Sadun et al.[k] Yamashita and colleagues,[h, f, l] Webster & Cameron, [m] etc. Yamashita et al.[h] have demonstrated the specific and strain differences in susceptibility to primary multilocular hydatid infection. Albino hamsters showed a complete resistance to multilocular hydatid. Other animals, including 5 species of voles, the Asiatic chipmunk and 10 strains of mice, however, were susceptible to the parasite, although infection rates varied according to the species or strain. Three species of voles belonging to the genera *Clethrionomys* and *Microtus*, one species of gerbil (*Meriones* sp.) and 3 strains of mice (AKR, dba and CF1) did not show resistance to the infection, and the lesions—except in CF1—were of type 1. Seven strains of mice (dd, C57BL/6, b, CFW, A, BALB/C and C3H/He) were relatively resistant to the infection, and the rates of infection were 8 %–79 %, and their lesions were of type 2. Yamashita et al.[f] described the results of the primary infection of multilocular hydatid in 5 strains of mice (fm, NC, SM, gcp and KK). The strains fm and KK did not show any resistance to infection. However, NC showed a relatively high resistance and SM and gpc were intermediate in this respect. Strains fm, NC SM and gpc manifested the type 2 lesions.

Webster & Cameron [m] examined the susceptibility of strains DBA/1J and C57BL/6J to *E. multilocularis*, with results obtained differing from those of Yamashita et al.[h] However, there is full agreement that Webster & Cameron [m] attach importance to the genetic constitution of experimental animals.

Ohbayashi [n] discussed the types of lesions of multilocular echinococcosis from the viewpoint of histogenesis and pathogenesis, and suggested that the difference in susceptibility or resistance depends upon the histological difference of the host animals. Smyth,[d] as mentioned above, suggested that the digestive physiology of an animal probably determines its suitability as a host for *Echinococcus* and that some responsible physiological factors might be subject to dietary influences. From a report of Sweatman & Williams,[a] it has been known that the infection of intermediate hosts is partially related to the exposure level of infective ova. It is still unknown whether or not humoral factors in different animals are responsible for host-specificity in hydatid infection.

[k] Sadun, E. H., Norman, L., Allain, D. S. & King, N. M. (1957) *J. infect. Dis.*, **100**, 273-277.

[l] Yamashita, J. (1960) *Parassitologia*, **2**, 399-405.

[m] Webster, G. A. & Cameron, T. W. M. (1961) *Canad. J. Zool.*, **39**, 877-891.

[n] Ohbayashi, M. (1960) *Jap. J. vet. Res.*, **8**, 134-160.

Safe Handling of Infected Definitive Hosts and Eggs of *Echinococcus* spp.

by M. A. GEMMELL, *Director, Hydatid Research Unit, New Zealand Medical Research Council, University of Otago Medical School, Dunedin, New Zealand*

In drafting safety precautions for the handling of infective material, the public health official must define parameters, taking into account virulence, relative infectivity and transmissibility, in relation to specific situations in which precautions can be and should be taken.

Echinococcus granulosus and *E. multilocularis*, the etiological agents of hydatid disease in man, might be described subjectively as parasites of high pathogenicity but moderate or low infectivity under normal conditions.

No precautions are likely to give complete protection, but it is axiomatic that strict measures are needed wherever carrier hosts and cultures of organisms, which may cause disease or severe incapacity, are handled knowingly.

Some factors influencing concentration and dispersal

Availability. Sweatman & Williams [a] sprayed eggs of *E. granulosus* and *T. hydatigena* on adjacent pastures in two areas of New Zealand with extreme climatic conditions. They found that in an oceanic climate with an annual rainfall of 120 in (305 cm) eggs remained available throughout the 4-month winter period, but were carried deep into the soil profile within the year, the high rainfall being an important factor in limiting the availability of the eggs. In an area with a quasi-continental climate, eggs were still available during the 4-month winter period with a $\frac{3}{4}$-in (7.6 cm) rainfall. After the cold,

[a] Sweatman, G. K. & Williams, R. J. (1963) *Res. Vet. Sci*, **4**, 299-316.

dry winter, followed by a hot, dry summer, eggs were no longer available to animals at the site of deposition.

Methods of spread. Eggs of *Echinococcus* spp. adhere to the coat of dogs and can be found concentrated, particularly, on the hair near the mouth and anus.[b] Eggs of tapeworms may be found in dust and on vegetation, with a particularly high concentration around kennels, but in water they sink rapidly. There are several agencies, apart from those associated with the activities of dogs, by which eggs may be spread mechanically. These include wind,[a] flies,[c] beetles [d] and, as suggested by Parnell,[e] boots and clothing.

Survival of eggs. Eggs of *E. granulosus* may remain infective for several weeks.[f] Although sunlight and heat have been reported to be lethal within 3 weeks,[g] eggs may survive for several months in hay.[h] Sweatman & Williams [a] observed that eggs were still infective after they had been kept in tap-water at 6°C for 7½ months.

Eggs of *E. multilocularis* were still infective after being kept in pond-water at room-temperature (unstated) for 22 days [i] or at −26°C in the frozen semifluid intestinal contents of foxes for 60 days.[j]

Jepson & Roth [k] produced infections of *T. saginata* in cattle with eggs maintained for 23 weeks under winter and spring conditions in Denmark. Considerably more eggs survived in fluid media than on grass in summer when desiccation was, apparently, an important limiting factor.

Similar observations on the lengthy survival of tapeworm eggs have been recorded by Silverman,[l] who was able to activate the embryos of *T. saginata*

and *T. pisiformis* artificially after maintaining them at 4°C for 335 days and 187 days, respectively, although embryos of neither tapeworm could be activated after 60 days at room-temperature (20°C). Gemmell (unpublished data) observed that no eggs of *T. hydatigena* or *E. granulosus* could be activated artificially after they had been maintained in unfiltered tapwater at 37°C for 9 days, but some, after being kept at 6°C, could still be activated after 1 year. The survival time of eggs between these extremes was related to temperature. Data from *in vitro* hatching studies, however, cannot be taken as more than a guide without confirmation from *in vivo* studies, since hatching requirements alter when eggs are stored. The longevity of eggs of *Echinococcus* spp. still requires evaluation under the wide variety of conditions to which they may be exposed in the field and laboratory.

Factors influencing infection in man

Mode of transmission and portal of entry. The view that hydatid disease in man results only from ingestion of eggs is generally accepted; certainly, animals can readily be infected by this route. The mode of transmission is usually taken to be a direct transfer of eggs by the hand, or *via* unwashed vegetation, to the mouth. However, experimental evidence has been reported that *E. granulosus* can become established in the lungs of sheep from eggs without their prior ingestion.[m] It is possible, then, that recommendations to wash hands, boil vegetables and take other general hygienic measures after handling infected dogs may be an oversimplification of the hygienic requirements needed to preclude all chances of infection.

Size of dose and capacity of host to resist. Normally, a population of *E. granulosus* is genetically heterogeneous, although repeated passage in a particular cycle may induce greater genetic homogeneity and reduce their infectivity for other hosts. Man is an abnormal host and it seems likely, therefore, that in highly homogeneous strains only one egg in several thousand may be capable of establishing an infection. In consequence, the size of the dose received may be very important in determining the chance of an infection becoming established.

Many infections are established in childhood and this information has been used to suggest that adults are more resistant. It is possible, then, that both the

[b] Nosik, A. F. (1952) [*Proc. Kharkov. vet. Inst.*], 21, 264-270.

[c] Schiller, E. L. (1954) *Exp. Parasit.*, 3, 161-166.

[d] Fontana, V. P. (1958) Unpublished communication to WHO.

[e] Parnell, I. W. (1965) *J. Helminth.*, 39, 257-272.

[f] Batham, E. J. (1957) *N. Z. vet. J.*, 5, 74-76.

[g] Clunies-Ross, I. (1929) *Bull. Coun. sci. ind. Res. Aust.*, 40, 1-63.

[h] Nosik, A. F. (1952) [*Proc. Kharkov. vet. Inst.*], 21, 304-311.

[i] Rausch, R. & Schiller, E. L. (1956) *Parasitology*, 46, 395-419.

[j] Schiller, E. L. (1955) *J. Parasit.*, 41, 578-582.

[k] Jepson, A. & Roth, A. (1949) *Epizootiology of Cysticercus bovis—resistance of the eggs of* Taenia saginata. In: *Proceedings of the 14th International Veterinary Congress*, vol. 2, p. 43.

[l] Silverman, P. H. (1956) *Trans. roy. Soc. trop. Med. Hyg.*, 50, 7.

[m] Borrie, J., Gemmele, M. A. & Manktelow, B. W. (1965) *Brit. J. Surg.*, 52, 876-878.

number of eggs ingested and an age factor may influence the chance of infection in man.

Parameters for determining the need for precautions

The ubiquity of the definitive hosts of *Echinococcus* spp. (vulpids, canids and felids) means that there are a number of situations where specified precautions cannot be taken. There are, however, some occasions when specific methods of handling infected hosts may be needed, and other occasions when they must be applied rigidly.

Situations in which safe handling may be needed. Some examples of some of these situations are in the handling of:

(1) definitive hosts in zoos and on fox-farms;

(2) dogs on farms and in slaughter-houses;

(3) dogs in veterinary clinics and hospitals;

(4) carcasses in diagnostic laboratories.

In such situations, specific precautions are not usually applied against *Echinococcus* spp., primarily because infections usually remain undetected. However, the need to apply specific methods for safe handling may arise as a result of detection either *post mortem* or following treatment with arecoline hydrobromide.

Situations where safe handling methods must be applied. Some examples of these situations occur at the following places:

(1) research establishments maintaining infected dogs or egg cultures;

(2) sites where dogs are regularly treated in hydatid-control schemes;

(3) laboratories where faecal samples are examined for *Echinococcus* spp.

In these situations, infective material is known to exist and high concentrations of eggs may be present.

Methods of killing eggs

Chemical. Nosik [h] first reported that eggs of *E. granulosus* were resistant to formol. Since this report, further data involving both formol and a wide range of other substances [n-u] have revealed no compound that can effectively kill the eggs.

Parnell [e] pointed out that, unlike other helminths which depend on numbers to produce disease, hydatidosis may develop if only 1 embryo becomes established. He concluded, therefore, that before any compound can be recommended, it should be 100% effective, because if it is unreliable, it may mislead the user into the neglect of other precautions, such as the use of heat.

Physical. Eggs of *E. granulosus* are rendered harmless by boiling water [p] but heat penetrates dog faeces slowly and infective material should be boiled for at least 5 minutes to render the eggs harmless. [v]

Laws, [w] using Silverman's *in vitro* hatching techniques, reported that eggs of *E. granulosus* are susceptible to desiccation. Further work on the lethal effects of desiccation, including *in vivo* studies on eggs enclosed in proglottides, may reveal a practical method, other than heating, for decontaminating laboratories and kennels.

Measures for protection of staff and safe-handling of infective materials

Research kennels. Facilities for holding about 150 dogs with patent infections of *E. granulosus* are provided in the Hydatid Research Unit laboratories. The pertinent measures taken to protect staff are summarized below.

(1) The kennel accommodation is inside a fly- and dust-proof brick building, 100 ft by 21 ft (approximately 30.5 m by 6.5 m); ventilation is maintained under slight negative pressure using extractor fans with outlet ducting screened and extending upwards for 30 ft (9 m).

(2) Entry to the kennel accommodation is approached through a changing-room and a sterilization laboratory.

(3) Dogs are kept in 3 ft by 3 ft (1 m by 1 m) wire cages in two banks. Each cage has an individual tray to collect waste.

(4) There is a steam sterilization plant in the building and also

(5) a boiling tank and garbage disposal unit.

[n] Boray, J. (1954) *Acta vet. Acad. Sci. hung.*, 4, 93-109.
[o] Meymerian, E. & Schwabe, C. W. (1962) *Amer. J. trop. Med. Hyg.*, 11, 360-364.
[p] Williams, R. J. (1963) *Res. vet. Sci.*, 4, 550-555.
[q] Hercus, C. E., Williams, R. J., Gemmell, M. A. & Parnell, I. W. (1962) *Vet. Rec.*, 74, 1515.
[r] Parnell, I. W. (1965) *J. Helminth.*, 39, 257-272.

[s] Mackie, A. & Parnell, I. W. (1967) *J. Helminth.*, 41, 167-210.
[t] Laws, G. F. (1965) *Proc. Univ. Otago med. Sch.*, 43, 11.
[u] Laws, G. F. (1967) *Exp. Parasit.*, 20, 27-37.
[v] Gemmell, M. A. (1968) *Bull. Wld Hlth Org.*, 39, 73-100.
[w] Laws, G. F. (1966) *Proc. Univ. Otago med. Sch.*, 44, 23-25.

Working procedures are as follows:

(6) Authorized personnel only are permitted to enter the building.

(7) Full protective clothing, including overalls, masks, caps, rubber gloves and boots, are put on in the sterilization room.

(8) Waste trays are emptied daily and scrubbed in boiling water. Waste materials are boiled before their disposal *via* the garbage disposal unit into the sewerage system.

(9) Carcasses are placed in waxed paper bags and buried.

(10) Communication with the laboratory is maintained by an alarm-bell system.

(11) Protective clothing is removed in the sterilization room before staff enter the changing-room to leave the building; the used clothing is boiled before it is used again.

Research laboratories. Similar precautions are taken to provide protection for staff in the Hydatid Research Unit laboratories when they are working with infective material. A special laboratory is provided solely for work with *E. granulosus* eggs. This laboratory is also entered through a changing-room and a sterilization laboratory. All examinations of faeces are made over a sink. An immersion heater is placed in each sink and the material is boiled before it is passed into the sewerage system. Eggs required for cultures are stored in a refrigerator in bottles which are placed in sealed plastic bags or in large well-marked jars containing cotton-wool.

Accidents. It may happen that a jar containing worms is broken and the contents are scattered over a wide area. The use of boiling water to sterilize the area cannot be recommended since it may cool too rapidly on contact with a large surface to destroy all the eggs. Decontamination can be carried out with the aid of a flame-torch; and for this reason no inflammable materials should be kept in isolation laboratories.

Field control schemes. During the course of a hydatid control scheme, dog faeces, induced by treatment with arecoline hydrobromide, may be required for diagnostic purposes.

The following operating procedure has been developed by the Tasmanian Department of Agriculture:

(1) A mobile diagnostic laboratory caravan (or trailer) is provided.

(2) Specific enclosed sites, isolated from habitation, are maintained. Dogs are brought into these areas for treatment at specified times.

(3) Following their identification and treatment with arecoline hydrobromide, the dogs are chained-up for about 3 hours.

(4) Treatment is undertaken by a technician wearing protective clothing.

(5) Faeces induced by the treatment are passed to a second technician working inside the mobile laboratory and are immediately boiled. Subsequently the faeces are examined for *E. granulosus.*

(6) Dogs found to be infected with *E. granulosus* are given a second treatment and are then allowed to walk home; they may then be quarantined on the farm until they have been shown by subsequent treatments to be free from worms.

(7) The site where infected faeces have been deposited is sterilized with a flame-torch.

The laboratory aspects of this operation are probably almost ideal, but not all risks can be avoided in practical field operations. For example, faeces may be passed by the roadside after the dog has left the site, or dogs, while chained, may lie in the faeces induced by arecoline hydrobromide and grossly contaminate their coats with *E. granulosus* eggs.

Conclusions

The interval between infection and the appearance of clinical disease in man, together with the belief that hydatidosis is mainly contracted in childhood, may lead to carelessness in the handling of *Echinococcus* spp. in endemic areas. Some justification for this attitude is also provided by the fact that no excessive occupational risk for those handling infective material (e.g., veterinarians) has ever been demonstrated.

On the other hand, research institutes and public health authorities cannot operate without the provision of special facilities and high standards of supervision. Even those methods being developed, and those in daily use in the laboratory and in the field, are exploratory and not ideal, since they are seriously handicapped by the lack of an effective chemical ovicide for use in a wide variety of situations, where physical methods are difficult to apply.