction in surgical incidence. In the measurement of incidence, it is important to obtain information on the birth place and the place of residence and age of the patient at the time of admission.

In a developed country, where the majority of people who need surgical treatment can have it, the surgical incidence rate is a reliable index of the true incidence. However, since infection with this parasite is not always synonymous with disease, the surgical incidence should be regarded as the figure that counts. Where, however, surgery can be given only to a proportion of those in need of it, the surgical incidence becomes less reliable as an indicator of progress in control. The incidence figure should include inoperable and untreated fatal cases and must be based on a proper parasitological diagnosis (see chapter 4).

10.4.3 The value of surveillance

Experience in several control programmes has shown that the age-specific surgical incidence rate of echinococcosis decreased in the human population before the survival of the parasite population was jeopardized in the normal animal intermediate host. This reduction was observed in all age groups and probably results from the decrease in new infections with fast growing cysts.[3,4] It follows that control measures can be justified in the future by the expectation of an early benefit to the whole community including the elderly.

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CHAPTER 11

PREVENTION OF INFECTION AND REDUCTION OF PARASITE BIOMASS

11.1 Introduction

All control programmes regard the prevention of dogs gaining access to raw offal as a fundamental measure for the control of echinococcosis. The methods that can be applied are summarized in Fig. 19.[44,45,52]

FIG. 19. CONTROL MEASURES PRINCIPALLY DIRECTED TOWARDS PREVENTION OF INFECTION OF CANINE ECHINOCOCCOSIS (FROM 45)

Measure	Method
Disposal of offal:	
at abattoirs	Meat inspection
at local slaughterhouses	Restructure
on farms	Education
	Legislation
	Erection of killing facilities
	Burning or burial
	Sterilization

The reduction in the parasite biomass forms the second important measure in the control of echinococcosis. The methods that can be applied are summarized in Fig. 20.[44,45,52]

FIG. 20. CONTROL MEASURES PRINCIPALLY DIRECTED TOWARDS REDUCING THE TAPEWORM BIOMASS IN DOGS (FROM 45)

Measure	Method
Control of dogs	Registration (collar/tattoo)
Reduction of dog population	Banning of dogs from restricted areas
	Mass killing
	Test and kill
Reduction of tapeworm population only	Test and penalize
	Mass drug treatments

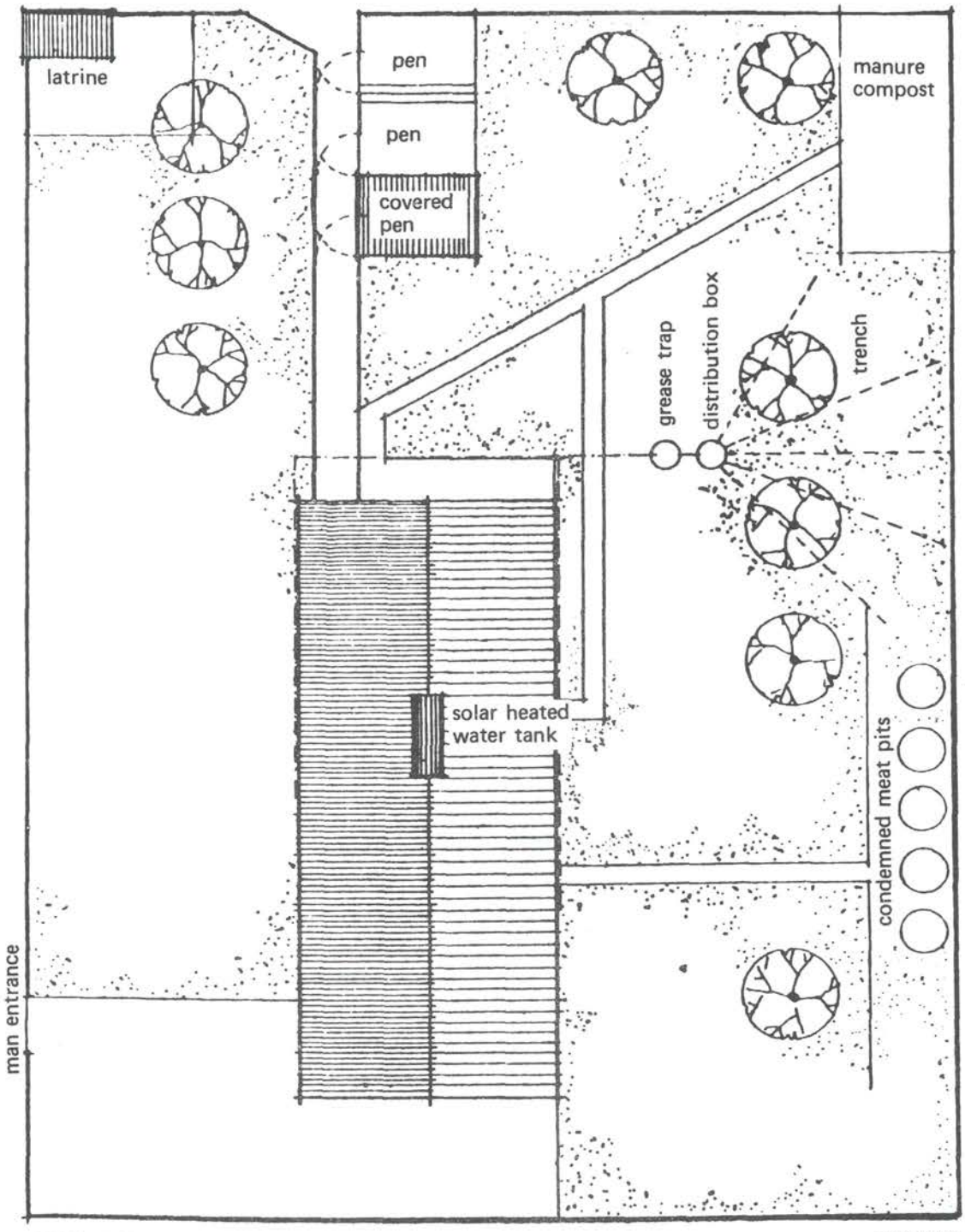
11.2 Prevention of infection from slaughtered animals

Priorities in the provision of animal slaughter facilities differ between endemic zones. In some programmes, (for example, Cyprus), priority has been given to the establishment and development of local (village) abattoirs,[92-95,97-99] whereas in others (for example, New Zealand and Tasmania), animal slaughter was centralized prior to the introduction of the control programme. In the latter, priority was given to the development of "on farm" killing facilities.[7,83] The choice depends on factors such as population density, frequency of slaughter and socioeconomic circumstances. This highlights the need for base-line data.

11.2.1 Safe slaughtering in large killing establishments

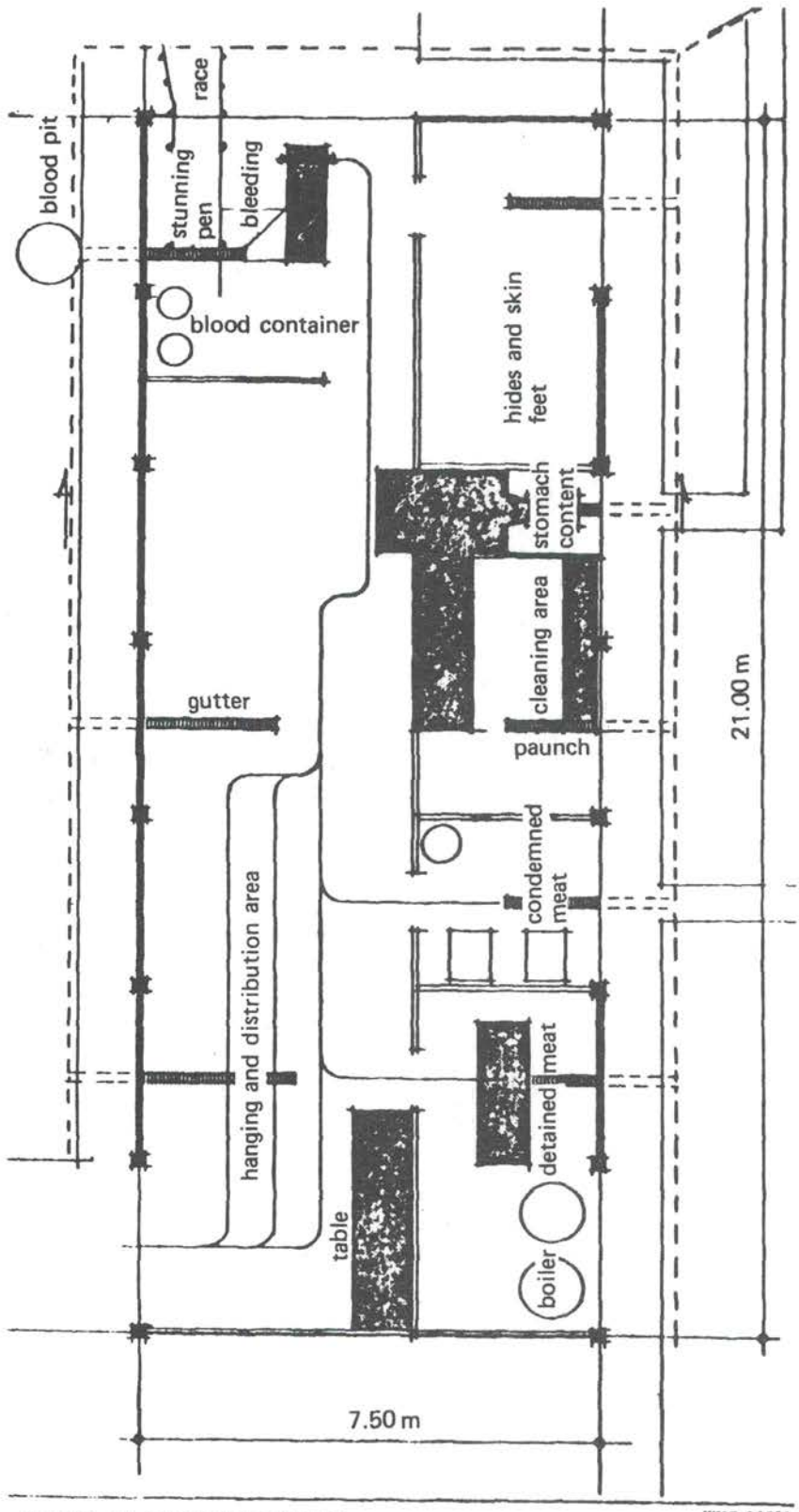
At most city abattoirs, condemned viscera are usually processed. However, precautions may be required to ensure that this material is not stolen or clandestinely used for dog

FIG. 21



RURAL SLAUGHTERHOUSE
SITE PLAN scale 1: 200

FIG. 22



RURAL SLAUGHTERHOUSE
PLAN scale 1 : 100

food. In smaller city abattoirs, which may or may not have refrigeration or processing facilities, the disposal of condemned offal may present real problems. The effective supervision of disposal of this material forms an important part of echinococcosis control, because if no control is applied, urban echinococcosis may continue.

11.2.2 Safe slaughtering for village communities

A village abattoir should meet the minimum standards for sanitary slaughter, including proper layout, effluent disposal and clean water supply (see Figs. 21 and 22).[114] There should be incinerating facilities within the slaughter compound. However, if there is no incinerator, there should be at least two condemned offal pits. The pit should be at least 20 m from the slaughter floor. It should be at least 5 m deep and have a diameter of 2 m. If the ground is too hard to allow pits to be dug, an incinerator then becomes essential. The pits should be sealed and new ones should be constructed when the contents reach 1 m below the ground level.

The abattoir should be situated at a reasonable distance from the village and to prevent entry by dogs, the whole compound should be fenced. The fence should be set well into the ground to ensure that dogs cannot burrow under it. The compound should be locked when not in use.

The construction of an abattoir compound may need the financial assistance of government. In one programme, half the cost was borne by the district commissioner (i.e. government) and the other half by the village community with encouragement from a long-term low interest loan from the government.[98] Only abattoirs fulfilling the above minimum requirements should be permitted to operate under licence. The appropriate campaign authority should cooperate closely with and assist the village authorities in planning and constructing the compound as well as in indicating improvements to existing slaughter facilities. If the village authorities fail to respond to the recommendations, slaughter at that village should be prohibited until the improvements have been made. The control authority should visit the village regularly to discuss any set-backs or delays in the construction programme.

11.2.3 Prevention of illegal slaughtering

Butchers at licensed village abattoirs should notify the control authority of the days and times when slaughter will be undertaken. The authority should supervise the slaughtering and should inspect all carcasses and ensure the correct disposal of infected offals. The dressed carcass should be stamped to indicate that it has been inspected. Unstamped carcasses may indicate illegal slaughter. Periodic inspections of butchers' shops and meat stores may be necessary to enforce these regulations.

11.2.4 Emergency slaughtering

Where emergency slaughter is necessary, the carcass and offal should be subjected to the same procedures of meat inspection laid down by the control authority.

11.2.5 Safe farm slaughtering

In most endemic situations, it is the rural dog that forms the important focus of echinococcosis. Diseased viscera unfit for human consumption are often regarded by rural workers as an economic, practical and important nutritional source of food for dogs. It is these attitudes and beliefs that require to be modified. Even when these have been changed, many breakdowns in control have been shown to have resulted from carelessness in offal disposal during the killing process.

Methods of disposal include the burial in dog-proof pits and burning under controlled conditions. Care is required to ensure that dogs cannot gain access to raw offal prior to or after apparent disposal. Effective dog-proof killing facilities on farms are recommended in some countries, while in others the disposal of offal with locally developed carcass burners forms an important part of control.[8]

FIG. 23. A DOG-PROOF KILLING ENCLOSURE[8]

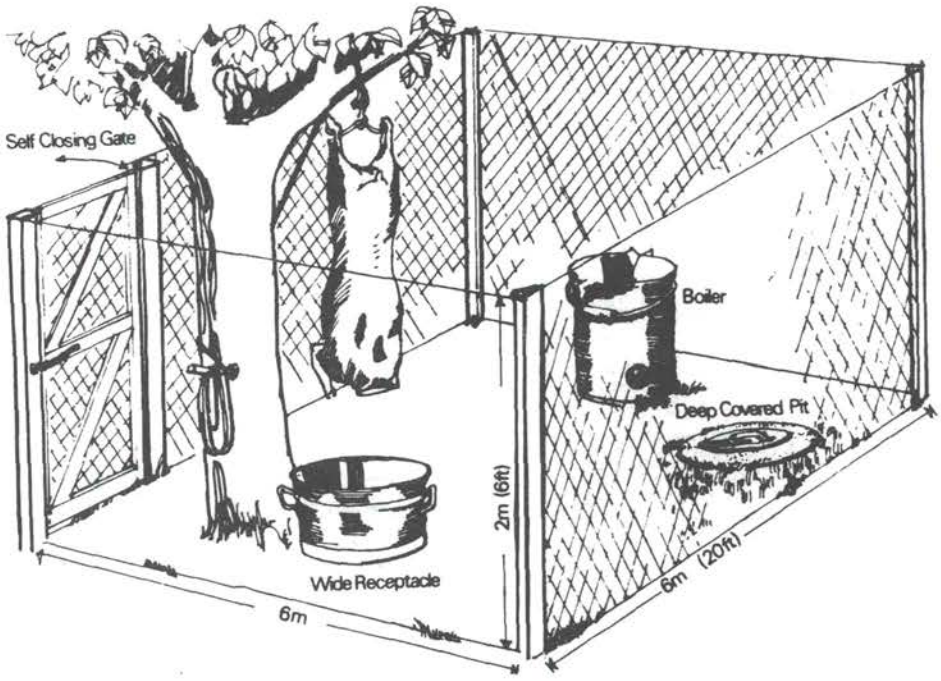
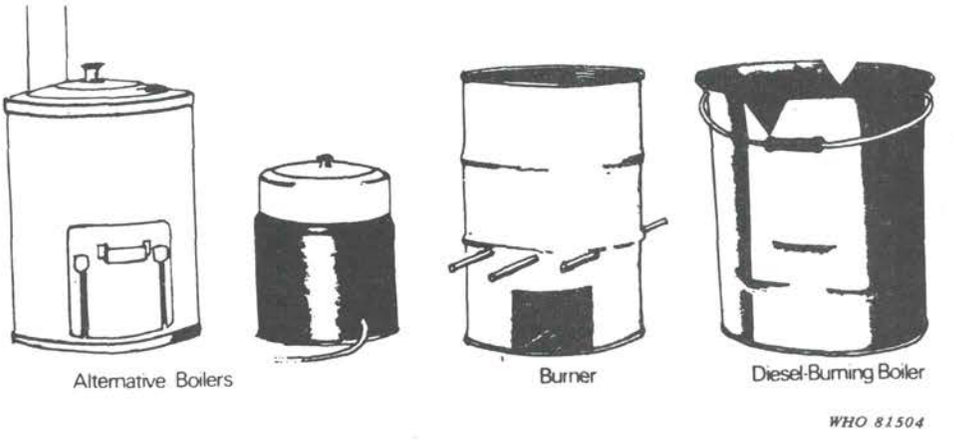


FIG. 24. EXAMPLES OF BOILERS AND A BURNER THAT CAN BE USED IN A DOG-PROOF KILLING ENCLOSURE[8]



As already implied, farm or home slaughtering of livestock is one of the main causes of the perpetuation of echinococcosis. This is due mainly to the improper disposal of offal, and in many cases, the deliberate use of offal as dog food. As a matter of principle, home slaughtering should be discouraged and every effort should be made to encourage individuals to use centralized slaughter facilities. However, when for socioeconomic reasons, home slaughtering cannot readily be prevented, special precautions must be taken. As a minimum precaution, an effective dog-proof enclosure within which offal and trimmings can be destroyed, must be constructed. The requirements of a dog-proof enclosure include:

(i) a wire netting fence high enough to be dog-proof and of a mesh size small enough to keep out pups (see Fig. 23); portable structures may be particularly useful where occasional slaughterings are carried out for social ceremonies;

(ii) gallows, mobile gantry hoist or tree from which a carcass can be suspended;

(iii) a receptacle for offal and trimmings;

(iv) a boiler or burner in which offal can be boiled or incinerated, or a pit in which they can be buried (see Fig. 24).

The following routine procedures are important:

(v) all dogs must be tied up during slaughtering operations and until cleaning-up is completed;

(vi) particular care must be taken not to rupture hydatid cysts when slaughtering sheep, cattle, camels, etc.

11.2.5.1 Sterilization of offal

It is recognized that raw offal may provide the only readily available source of protein in some areas. Its destruction, although justified on epidemiological grounds, may be hard to justify in areas where animal protein is scarce.[80,81] Hence, there is a need to ensure thorough sterilization of offal if it is to be used as dog food.

Sterilization by boiling for 40 minutes[37] has been permitted as a method of control in some programmes (see Fig. 24).[7] Deep freezing at -18 to -20°C for at least 48 hours has been reported to kill hydatid organisms[1,9] and some authorities recommend this as a method of sterilization. However, because of the possibility of super-cooling effects on the survival of protoscolices, some other control authorities do not accept this as a safe means for sterilization. Other methods, such as immersion in salt, should not be permitted, since it has been found to be unreliable.

11.2.5.2 Disposal of carcasses

Leaving dead carcasses and offal in the field should be made a legal offence, but the nature of the terrain may make this very difficult. The owner should be encouraged to burn or bury them immediately or dispose of them through a licensed felmonger (knacker). Where there is no felmonger and where deep burial is difficult, the local authority should provide a communal dog-proof carcass disposal pit. The authority or its authorized agents should inspect farms and village grazing areas regularly. They should ensure that carcasses are removed immediately. Felmonger establishments should come under the same strict control and supervision as those applied to slaughterhouses. Offal from such establishments destined for dog or hound food should be sterilized before they leave the premises.

Under special circumstances, predatory carnivores may have access to human corpses, thereby establishing a direct dog-man life cycle. Educational programmes are necessary to change such customs and practices where echinococcosis is endemic.

11.3 Dog control

11.3.1 Registration of dogs

All control programmes favour an effective scheme for the registration of dogs.[52] This should be undertaken by local government authorities. However, where this cannot be done, the control authority itself should undertake this task. Registration and notification of change of ownership should be obligatory, unregistered dogs being liable to collection and destruction. Restrictions on the numbers of dogs owned by an individual can be imposed and differential registration fees can be applied with particular reference to limiting the number of unspayed females. Special restrictions can be implemented for limiting the number of breeding bitches. For registration to be useful in echinococcosis control, it must be rigorously applied and it is fundamental that the register be accurate and up to date.[96] An alternative approach is the registration and licensing of owners to keep dogs.

Two main methods of identification of dogs have been used. The one involves a dog collar with an attached registration number and the other involves an ear tattoo. The latter permits more effective policing and prevents substitution.

11.3.2 Elimination of unwanted dogs

The reproductive capacity of bitches is such that the dog population can recover rapidly following discontinuous suppressive efforts. Without effective dog control, the surplus can become enormous. To overcome this in one control programme, it was found necessary to mount a special educational campaign to convince the community of the need to reduce the dog population drastically.[96] In the integrated programme involving both echinococcosis and rabies in Bulgaria, special attention has been given to the reduction of the dog population and registration has been strictly enforced.[90,91] In most circumstances, baited traps or nets permit a high capture rate. Methods applied have also included the shooting of "unwanted" and "stray" dogs. Usually, ordinary firearms are used in rural areas. However, the use of tranquillizing drug guns for capturing dogs for subsequent euthanasia can be applied in urban and suburban areas. The euthanasia of unwanted new-born puppies should be actively encouraged.

11.3.3 Control of dogs in selected areas

In Iceland, most of the larger towns use the legal option to ban the keeping of dogs altogether. In other control programmes, exclusion of dogs from the proximity of abattoirs has been rigidly enforced.[52] Alternate measures include the requirement that dogs working in close association with livestock must be muzzled. Working dogs should be tied up or kennelled when not in use. Pet dogs should be kept on a leash when outside the home.

11.3.4 Spaying of bitches

In some control programmes, an important aspect of dog population control is the spaying of bitches.[96] There is evidence that an active spaying programme in conjunction with a positive dog control policy helps to stabilize the dog population at a satisfactory level with a marked reduction in the number of "unwanted" and "stray" dogs. All teams in the control programme, particularly the educational team, should be involved in persuading owners of bitches to have them sterilized. For greatest impact, surgical sterilization should be subsidized by the control authority.

11.3.5 International regulation of dog movement

The movement of carnivores is controlled already by legislation in many countries (see International Zoo Sanitary Code - amended edition 1976 - published by International Office of Epizooties, Paris). It would be useful to take advantage of the rules of this Code to impose restrictions on the importation of carnivores from zones endemic for Echinococcus spp. In some countries, the authorities require the treatment of dogs with praziquantel prior to importation (see section 11.4).

11.4 Chemotherapy

11.4.1 Introduction

In this section, some of the drugs[a] reported in the literature over the last 20 years as having cestocidal activity are reviewed. An attempt is made to identify effective dose rates or treatment schedules, taking account of toxicity and other factors. The method of determining the dose rate is based on an estimate of the proportion of dogs cleared of tapeworms following a single treatment or multiple treatments. Briefly, the single doses ED_{50} and ED_{90} (with 95% confidence intervals) are estimates of the doses required to eliminate all tapeworms from 50% and 90% of dogs, respectively. Some of the reasons for using this method for defining dose rates and treatment schedules are discussed in reference 46. The drugs reviewed are divided into those which have no consistent echinococcicidal value, those which are moderately active, but require more than one treatment to reach the ED_{90} at safe dose levels, and those which are strongly active and the ED_{90} is achieved with a single treatment. The efficacy and limitations of the drugs described in this section have been extensively reviewed.[46]

11.4.2 Drugs with no or inconclusive activity against *Echinococcus* spp.

Dichlorophen (Anthipen, Didroxane, Diphenthane-70, Hyosan, Parabis, Plat-Lyse, Preventol G-D, Taeniathane, Taeniatol)

Conflicting evidence has been reported on the efficacy of dichlorophen against *Taenia* spp. and *Dipylidum caninum*. The ED_{50} for a single treatment against *Taenia hydatigena* lies well above the recommended dose rate of 150-300 mg/kg.[46] No consistent evidence can be found supporting activity against *E. granulosus* and *E. multilocularis* infections.[40,77,89,103,105,107,128] The drug cannot be recommended for the treatment of canine echinococcosis (see Tables 7 and 8).

Niclosamide (Cestocid, Lintex, Mansonil, Phenasal, Radeverm, Sagimid, Sulqui, Tredermine, Vermitin, Yomesan, Zemun)

Many studies have been reported on the use of niclosamide in the treatment of canine taeniasis and dipylidiasis.[46] One estimate of the single dose ED_{90} for the treatment of *T. hydatigena* was 8 (5-15) mg/kg,[55] indicating that the drug is strongly taeniacidal. Most writers agree that the drug is safe and without serious side effects, although vomiting and/or diarrhoea have been reported as relatively common sequelae at and above 32 mg/kg.

The drug appears to be very erratic in its action against *Echinococcus* spp. Some reports suggest that it is effective and recommend the drug for the treatment of canine echinococcosis, whereas others conclude that the drug is ineffective.[12,13,24,25,27-29,38,55,70,71,75-79,82,85,88,89,102,103,105,107] The possibility exists that the age of the tapeworms at the time of treatment may account at least in part for some of the conflicting results. The ED_{50} for 28-day-old *E. granulosus* does not lie within the dose range 75-200 mg/kg, and the number of treatments required to reach the ED_{90} is not known (see Tables 7 and 8).[55] The drug cannot be recommended for the treatment of canine echinococcosis.

11.4.3 Drugs with moderate activity against *Echinococcus* spp.

Antibiotics

Antibiotics with taeniacidal activity include *Streptomyces griseocarneus* strain S15-1.[46] This antibiotic complex was found to be strongly active against *T. hydatigena* infections in dogs and the single dose ED_{90} was estimated to be 14 (9-24) mg/kg. The drug was also active against *E. granulosus* and the single dose ED_{50} was estimated to be 84 (12-570) mg/kg.[59] The number of treatments required to reach the ED_{90} has not been determined (see Tables 7 and 8). In the absence of sufficient data, the drug cannot be recommended for the treatment of canine echinococcosis.

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[a] Arecoline, as a vermifuge, is described in chapter 6.

Benzimidazoles

The benzimidazoles as a group have a broad spectrum of activity, particularly against the gastrointestinal nematodes of man and animals. Fenbenzadol, parbendazole and oxibendazole were reported to be more active than cambendazole and tiabendazole against T. hydatigena infections in dogs, but several treatments were required to achieve strong activity.[54] Both mebendazole (micronized powder) and oxfendazole appear to be much more active and estimates of the ED_{90} for single dose treatments were 8 (3-18) mg/kg and 47 (25-84) mg/kg, respectively.[53,62]

Only two of this group have been studied for activity against E. granulosus. The single dose ED_{50} for oxfendazole was estimated to be 1 (1-165) mg/kg.[62] The single dose ED_{90} for mebendazole (micronized powder) was estimated to be 56 (14-218) mg/kg.[53] No estimate can be made for the commercial tablet (Telmin^{R}) from the literature, but it has been suggested that particle size effects involving agglomeration may reduce activity.[46] Mebendazole is a safe and virtually tasteless compound, and has been found to be active against E. granulosus when incorporated in food.[58] Further studies are required with this compound before its role in echinococcosis control can be defined (see Tables 7 and 8).

Bithionol and bithionol sulfoxide (Bitin, Bitin-S, Actamer, Lorothidol)

Bithionol and/or bithionol sulfoxide have been evaluated against canine taeniasis and dipylidiasis.[46] The drug is strongly active against T. hydatigena and an estimate of the single dose ED_{90} was 36 (24-55) mg/kg.[64] Vomiting and/or diarrhoea were relatively common sequelae, but deaths were not recorded at this dose level.

The drug is moderately active against Echinococcus spp. infections in dogs.[64,77,103,107,133] Three doses at 200 mg/kg of bithionol sulfoxide, but not bithionol, were reported to be effective against E. multilocularis.[107] An estimate of the ED_{90} for a single treatment of bithionol sulfoxide against E. granulosus was 152 (71-345) mg/kg. At this dose level, deaths were recorded.[64,107] The number of treatments required to reach the ED_{90} at safe dose levels has not been determined, and in the absence of such data, no recommendation can be made regarding its use in echinococcosis control (see Tables 7 and 8).

Bunamidine hydrochloride (Scolaban)

Many studies have confirmed the activity of bunamidine hydrochloride in the treatment of taeniasis and dipylidiasis.[46] The dose rate recommended was of the order of 25-50 mg/kg. Neither the dose rate nor the number of treatments required to reach the ED_{90} for the treatment of taeniasis can be determined from the literature. Diarrhoea and/or vomiting were relatively common sequelae at dose rates of 25 mg/kg and above.

Bunamidine hydrochloride has a moderate activity against E. granulosus.[4,11,15,16,19,29,39,41,51,65,71,75,101,125,131,132,136] The evidence suggests that at 25-50 mg/kg, the drug can usually be expected to reduce worm burdens by about 80-90%. The drug appears to be somewhat erratic in its action. This may be associated with a number of factors, including the age of the tapeworms at the time of treatment.[4,51] Two treatments of bunamidine hydrochloride at 40 mg/kg were reported to be effective in eliminating E. multilocularis infections.[107] The drug was regarded as having ovicidal properties,[132] but this was not the case against the eggs of E. multilocularis.[107] The number of treatments required to reach the ED_{90} at safe dose levels has not been determined (see Tables 7 and 8).

Bunamidine hydrochloride is relatively toxic to dogs. Field studies have shown that deaths (about 1/3000 doses) occurred at and above 25 mg/kg under circumstances where substantial amounts of catecholamines were liberated in the blood, as in excitement.[18,33-36,84,126] Deaths after bunamidine hydrochloride treatment have been recorded in veterinary practice.[130] This lethal effect restricted the use of bunamidine hydrochloride in, for example, the echinococcosis control programme in New Zealand to the treatment of dogs identified as harbouring E. granulosus by the "arecoline surveillance" programme. It is

important to recognize the limitations of compounds in regions where measures are operating to control echinococcosis.[125] Based on the experiences gained in practical field control trials and case history reports (but not on estimates of the ED_{90}), it seems that a single treatment with bunamidine hydrochloride given once daily for three days at 50 mg/kg has been found to be useful in the control of canine echinococcosis.

Bunamidine hydroxynaphthoate (Buban)

The drug has been relatively extensively studied for activity against E. granulosus.[47,48,68,69,71,72,87,101,102,112,134,135] The drug appears to be more active against mature than immature infections. Vomiting and/or diarrhoea were relatively common sequelae at 32 mg/kg and above. Deaths, however, were not recorded at this and higher dose rates. Estimates of the single dose ED_{50} were determined at 27 (9-83) mg/kg. It seems that at least three treatments may be required at the recommended dose rate of 64 mg/kg to ensure that most dogs are cleared of E. granulosus (see Tables 7 and 8). Bunamidine hydroxynaphthoate appears to have a greater cestocidal activity when given in food than when administered to fasted animals. Attempts have been made to incorporate the drug in food.[47,48,72] The drug has a bitter taste and difficulties have been experienced in masking this and making the food acceptable for continuing treatment schdeules.

Fospirate

Fospirate is strongly active against T. hydatigena and the ED_{90} has been estimated to be 4 (2-7) mg/kg.[49] In contrast, the estimate of the ED_{50} against E. granulosus was 101 (61-1688) mg/kg (see Table 7).[49,109] The number of treatments at 40 mg/kg required to reach the ED_{90} against E. granulosus has been estimated to be 2.6 (2.0-3.2). Vomiting and/or diarrhoea were relatively common sequelae at 20 mg/kg (Tables 7 and 8). This drug has not been evaluated for echinococcosis control and, therefore, cannot be recommended.

Nitroscanate (Lopatol, Canwerm, Echinon)

This drug has been carefully evaluated for efficacy against Taenia spp. and E. granulosus.[16,20-23,50,56,61,71,100,101,110] The drug administered as a powder (particle size 2-3 μm) was strongly active against T. hydatigena with an estimated single dose ED_{90} of 8 (5-12) mg/kg. Efficacy was lost when the drug was given as a powder (particle size 10-20 μm) and in tablet form (nominal particle size 2-3 μm). For these formulations, the estimates of the ED_{90} were 38 (19-74) mg/kg and 36 (23-60) mg/kg, respectively. Another estimate of the ED_{90} for the tablet was similar at 31 (21-46) mg/kg.[20] Particle size appears to be important and agglomeration of particles during the manufacture of the tablets has been suggested as a possible cause of loss of efficacy. A similar variation in efficacy was observed between the various formulations in the treatment of canine echinococcosis.[50,56,61] The number of treatments required to reach the ED_{90} with the micronized powder at 32 mg/kg was 3.7 (2.4-5.0), whereas with the nonmicronized powder given at four times (125 mg/kg), or eight times (250 mg/kg) that dose rate, the estimates of the number of treatments required were 3.0 (2.0-4.0) mg/kg and 2.0 (1.5-2.6) mg/kg, respectively (see Tables 7 and 8). Based on the probability that the efficacy of the tablet is similar to that of nonmicronized powder, three daily doses each at 250 mg/kg can be recommended for the treatment of canine echinococcosis.

Mild diarrhoea and/or vomiting occurred at and above 62.5 mg/kg and reversible tranquillizing effects were reported in a few dogs.[50,56,61] However, a group of dogs was treated at 1000 mg/kg every two weeks for one year without any clinical signs of toxicity.[20,23] A dose rate of 50 mg/kg given with food was recommended for the treatment of such common tapeworms as Taenia spp. and D. caninum. At this dose level, the number of treatments required to reach strong activity against E. granulosus has not been determined. It has been pointed out that an additional value of regular treatment with nitroscanate includes the control of Ancylostoma caninum, Uncinaria stenocephala and Toxacara canis.[20,23]

Uredofos (Diuredosan, Sansalid, RH 565)

Several reports were published indicating that uredofos is active against a wide range of cestodes and nematodes. The ED_{90} for T. hydatigena infections was estimated to be 17 (7-40) mg/kg.[60] Initially, it was considered that three treatments at 25 mg/kg were sufficient to control canine echinococcosis.[113] Subsequent studies suggest that the ED_{50} for a single treatment does not fall within the dose range 12.5 mg/kg to 100 mg/kg, and that the number of treatments at 50 mg/kg required to reach the ED_{90} was 3.6 (2.6-4.7) (see Tables 7 and 8).[60] Diarrhoea and vomiting were relatively common sequelae at 25 and 50 mg/kg, respectively. Because the large number of treatments (3-5) required to achieve strong activity and the toxicity problems, this drug cannot be recommended for use in canine echinococcosis control.

11.4.4 Drugs with strong activity against Echinococcus spp.

Praziquantel (EMD 29810, Embay 8440, Droncit)

Many reports are available confirming a consistently strong lethal effect of the compound against taeniasis and dipylidiasis, as well as other tapeworm infections of dogs and cats.[46] Estimates of the single dose ED_{90} for T. hydatigena infections varied slightly from 0.5 (0.3-1.1) mg/kg for 28-day-old tapeworms to 1.8 (1.0-3.1) mg/kg for 2 to 28-day-old parasites.[14,57]

Special attention has been given to the evaluation of praziquantel against E.granulosus infections[2,3,10,16,17,26,30,57,63,66-68,73,74,117-119,124,129,137] and E. multilocularis infections.[104,106,108,120,123] The drug is equally effective against E. granulosus when administered by the intramuscular and by the oral routes,[2,17,63] but less effective when injected by the subcutaneous route (see Tables 7 and 8). Preliminary evidence indicates that the drug is effective when incorporated in biscuits.[46] Praziquantel is not ovicidal.[119] Estimates of the single dose ED_{90} for naturally acquired and experimentally induced 28-day-old infections were similar at 2.3 (1.5-3.7) mg/kg and 2.7 (2.1-3.5) mg/kg, respectively.[30,57] An estimate (from 106) for E. multilocularis at 4.6 (2.1-10.1) mg/kg suggests that this species may be slightly less susceptible to the drug than E. granulosus. This is the only compound that achieves the ED_{90} at safe dose levels with a single treatment against canine echinococcosis.

Extensive studies have now been made on the pharmacokinetic and other properties of the compound in vivo and in vitro.[5,31,32,111,115,116] Investigations on acute, subacute and subchronic toxicity, including teratogenicity, embryotoxicity, perinatal and postnatal toxicity and mutogenicity have shown that the drug has no known side effects.[6,86] The high efficacy and lack of toxicity indicate that at present this is the drug of choice for the treatment of echinococcosis in carnivores.

11.4.5 Mass treatment of dogs

Several dog-dosing trials and pilot control programmes have been reported in the literature.[42-46,52,127] These should be consulted before the decision is made to implement mass drug treatment programmes, as they describe some of the limitations and problems encountered during their course. Generally, the interval adopted between treatments for E. granulosus control has been six weeks.

With the introduction of new anthelmintics with improved efficacy against E. granulosus, there has been a shift in emphasis from "arecoline surveillance" to the mass treatment of dogs. Where this is the elected approach, it should be recognized that continuing surveillance of echinococcosis in livestock becomes even more essential to the success of the programme. This is because "arecoline surveillance" no longer applies as a control marker.

Mass treatment programmes are justifiable to achieve a quick break in transmission in highly endemic areas.[46] It is important to stress that it may not be the number of dogs that are successfully treated, but rather the number that are unsuccessfully treated (for example, vomiting tablets), or not treated at all (for example, unregistered wandering and vagabond dogs), that determines progress in control. It follows that a fully effective dog registration scheme should precede the introduction of the mass treatment programme.

There may also be a need to legislate to restrict the number of dogs per household. Long-term funding should be obtained before the programme is introduced. This is because breaks in the dosing programme may quickly nullify early successes. As the control of the parasite improves, demands may be made to restrict the treatments to the animals most at risk. There is also the possibility that a degree of noncooperation may develop if the mass treatment programme is unduly prolonged. This is most likely to occur if the data from the surveillance programme do not show sufficiently encouraging signs of success in control. This highlights the importance that should be attached in the planning stages to ensure that the surveillance programme is given priority. There is also evidence that dog owners tend to see the dog-dosing programme as the only important measure required in echinococcosis control. The introduction of highly effective drugs, such as praziquantel, does not reduce the need for other control measures.[46]

11.4.6 Contamination of environment with eggs following drug treatment

Praziquantel is strongly echinococcicidal, but not ovicidal. Thus, for several days after treatment, eggs will continue to be shed and contaminate the environment. Some of these situations are described below:

(i) where an infected dog is treated with praziquantel and returned to its home;

(ii) where an infected dog is treated with praziquantel at the time of its introduction to a new home;

(iii) where a mass drug treatment programme with praziquantel has just been introduced and large numbers of worms are expelled.

These are potentially hazardous occasions, because more eggs than usual may be suddenly introduced into the environment. It is necessary to take extra care in the handling of these dogs for a few days after treatment. However, the concept that mass dog treatment programmes may be contraindicated because of the heavy contamination of the environment with eggs is not justifiable. This is because the extra eggs expelled would, in any case, be deposited within a few days if no treatment had been administered; furthermore, at the time of expulsion following drug treatment, many of the eggs would be too immature to be infective and only a few of them would mature in the environment.

One way to avoid a situation that might cause a temporary increase in eggs is to use praziquantel and arecoline hydrobromide simultaneously. The faeces from the purgation can then be picked up and disposed of in the normal way (see section 6.2.7). In zones where dogs have never been tied up, and cannot readily be restrained for treatment, they should be followed and the purged material should be collected or be burnt on the spot with a flame thrower. Treatment of dogs in closed units (for example, cages) which can be effectively sterilized may be useful under certain conditions.

11.5 Limiting of risk from *E. multilocularis* and *E. vogeli*

Although these two parasites are known to cause severe disease or even death in man, no attempts have been made to establish control programmes. This is mainly due to the lack of detailed epidemiological information, and of effective control methods applicable to the sylvatic cycles of these parasites.

As domestic dogs and sometimes cats may acquire *E. multilocularis* infections from rodents in endemic areas, they represent a possible source of infection for man. It is recommended that all such animals having access to intermediate hosts (rodents) should be treated monthly with praziquantel. If persons living in areas where the infection is enzootic are educated about the dangers, they may be motivated to take effective measures to prevent their pets from eating rodents and to practise better personal hygiene. Persons handling foxes and other definitive hosts should carry out the safety precautions described in chapter 13.

TABLE 7. THE COMPARATIVE EFFICACY OF A SINGLE TREATMENT OF SELECTED COMPOUNDS AGAINST ECHINOCOCCUS GRANULOSUS INFECTIONS IN DOGS*

Compound	ED_{50} (mg/kg)	ED_{90} (mg/kg)	Reference
Praziquantel[1]	0.2 (0.1-0.7)	2.3 (1.5-3.7)	57
Praziquantel[1,6]	(a)	2.7 (2.1-3.5)	30
Praziquantel[3]	(a) Similar to oral	(a) Similar to oral	63
Praziquantel[4]	-	7.6 (3.1-18.6)	63
Mebendazole[2a]	3.0 (0.4-20.4)	55.9 (14.3-218.0)	53
Nitroscanate[2a]	(a)	(a)	56
Bithionol sulfoxide[2]	31.9 (16.2-63.0)	151.5 (70.7-344.5)	64
Nitroscanate[1]	89.0 (56.0-142.0)	365.0 (132.0-1010.0)	61
Nitroscanate[2]	115.3 (66.3-200.5)	519.8 (246.3-1364.9)	50
Oxfendazole[2]	0.9 (0.0-164.7)	(b)	62
Bunamidine hydroxynaphthoate[2,5]	27.4 (9.0-83.1)	(b)	47
Bunamidine hydrochloride[2]	(a)	(a)	51
Streptothricin[2]	83.5 (12.2-570.0)	(b)	59
Fospirate[2]	101.1 (60.9-1687.8)	(b)	49
Mebendazole[1,5]	(b)	(b)	58
Mebendazole[2a,5]	(b)	(b)	58
Uredofos[2]	(b)	(b)	60
Niclosamide[2]	(b)	(b)	55
Dichlorophen[1]	(b)	(b)	40

[1] Oral tablet

[2] Oral powder

[2a] Oral powder micronized

[3] Intramuscular

[4] Subcutaneous

[5] With food

[6] Natural infection - all others treated 28 days post infection

* Based on the proportion of dogs cleared of worms (see section 11.4.1)

(a) Probit model does not fit data

(b) Not obtainable (outside dose range tested)

Figures inside brackets are 95% confidence limits

For E. multilocularis, the ED_{50} was estimated to be 1.63 (0.97-2.74) mg/kg and ED_{90} 4.6 (2.1-10.1) mg/kg[106]

(Data from Ref. 46)

TABLE 8. GUIDE TO TREATMENT SCHEDULES AND DOSE RATES REQUIRED TO ACHIEVE STRONG LETHAL EFFECTS* AGAINST ECHINOCOCCUS GRANULOSUS INFECTIONS IN DOGS

Drugs	Number of treatments	Dose rate (mg/kg)	Side effects
Praziquantel[a]	1	5	None
Praziquantel[b]	1	5	None
Praziquantel[c]	1	20	None
Bunamidine hydroxynaphthoate[a]	3	64	[d]
Bunamidine hydrochloride[a]	3	50	[d], [e]
Nitroscanate (nonmicronized powder)[a]	3	250	[d], [f]
Fospirate[a]	4	40	[d]
Uredofos[a]	5	50	[d]
Bithionol sulfoxide[a]	?	?	[d], [e]
Streptothricin[a]	?	?	4
Oxfendazole[a]	?	?	None
Mebendazole[a]	?	?	None
Niclosamide[a]	N.A.	N.A.	-
Dichlorophen[a]	N.A.	N.A.	-

[a] Oral

[b] Intramuscular

[c] Subcutaneous

[d] Vomiting and/or diarrhoea of a mild and transient type

[e] Occasional deaths

[f] Occasional tranquillizing effects

* Based on the upper limit of the 95% confidence interval of the ED_{90} (except for bunamidine hydrochloride) for which no data are available

? - Active, but treatment schedules and dose rates not determined

N.A. - Not active at reasonable dose rates against immature infections

(Data from Ref.

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CHAPTER 12

EVALUATION OF ANTHELMINTICS AGAINST LARVAL *ECHINOCOCCUS* INFECTIONS

12.1 Introduction

Although anthelmintics against larval *Echinococcus* spp. are not yet available for use in control programmes, some progress has been made on their development. In view of their potential future importance and the paucity of published information on testing procedures, a brief review is given below. Several compounds of the benzimidazole group have certain anthelmintic effects against metacestodes of various cestode species, including those of *Echinococcus*.[3,11,12,16-21,24,25,27,28,32,44] These encouraging results have stimulated the search for drugs active against larval cestodes and the need for suitable testing models. Some of the drugs have already been used in the treatment of a limited number of cases of cystic and alveolar hydatidosis in man (see section 12.4). It has to be stressed here that this field of chemotherapy is still in an early stage of development. Therefore, research activities should be encouraged.

12.2 Evaluation in laboratory animals

12.2.1 Present status

According to recent studies[3,16,17,18] metacestodes of *E. granulosus* in rodents (*Meriones unguiculatus*) can be severely damaged or killed by treatment with mebendazole or fenbendazole for 80 days in the food at dose rates of 500 ppm (corresponding to about 30-50 mg/kg body weight per day). Long-term treatment with 500 ppm mebendazole or fenbendazole for 167-300 days resulted in prolonged survival of *Meriones* infected with metacestodes of *E. multilocularis*. The parasites were significantly inhibited in proliferation, and formation of metastases was completely suppressed during therapy. However, in most instances complete destruction of the metacestodes was not achieved. After termination of treatment they resumed growth as demonstrated by transplantation experiments.

12.2.2 Drug testing

The potential of metacestodes of *Echinococcus* spp. to proliferate asexually has to be considered in chemotherapeutic studies. Therefore, special methods for viability testing of the parasites after chemotherapy are essential. Besides the metacestodes of *Echinococcus* spp., some other larval cestodes have the capability of asexual reproduction and may be used as models in drug screening programmes. Although no definite recommendations can be given for models and procedures for screening, some examples are described below.

12.2.2.1 Screening with *Mesocestoides corti* and/or *Taenia crassiceps*

It has been shown[3,12,17,24] that metacestodes (tetrathyridia) of *Mesocestoides corti* and metacestodes of *Taenia crassiceps* inhabiting mice are highly sensitive to drugs that also show activity against the metacestodes of *E. granulosus* and *E. multilocularis* in laboratory rodents. These metacestodes can be maintained easily and at low cost and without danger for man. Consequently they provide useful primary screening models.

Maintenance of tetrathyridia of *M. corti*

The maintenance of metacestodes of *T. crassiceps* is similar to those of *M. corti*, which is described below.

Tetrathyridia are maintained by intraperitoneal serial passages in white mice. Parasites obtained from an infected animal are washed three times in sterile physiological

saline or Tyrode's solution (with 500 I.U. penicillin and 500 µg streptomycin per ml). Normally 0.1 ml of the packed organisms are injected into the peritoneal cavity of a mouse with a body weight of 25-30 g. Large quantities of tetrathyridia (several ml) can be harvested about three to five months post-infection (p.i.).

Screening procedure

Adequate groups of mice (at least five per group) are infected by the intraperitoneal route with 0.1 ml tetrathyridia suspension. Ten days p.i. treatment is initiated for the specified period. Sixty days p.i. the treated and the untreated (control) animals are killed and examined. Mebendazole can be used as a reference drug.

Evaluation of efficacy

The determination of drug efficacy is based on the following criteria:

(i) volume of tetrathyridia: all tetrathyridia are carefully washed out of the peritoneal cavity with physiological saline into graduated conical centrifuge tubes and subsequently washed free of adhering material. After sedimentation the volume of the sediment is recorded. Comparisons are then made between treated and untreated animals;

(ii) motility of tetrathyridia: after determination of the volume, the tetrathyridia can be transferred into culture flasks with sterile Tyrode's solution (maximum of 0.1 ml packed tetrathyridia per 10 ml Tyrode).

After incubation at +37°C overnight the motility of - if possible - 100 organisms per flask is determined using a dissecting microscope. At the same time dividing forms and morphological changes can be observed. In animals treated with highly active compounds no motile tetrathyridia should be present;

(iii) histological examinations: after intraperitoneal infection, a proportion of the tetrathyridia penetrate the liver. Representative livers are fixed in 4% formalin, and sectioned and stained with haematoxylin-eosin or PAS. The organisms are classified as follows:

- intact organisms: with normal, intact tegument and well-stained nuclei of tegumental and parenchymal cells;

- damaged organisms: outlines of the organisms indistinct, tegument interrupted, structure of parenchyma unclear and sometimes vacuolated.

- dead organisms: only remnants of tetrathyridia visible, especially calcareous corpuscles. Only foci with remnants of dead tetrathyridia should be counted, cell infiltrated migration tracts in the liver should be excluded;

(iv) remarks: in many cases, methods (i) and (ii) may be sufficient for a pre-screening evaluation.

12.2.2.2 Screening with metacestodes of Echinococcus spp.

Since eggs of E. granulosus and E. multilocularis are infective for man routine screening against metacestodes of Echinococcus species should preferably be carried out with parasite stages, which can be handled without risk for laboratory workers. This can be achieved by the serial passage of larval E. multilocularis and by the induction of secondary E. granulosus infections in laboratory rodents.

E. multilocularis[3,12,17,18,25,32]

Parasite strain and maintenance

In various geographical regions metacestodes of E. multilocularis are maintained in laboratory rodents. Preferably strains should be used that produce an appreciable number of protoscolices in laboratory rodents. Suitable laboratory hosts for larval E. multilocularis in drug testing experiments are for example the jird (Meriones unguiculatus), the white mouse (Mus musculus) and the cotton rat (Sigmodon hispidus). In Meriones the metacestodes exhibit a rapid growth, the host reaction is comparatively weak, and most of the animals with medium or high initial infections will die within four to five months. Various strains of mice may differ markedly in their susceptibility for metacestodes of E. multilocularis. Therefore, the selection of the mouse strain for the maintenance of this parasite is important. For maintenance of the strain of parasite the animals are infected as follows: metacestode tissue taken from the peritoneal cavity of an infected donor animal is washed several times in sterile Tyrode's solution containing antibiotics. The tissue is ground up in Tyrode's solution (1 g in about 10 ml) by means of an electric mixer, to a crude homogenate. This is then strained through one layer of gauze into a cylinder. After two hours the sediment is injected by intraperitoneal route into mice or Meriones in 0.3 ml or 0.5-1.0 ml quantities respectively. This technique may result in wide variations in parasite growth. More uniform results may be obtained with a transplantation technique. Meriones or mice are infected intraperitoneally with 0.1 g metacestode tissue transplants by surgical means.

Screening procedure

Seven or 40 days after infection therapy can be initiated. Autopsy can be carried out usually 10 days after the termination of treatment.

Criteria used in determining efficacy

The determination of the anthelmintic effect may be based on the following criteria: (i) determination of metacestode weight and comparison between treated and untreated animals; (ii) histological examinations of parasite tissue (number and structure of protoscolices, morphology of cyst wall and especially of the germinative layer and other criteria); (iii) transplantation of metacestode tissue (0.1 g) from treated and untreated animals into the peritoneal cavity of helminth-free animals, which then are examined 10-12 weeks later. This is a reliable method for the determination of viability; (iv) survival rates of treated animals in comparison to untreated controls.

Echinococcus granulosus[14,15,18,19,24,27,28,31]

Parasite strains and maintenance

Since several strains of E. granulosus exist[19,34] (see section 1.5.2) the selection of a susceptible strain of rodent is important. For example, the European "horse strain" which also occurs in cattle is infective for Meriones and mice[10,14,15,45,46] and protoscolices from "sheep strains" have been successfully used for the infection of mice.[28,31] The serial passage of E. granulosus strains in laboratory rodents is possible,[10,34] but time consuming. Therefore, in endemic areas it is convenient to use protoscolices isolated from slaughtered domestic animals for the induction of secondary hydatidosis in laboratory rodents.

Screening procedure

Mice or Meriones are infected intraperitoneally with a single dose of 2000-10 000 protoscolices that have been washed and suspended in physiological saline or Tyrode's solution containing antibiotics. Cyst development is slow and the formation of larger cysts requires at least five to eight months.

Criteria used in determining efficacy

The determination of the anthelmintic effect is based on the following criteria (see also section 12.3):

(a) cysts from untreated animals are filled with clear hydatid fluid, whereas cysts from effectively treated animals are collapsed and shrunken;

(b) the weight of the cyst masses can be used as a further parameter. However, the weight of the treated cysts may be relatively high as the laminated membranes are neither destroyed nor eliminated;

(c) histologically, in successfully treated cysts the germinal layer should have been destroyed, whereas in untreated cysts this layer should show nuclei that stain clearly. Additionally, brood capsules and protoscolices may be present in untreated cysts;

(d) in viability tests, protoscolices from untreated and from treated animals are incubated in vitro overnight (see sections 12.2.2.1 and 12.3.2) and subsequently the mobility of the organisms is recorded. The infectivity can be determined by the oral transmission of protoscolices to dogs and/or by the intraperitoneal injection into suitable laboratory rodents. The intraperitoneal transmission to naive rodents of cysts and parts of the cyst wall can be used to determine regeneration potential.[6] Examination of the animals should be delayed until at least six months after injection of the material.

12.3 Evaluation in farm animals[20,21,23,38,47]

12.3.1 Experimental design

Observations on dose rates and treatment schedules for drugs against E. granulosus evaluated in laboratory animals (see section 12.2) provide the information required for designing trials using large animal models. Ideally, the trials should be designed to determine the 50% and 90% effective dose rates with 95% confidence limits for dose rates and/or treatment schedules of the candidate compound (see section 11.4). The experiments can best be carried out by using sheep or pigs from premises known to be heavily infected with E. granulosus. Each animal is weighed and randomly allocated to a specified dose rate or treatment schedule. The animals are autopsied about three to six months after the end of treatment. Those animals with no metacestode infections (viable or dead) should be regarded as uninfected and excluded from the evaluation.

12.3.2 Criteria of viability

(i) Viability of hydatid bladder: cysts should be measured and the volume of fluid should be determined. Those that contain some fluid should be considered possibly viable. Collapsed cysts can be considered to be dead if they lack fluid and have extensive caseation and/or calcification.

Samples of cysts can be preserved in modified Karnovsky's fixative[29] (1% formaldehyde, 1.35% gluteraldehyde) in 0.1 M cacodylate buffer (pH 7.2) at room temperature. One sample from each selected cyst can be dehydrated in ethanol and embedded in paraffin wax. This can be sectioned at 2 µm - 7 µm and stained either with haematoxylin and eosin, PAS and/or toluidine blue. A second sample from each cyst can be post-fixed in 1% osmium tetroxide, 1% sucrose in 0.1 M sodium cacodylate buffer (pH 7.1), stained en bloc in 2% aqueous uranyl acetate (pH 3.8), dehydrated in acetone and embedded in either Spurr's or Epon 812 epoxy resins. Sections (1-2 µm) can be stained with methylene blue-Azure II - and basic fuchsin[26] and examined with a light microscope. Ultrathin sections can be post-stained with 1% uranyl acetate in 50% ethanol and with lead citrate.[48]

Where the germinal layers have intact borders and contain well-stained nuclei, the bladders can be regarded as viable. Bladders with fragmented germinal layers containing extensively vacuolated or granular cytoplasm and with pycnotic nuclei, can be regarded as dead. Supportive evidence of viability or death of cyst material can be made by transplant to susceptible laboratory animals (see section 12.2.2.2).

At the ultrastructural level, cysts can be regarded as viable if: (a) the germinal layer includes a syncytium with a vacuolated distal cytoplasm bounded by an intact and continuous limiting membrane; (b) the membrane has discrete microtriches on the distal surface; (c) the distal cytoplasm contains mitochondria, glycogen and membrane - bound vesicles; (d) the tegumental cytons contain mitochondria, golgi apparatus and nuclear membranes. Studies on the normal ultrastructure should be consulted.[6-8,33,35]

(ii) Viability of hydatid protoscolices: protoscolices can be considered to be possibly viable if they are ovoid, invaginated and have intact rostellar hooks and calcareous corpuscles. They should be examined for peristaltic-like movements in hydatid fluid and also in Krebs Ringer Bicarbonate (KRB) at 37.5°C. Their ability to evaginate should be tested in an artificial fluid consisting of 1% pancreatin, 1% trypsin and 5% dog bile in KRB.

Morphologically "normal" protoscolices that are non-motile and do not evaginate in vitro should be subjected to a modified dye exclusion test.[13] A drop of protoscolices in hydatid fluid can be mixed with an equal volume of 0.03% methylene blue in KRB on a microscope slide. Viable protoscolices should completely or partially exclude the dye. Flame cell activity can be observed in living protoscolices with an objective X 90-100.

(iii) Infectivity of hydatid protoscolices: protoscolices recovered from all cysts in each animal should be pooled in hydatid fluid. After sedimentation, the fluid should be replaced with 5-10 ml of sterile KRB containing an antibiotic. The volume of the suspension should be adjusted so that an aliquot of 0.5 ml contains about 500 organisms and mice should be given an aliquot by intraperitoneal injection. At autopsy, which can be carried out three months later, secondary cysts should be counted, measured and examined for viability. Protoscolices should also be fed to dogs. These should be necropsied about 28 days later and tapeworms should be counted. An alternative method includes in vitro culture of protoscolices and daughter cysts (see sections 12.5.2).

(iv) Assessment of efficacy: In any naturally infected flock, some animals will be strongly resistant to metacestode infections and others will overcome the parasites or limit their natural growth. In only a proportion of the flock will metacestodes grow well and survive indefinitely. Thus, because death of metacestode could be due to other causes, it is not possible in this type of population study, to demonstrate lethal effects due to a drug on any individual organism in any individual animal. It is only possible to demonstrate that the proportion of parasites expected to have normal structure and function decreases as the dose rate is increased or the duration of treatment is extended. It is possible, however, to identify individual organisms that survive at each dose rate and treatment schedule.

There have only been a few reports in farm animals on short- or long-term treatment schedules of benzimidazoles including mebendazole in pigs and lambs with experimentally induced infections as well as in aged sheep with naturally acquired infections.[20,21,23,38,47] The evidence suggests that mebendazole at 50 mg/kg daily for three months may kill naturally acquired thick-walled hydatid cysts in aged sheep. Such studies should be encouraged as they may have relevance to treatment schedules for E. granulosus in man.

12.4 Evaluation in man

Preliminary results of chemotherapy in more than 50 cases of E. granulosus or E. multilocularis infections in man have been reported.[1,2,4,5,9,22,30,36,37,49,50] Regression or inhibition of metacestode growth has been described in some cases treated with high doses of mebendazole, although recurrence has been apparent in some. One of the difficulties encountered in these studies has been the evaluation of the effects of treatment because knowledge of the natural history of untreated cases of cystic and alveolar hydatid disease is incomplete.

It is essential that future clinical trials should be coordinated and carried out under strict supervision. They should follow standardized protocols; patients' progress should be monitored carefully. Progress in evaluation of chemotherapy of hydatid disease would be best achieved by coordination through an international agency.

12.5 Further tests for viability

The procedures described below refer to complete E. granulosus cysts or their contents, but may also be applied to suspected material from surgery. The protocol is summarized in Fig. 25.

12.5.1 Procedures

(i) Surgically remove the cyst(s).

(ii) If the cyst is intact, withdraw some hydatid fluid (to reduce pressure) using a hypodermic syringe, transferring the fluid to a 20-ml vial. Brood capsules and/or free protoscolices should be immediately apparent if the cyst is highly fertile. They will sink to the bottom of the vial, from where a sample can be transferred to a microscope slide and examined.

(iii) Open the cyst using sterile instruments. (At this stage some material may be fixed for later histological examination, especially portions of cyst wall and germinal membrane.) If brood capsules are present, they will be found attached to the germinal membrane, or may occur free, as hydatid "sand" at the bottom of the cyst. If they are immediately visible, remove the brood capsules to a vial, with a sterile pipette. Sometimes they are contained within daughter cysts.

(iv) Transfer the remaining contents of the cyst to a sterile beaker, or similar container, with the addition of some warmed (37°C) saline (Hanks or BSS). It should be possible to identify germinal membrane, daughter cysts, brood capsules and protoscolices at this stage.

(v) If brood capsules and/or free protoscolices are apparent, either in the hydatid fluid removed initially, or within the contents of the cyst, examine a sample microscopically to determine viability:

(a) flame cell activity: this will be seen in living protoscolices, at least with X90-100 objective, if not with X40. It produces a faint fluttering movement, readily recognizable once seen;

(b) vital staining: dead protoscolices stain readily with eosin. A few drops of 0.1% aqueous eosin added to specimens on a slide is, therefore, a reliable test for viability. Living protoscolices will not take up the stain.

(vi) If protoscolices are not evident, the cyst may be sterile, or dead, or the hydatid organisms may be concealed within the cyst tissue, especially in daughter cysts. (The latter is often the case with human hydatids.) Therefore, chop the contents of the cyst with scissors in saline, and pass the whole through a tea-strainer type sieve. If the filtrate contains brood capsules and/or protoscolices their viability may be tested as in (v). However, it may be necessary to treat the residual tissue with digestive enzymes before determining whether any living hydatid organisms are present:

(a) pepsin: treatment with pepsin digests most of the dead material, or at least "clears" it, with the result that living protoscolices become apparent. It is suitable for small samples of 10-20 ml. Allow the material to settle in a vial,

and remove as much supernatant fluid as possible. Add 20 ml of 0.2% pepsin (Sigma 1:10 000; activity 1200-2000 units/mg protein), pH 2.0, in Hanks (or BSS) and incubate at 37°C for 15 minutes (or longer in some cases). A rotator or shaking water bath aids digestion, but is not essential. The brood capsules should be digested, along with dead material, thus releasing viable protoscolices;

(b) trypsin: this enzyme is more suitable for larger samples as the pH is not so critical (pH 7.4-7.8), but the treatment may take longer, up to two to three hours in some cases. 0.5% trypsin (Sigma Type II; activity 1000-1500 BAEE units/mg protein) in Hanks or BSS will not digest as much material as pepsin, and thus it may be necessary to pour the digest through a strainer before examining the liquid for protoscolices.

The viability of the latter may be tested as before (see v), but treatment with these enzymes usually causes any living protoscolices to become active. However, the addition of a few drops of 5% dog's bile or 0.2% sodium taurocholate can stimulate body contractions (often resulting in scolex evagination), thus confirming their viability.

(vii) In vitro culture of hydatid organisms is not a recommended test for their viability, as the procedures are somewhat complex, but it may provide additional valuable information on the growth rate and developmental pattern of human hydatids, and the occurrence of different strains from different secondary hosts.[39]

(viii) As living germinal membrane is capable of regeneration, causing secondary hydatidosis, its viability must be tested, even if no living protoscolices have been detected in the cyst material. Inject 0.2-0.5 ml chopped hydatid material containing germinal membrane (in Hanks or BSS) i.p. into laboratory hosts, such as Meriones or mice, then examine for cysts at a later date. Small, positively developing cysts in Meriones have been obtained six months after infection with human material. It is, therefore, a slow procedure, but should be regarded as an important confirmatory diagnostic technique, especially if no living protoscolices have been detected in the suspected material.

12.5.2 In vitro culture

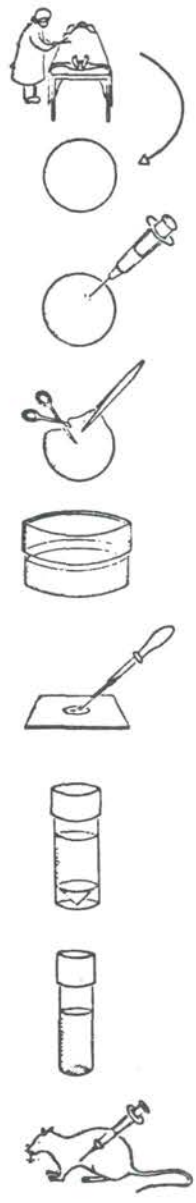
12.5.2.1 Protoscolex to adult worm

In vitro culture of human E. granulosus is only likely to be successful if (i) the material has been removed and stored under sterile conditions and (ii) at least 0.3 ml of settled brood capsules/protoscolices is available.

The culture procedures, modified from references 39 and 42 are detailed below, and summarized in Fig. 26. Aseptic techniques should be used throughout - all equipment and media must be sterile.

(i) Collect brood capsules and/or protoscolices aseptically and store in a vial, preferably in hydatid fluid, but saline would suffice (Hanks or BSS). Transfer a sample to a microscope slide and check the percentage viability of the protoscolices. Living organisms should be readily discernible from dead ones, the latter being somewhat shrivelled and yellow-brown in colour; the addition of a few drops of eosin may provide a more reliable result (see section 12.5.1 for details). The higher the viability, the more successful in vitro culture is likely to be. Check also for the presence of bacteria. If the material is infected initially, it is unlikely that the cultures will be sterile. Material from more than one cyst may be pooled, if this is necessary to obtain enough protoscolices for culture.

FIG. 25. OUTLINE OF PROCEDURES FOR THE DETECTION OF LIVING HYDATID ORGANISMS (FROM REFERENCE[40])



1. Surgically remove cyst.

2. Extract hydatid fluid to reduce pressure.
   – initialy check for presence of protoscoleces

3. Dissect cyst.
   – fix some material especially cyst wall

4. Remove contents to warmed saline.
   – check for presence of germinal membrane, brood capsules, protoscoleces and daughter cysts.

5. If protoscoleces immediately evident, test viability
   – check (i) flame activity. (ii) uptake of vital stains.

6. If protoscoleces not evident (or a apparently "dead"), use enzyme digestion technique.
   –treat with 0.2% pepsin, pH 2.0 (live protoscoleces should survive). treat with 0.5% trypsin (live protoscoleces should survive, as well as brood capsules and laminated membrane).

7. If aseptic protocol used, test development in vitro.
   – culture live protoscoleces, brood capsules and daughter cysts.

8. Infect laboratory animals with suspected material.
   – inject intraperitoneally, and examine for cysts 6 monts later.

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(ii) Allow the brood capsules/protoscolices to settle, and remove the supernatant liquid. Add 20 ml of 0.2% pepsin, pH 2.0, in order to digest the brood capsules and dead protoscolices. If the tissue is heavily calcified, it may be necessary to add some HCl (5 N HCl) to maintain the pH at 2.0 after the first few minutes, and possibly again later. The process takes approximately 40 minutes (preferably on a rotator), in an incubator (37°C).

(iii) When the protoscolices are free, allow them to settle, and replace the pepsin with a similar amount of Hanks (or BSS). Return the vial to the rotator (or a shaking water bath) for 15 minutes. This should be repeated three times in order to wash the pepsin from the protoscolices, and to remove any debris.

(iv) After the final wash, remove the saline and transfer the protoscolices to a Milk Dilution (M.D.) bottle containing evaginating medium: CMRL 1066 + 1% dog bile. (The quantity depends on the amount of protoscolices - usually 40-50 ml is sufficient.) Incubate overnight in a shaking water bath (37°C).

(v) The following day, transfer the protoscolices to the culture bottles, usually 0.05-0.1 ml to each. These M.D. bottles should contain a newborn calf serum base (20 ml coagulated for 90 minutes at 76°C), and 20-ml liquid culture medium - S1OE.H,

S1OE.H

130 ml CMRL 1066
50 ml foetal calf serum
18 ml 5% yeast extract in Hanks
2.8 ml 30% glucose in distilled water
0.7 ml 5% dog bile in Hanks

+ 20 mM Hepes, and 0.85 g $NaHCO_3$ per litre. Incubate in a water bath (discontinuous shaking) at 38°C. The liquid media should be renewed every three days and gassed with 10% CO_2, 5% O_2 in N_2 for 30 seconds at the same time. The development of the organisms may be studied under an inverted microscope, at intervals, as long as they are not allowed to cool.

Sexually mature adult worms will develop in 40-60 days, but fertile eggs have not yet been obtained.

12.5.2.2 Daughter cysts

As with protoscolices, in vitro culture of daughter hydatid cysts will only be successful if they have been removed and stored under sterile conditions.

Culture of daughter cysts is at present being attempted for the first time, and therefore the procedures detailed below will no doubt be improved upon with experience; aseptic techniques should be used throughout.

Procedures

(i) Remove daughter cysts from the contents of the hydatid cyst (carefully - as they burst very easily), and place in a suitable sterile container with some saline (Hanks or BSS), so that they may be viewed with a microscope. In young cysts, little will be discernible, whereas in older ones, brood capsules and/or protoscolices may be evident.

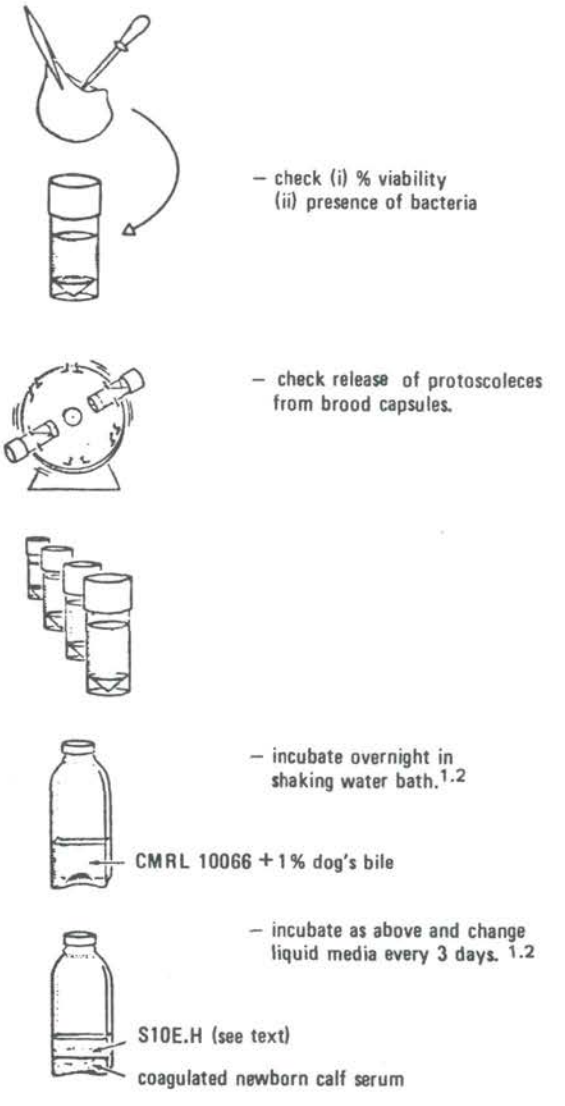
(ii) Wash the cysts several times (at least four times) in Hanks or BSS, by adding and removing the saline rapidly, then adding fresh saline, using a fresh sterile pipette each time.

(iii) Transfer the cysts to a culture vessel, such as a large Leighton tube, along with some liquid culture medium - S1OE.H. Details of the amount of medium to use, the type of vessel, or the number of cysts optimal for each culture etc., cannot be given as yet. However, in a large Leighton tube, containing 40 ml S1OE.H, in a discontinuous shaking water bath, three daughter cysts have been cultured for several weeks. The medium has been changed and gassed with 10% CO_2, 5% O_2 in N_2, every four days. Development will no doubt be slow, but brood capsules and protoscolices can clearly be seen in the two largest cysts, the protoscolices sometimes being active.

Comments

From in vitro culture of both protoscolices to adult worms (already successfully carried out), and daughter cysts (now in progress) basic information on the developmental characters of different strains is emerging. The sheep strain readily segments and becomes sexually mature in vitro, as did the only sample of human material yet cultured.[43] In contrast, protoscolices of horse and cattle origin do not segment in vitro; it is not known if either of these is infective to man.[41]

FIG. 26. OUTLINE OF PROCEDURES USED FOR IN VITRO CULTURE OF HUMAN HYDATID ORGANISMS: PROTOSCOLEX TO ADULT WORM (FROM REFERENCE[40])



([1]temperature: 37°C; [2]gas phase: 10% CO_2, 5% O_2 in N_2)

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CHAPTER 13

SAFETY PRECAUTIONS

13.1 Introduction

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.[5] This applies with special force to personnel involved in echinococcosis surveys and control programmes. They should be encouraged to regard all definitive hosts as potentially infected. They should always treat any faeces shown to contain taeniid eggs as if they were Echinococcus spp.

Human infection usually results from the ingestion of eggs of Echinococcus. However, there is also evidence that the hatching and activation of embryos can occur in extra-intestinal sites.[3,18] 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] Furthermore, secondary echinococcosis may possibly follow contamination of the conjunctiva with protoscolices.

Ingestion of eggs by man may result from:

(i) handling infected definitive hosts or egg-containing faeces followed by direct hand to mouth transfer. It has been shown that eggs of Echinococcus adhere to the coat of dogs,[13] particularly to the hairs around the anus, on the thighs, muzzles and on the paws.[10] The same may apply to foxes infected with E. multilocularis;

(ii) handling or ingestion of unwashed vegetables[5] which have become contaminated directly with Echinococcus eggs. Other food stuffs or surfaces may also be secondarily contaminated via agents such as wind, birds, beetles and flies (see section 1.9);

(iii) the ingestion, or possibly inhalation of eggs in dust[5] or drinking-water.

All field and laboratory personnel possibly exposed to Echinococcus infection should be subjected to a serological examination every six months (see chapter 3).

13.2 Resistance of eggs

The eggs are highly resistant, and may remain infective for about one year in a suitable environment. Desiccation and high temperatures are the two most important factors reducing the longevity of the eggs (see section 1.9).

13.3 Ovicides and disinfection

Although several studies have assessed the action of numerous chemicals on the eggs,[1,6-9,11,14-16,19] heat remains the only reliable and effective method for killing the eggs of Echinococcus. The eggs are killed by boiling water or dry heat and incineration. They are killed in 10 minutes at 60°C and instantaneously at 100°C. It has to be stressed, however, that the length of time for which contaminated materials should be boiled will vary. For example, heat penetrates dog faeces slowly and such material should be boiled for at least five minutes to ensure killing of the eggs.[4] Most sewage treatment processes do not totally eliminate taeniid eggs.[12,17]

13.4 Precautions for field workers

Ideally, personnel engaged in echinococcosis surveys should at all times wear appropriate protective clothing, i.e. impervious boots, gloves and coat or apron. In situations in which faeces 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 dug over or thoroughly decontaminated by burning. Faeces should either be rendered safe in the field by being boiled, or by being packed in secure leak-proof containers for transport. Where possible the standard precautions described for dog-dosing sites in section 10.2.4 should be carried out.

Animals autopsied in the field should be deeply buried or incinerated. 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 ligated 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.

13.5 Precautions in laboratories

Laboratories in which infected animals and/or faecal or intestinal material is regularly handled should be adjacent to a changing room. Protective clothing, including overalls, masks, caps, gloves and boots should be put on before entering the laboratory. Facilities should be available for the decontamination of protective clothing. They should never be sent to a laundry without first being sterilized.

Infective material may be examined over sinks, in which an immersion heater can be placed to enable material to be boiled before it is passed into the sewer system.[5] In situations where the intestines of definitive hosts have to be examined, this should be carried out on large metal trays. Following examination, the tray containing all the infective material should be sterilized, ideally by steam sterilization in an autoclave. If a flame torch is to be used for small-scale decontamination, inflammable chemicals and other materials must not be kept in the laboratory in which infective material is processed.

Personnel involved in the examination of larval material from intermediate hosts should wear safety glasses. This will eliminate the possibility of protoscolices being squirted into the eyes of the operator, with the risk of conjunctival hydatidosis. Remnants of metacestode material and infected intermediate hosts should be heat sterilized, incinerated or decontaminated by treatment with 4% formalin.

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