# Studies in the epidemiology and control of Echinococcus granulosus in Kenya

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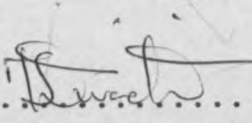
is thesis is submitted in fulfilment of the degree of Doctor
Philosophy in the Faculty of Veterinary Medicine, University
of Nairobi

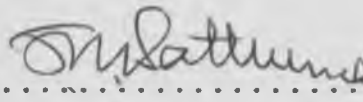1988

# DECLARATIONS

This is my original work and has not been presented for a degree in any other university
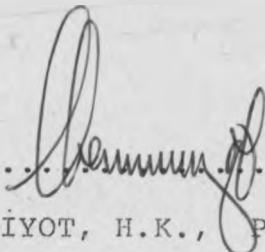
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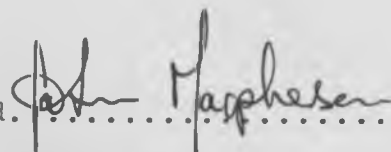
This thesis has been submitted for examination with our approval as University supervisors.

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To


**My beloved wife Njeri**

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# ACKNOWLEDGEMENTS

## ABSTRACT

Hydatid disease (hydatidosis, echinococcosis) caused by the larval stage of the taeniid, *Echinococcus granulosus* (Batsch, 1786), is a cyclozoonosis of major medical and veterinary importance in most countries of the world. In nature, the disease is primarily transmitted between wild carnivores and herbivores, but of importance to man is the domestic cycle which involves dogs and domestic livestock.

Control of the disease has been instituted in more than twelve countries. In Kenya, a pilot control programme began in 1983 in the northwestern part of Turkana district, a region with the highest known incidence of human hydatidosis in the world. However, a number of areas in the epidemiology, biology, transmission and stability of the parasite in Kenya were still unclear and yet were important for effective control of the disease. The present study was therefore, initiated in an effort to bridge the gaps in the epidemiology of *E. granulosus* in Kenya, leading to a more strategic and effective control programme against the parasite in the country.

Included in the objectives of the study, were investigations in the prepatent period and the existence and extent of *E. granulosus* strains in Kenya, the infection pressure of the parasite to dogs and hence to humans, the number, survival and release of *E. granulosus* eggs in the environment. The application of regulated release encapsulated praziquantel in

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the mass dog dosing programme, in the control of the disease
in Kenya and the effect of drought on the prevalence and
intensity of *E. granulosus* infections in dogs, were also
evaluated.

In an attempt to characterize *E. granulosus* in Kenya, the
rate of development of the parasite from human and domestic
hosts in experimentally infected dogs, was studied at days
14, 17, 21, 26, 35 and 42 post infection.  Worms of different
intermediate host origin harvested at day 35 post infection,
were also compared morphologically among themselves and with
worms of similar infection age from Australia, Britain,
Canada, Switzerland and South Africa.  In addition, Sau 96 I
digest fragments of DNA material, extracted from
protoscoleces obtained from hydatid cysts of goat, sheep,
cattle, human and camel origin were compared.  The DNA
fragments were separated on horizontal gel electrophoresis.

Two strains of the Kenyan parasite were identified, one
infecting cattle, sheep, man and goat, while the other which
occurred less frequently, infecting goat and camel.  The
strains were differentiated by electrophoretic separation of
DNA material after a complete restriction endonuclease
digestion.  The results thus obtained were similar to those
reported from isoenzyme profiles of parasite material of
similar origin (Macpherson and McManus, 1982).  The strains
were found to be developmentally and morphologically similar.
However, the rate of development of the parasite was shown to
be faster in Turkana dogs than in Nairobi dogs.
The Kenyan parasite was found to closely resemble *E. g.*

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*granulosus* (Verster, 1965) from South Africa, both in morphology and rate of development, but significantly different from the widespread domestic sheep/dog strain (Thompson and Lymbery, 1988). *E. granulosus* in Turkana dogs was shown to have short prepatent period comparable to *E. granulosus* of cattle/dog cycle both from South Africa and Switzerland.

The incidence of echinococcosis in praziquantel treated and untreated dogs in the hydatid pilot control area, was investigated over a period of four years (1984 - 1988) using autopsy and arecoline hydrobromide purge methods. Purging of dogs with arecoline hydrobromide as a method of diagnosis of tapeworm infections, was found to be ten times less sensitive compared to autopsy. Of 58 undosed dogs killed and autopsied, 63.8% were found infected with *E. granulosus*, an infection level similar to that recorded in the same area, in the previous four years. However, significant increase in *E. granulosus* infection intensity in dogs was shown to have occurred at the end of 1978/82 drought in Turkana. The natural infection rate in dosed dogs was found to increase with time and reverted to pre-control level by six months. *Taenia hydatigena* was shown to be the only other commonly found dog taeniid in Turkana.

The number of *E. granulosus* eggs in undetached and detached segments in infected dogs was counted and compared in heavy, medium and light infections. Survival of the eggs in

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different Turkana and Nairobi environments was also studied. It was found that the average number of eggs in *E. granulosus* gravid segment was 825; this was not dependent on the intensity of infection. The majority of the eggs (70%) were released in the host gut, and contrary to earlier reports, proglottid movements outside the definitive host were found to have no significant role in egg dispersal under the Turkana environment. *E. granulosus* eggs lost viability in less than three hours in the open ground heat in Turkana, but survived for three days in the shade and for more than two weeks in water. In contrast the taeniid eggs survived for more than four weeks in the shade in Nairobi.

In an effort to find an effective treatment that would provide prolonged protection against *E. granulosus* reinfection in dogs, an experiment was carried out to test the application of controlled release glass encapsulated praziquantel in dogs infected with *Taenia hydatigena*. Four weeks following capsule implantations, seven of eight experimental dogs were found on autopsy to have been cleared of the tapeworm infections, while control dogs were still infected. No side effects were observed.

The results of the present study had a number of implications on the hydatid control programme in Kenya. *T. hydatigena*, being the only other common dog taeniid in Turkana and having a similar life cycle to *E. granulosus*, will be of value to the education programme as it can serve as an indicator of dogs having been fed on raw offal. In addition the large size of the *T. hydatigena* parasite would help the people to

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appreciate it better than the small *E. granulosus* tapeworm.

Surveillance using arecoline hydrobromide is not a reliable technique, as often dogs fail to purge and when they do, occasionally the worms are not expelled and young or light infections can be missed.  It is also a cumbersome method and presents a greater risk of infection to personnel.  Although autopsy was found to be a highly sensitive method of diagnosis of *E. granulosus* in dogs, it has limited use as a surveillance technique in a hydatid control programme. Development of a safe and appropriate diagnostic method, therefore, requires further research.

From studies of the prepatent period of the parasite, it was shown that gravid segments were shed by dogs less than six weeks following infection.  In addition the infection pressure to dogs was shown to be high.  Therefore, to reduce transmission as much as possible between dogs and the intermediate hosts, the dosing interval ought to be reduced to every five weeks.  The need for this was found to be more critical towards the end of a drought when infection pressure is thought to be highest.  With about 200 dogs spread over 9,000 sq. km., this would be an expensive exercise and the long term answer, therefore, lies in the intensification of the education programme, teaching people not to feed cysts to dogs and the basic elements of hygiene that will reduce the currently exceptionally high level of man-dog contact.

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The field application of the controlled release glass encapsulated praziquantel would be of great value to the control programme. Further studies are needed in the development of the capsules of varied digestibilities in dogs which would allow for a prolonged dosing interval.

# Chapter 1

## GENERAL INTRODUCTION

### 1.1 PREAMBLE AND AIMS

Hydatid disease (hydatidosis, echinococcosis) caused by the larval stage of the taeniid, *Echinococcus granulosus* (Batsch, 1786), is a cyclozoonosis of world wide distribution (Matossian *et al.*, 1977).  In nature the disease is primarily transmitted between wild carnivores and herbivores, but of importance to man is the domestic cycle which involves domestic dog and livestock.

The definitive host of the parasite, which is invariably a carnivore, becomes infected by ingesting larvae (protoscoleces) which are asexually produced in a hydatid cyst.  Each protoscolex is capable of developing into a sexually mature worm in the small intestine of a definitive host, where it produces thousands of eggs.  The eggs are released in the faeces of the definitive host.  Intermediate hosts which include various ungulates, primates and marsupials are infected through ingestion of viable eggs of the parasite.  The eggs hatch in the intestine of the intermediate host, each releasing a single embryo (oncosphere) which penetrates the intestinal wall and enters the circulatory system.  Although oncospheres may localize and develop in any tissue, this usually takes place in the

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liver and/or the lungs.  Each oncosphere develops into a
hydatid cyst.

Hydatidosis is of particular importance to man due to lack of
an effective chemotherapeutic treatment of infected
individuals.  Surgery is presently the main form of treatment
in man but, it often results in a poor prognosis.  The
disease is widely distributed in Kenya, both in man and
livestock but, it shows marked regional variation
(Macpherson, 1981, Wamae and Cheruiyot, 1983).  The economic
importance of hydatid disease in Kenya, attributed mainly to
the condemnation of livers at slaughter, is shown by the high
prevalence of the disease reported in domestic livestock
(Mango, 1971; Eugster, 1978; Macpherson, 1981; Cheruiyot,
1983).

Human hydatidosis in Kenya, is endemic in Turkana district
and to some extent in Maasailand (Eugster, 1978; Macpherson,
1981).  Following a number of reports on the high incidence
of human hydatid infections among the Turkana people (Wray,
1958; Schwabe, 1969; Rottcher, 1973; Irvin, 1974; O'Leary,
1976), a Hydatid Research Unit, charged with the
responsibility of finding out more about the disease in
Turkana and eventually leading to a control programme, was
established by the Africa Medical and Research Foundation
(AMREF) in 1976 (AMREF 1977).  Thus in the period between
1977 and 1982, a considerable amount of information was
accumulated and formed the subject of many publications on
the human distribution of the disease (French and Nelson,
1982) and hypotheses to account for the remarkably high

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prevalence of the disease in man (French *et al.*, 1982), the role of man (Macpherson, 1983), wildlife (Macpherson *et al.*, 1983), dogs (Macpherson *et al.*, 1985) and livestock (Macpherson, 1981) in maintaining the life cycle of the parasite in Turkana. The biology of the parasite (Macpherson and McManus, 1982; McManus, 1981; Macpherson and Smyth, 1985) and possible mode of its transmission in Turkana (Stevenson and Macpherson, 1982) were also studied. With this information, options for control strategies were critically evaluated (Macpherson *et al.*, 1984b), and in 1983 a pilot hydatid control programme was started by AMREF in conjuction with the Kenya Ministry of Health in the northwestern Turkana, a region with the highest prevalence of the disease (Macpherson *et al.*, 1984a).

A number of gaps, however, remained in our knowledge of the epidemiology of the parasite in Turkana. The knowledge is essential for effective control of the disease; this formed the basis of the present study. Areas needing investigation included the study of possible strain variation, natural infection pressure to dogs and prepatent period. The source of infection to man with hydatid disease was also not clearly understood. Although an effective chemotherapeutic treatment against *E. granulosus* in dogs was available and being used in the control programme, the drug (praziquantel, Droncit[R], Bayer, West Germany) provides no protection against reinfection. There was therefore a need for development of a sustained release formulation of praziquantel, or a vaccine which would provide prolonged protection against reinfection with *E. granulosus*.

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## 1.2 AREA OF STUDY

The study was carried out in Turkana district of Kenya (Fig. 1.1) but as *E. granulosus* is distributed in various regions of the country, collection of the parasite material was not restricted to the district.

Turkana district which lies between climatic zones five and six, is a semi-desert with seasonal grass cover and scattered dwarf shrubs (Survey of Kenya, 1970). Large shrubs may be seen along the common dry river beds found in the region. The mean annual rainfall is 300 - 600 mm (Survey of Kenya, 1970) but greatly varies from year to year, with droughts occurring every ten years or so (Appendix 1.1). The region has maximum annual mean temperature range of $30^{o}C$ - $34^{o}C$ and a minimum of $18^{o}C$ - $22^{o}C$ (Survey of Kenya, 1970).

Turkana people, who inhabit the land, have a nomadic life style. They move from one region to the other, depending on season and availability of pasture for their livestock. They herd cattle, camels, sheep and goats. They also keep donkeys for transport. Dogs in Turkana are kept mainly for guarding against intruders and as "nurse dogs", cleaning up babies' vomit or faeces (French *et al.*, 1982). The small Turkana breed of dog is found throughout the district, north western Uganda and southern Sudan (French *et al.*, 1982; Macpherson *et al.*, 1985).

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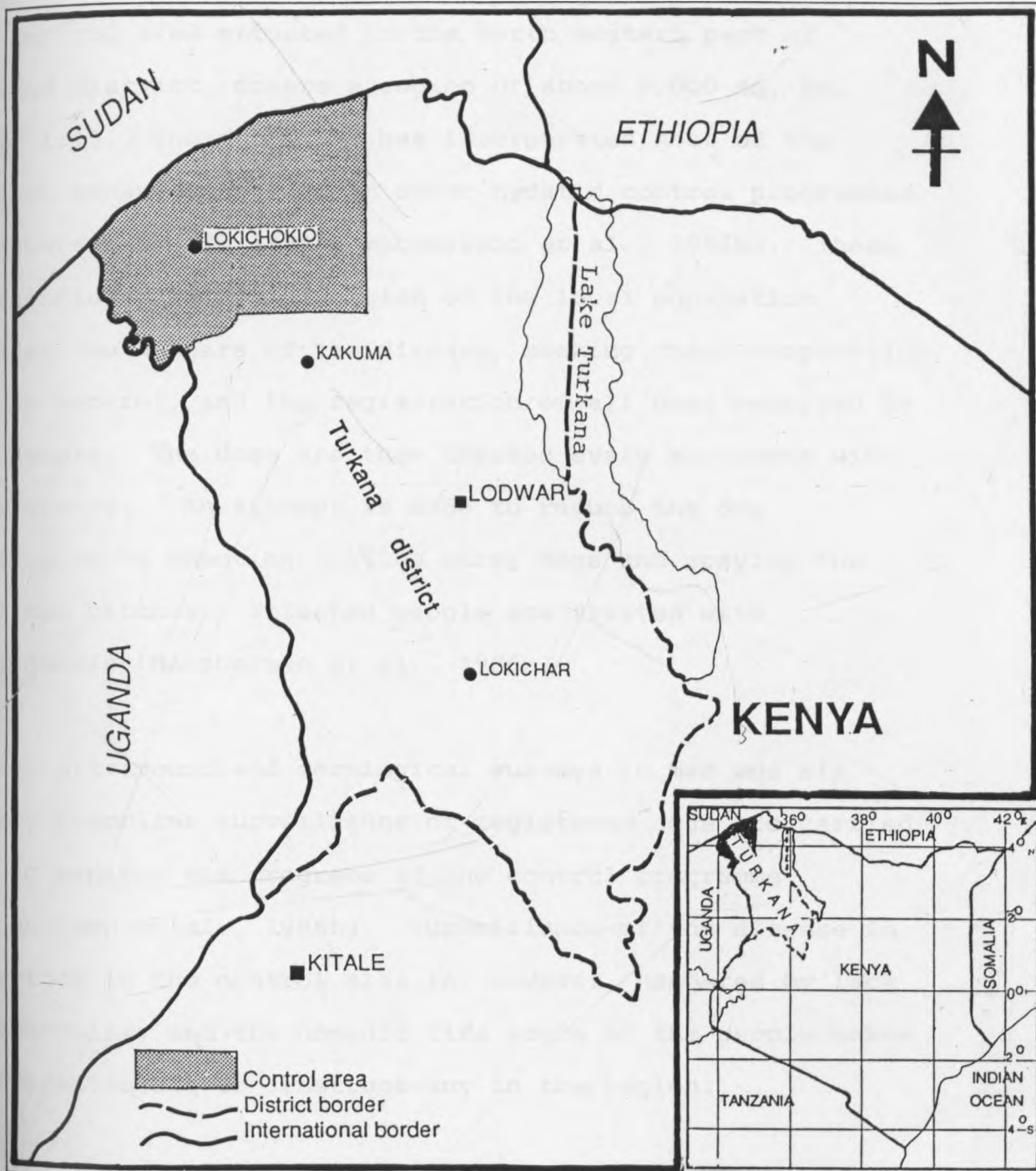
FIGURE     1.1

Map of Turkana District of Kenya showing the hydatid
control area

## 1.3 The Turkana pilot hydatid control programme

The control area situated in the north western part of Turkana district, covers a region of about 9,000 sq. km. (Fig. 1.1). The programme has incorporated most of the control measures applied in other hydatid control programmes (Macpherson et al. 1984b; Macpherson et al., 1986b). These have included mainly education of the local population against the dangers of the disease, seeking their cooperation in the control, and the registration of all dogs required by the people. The dogs are then treated every six weeks with praziquantel. An attempt is made to reduce the dog population by shooting all the stray dogs and spaying the required bitches. Infected people are treated with albendazole (Macpherson et al., 1986b).

Annual ultrasound and serological surveys in man and six weekly arecoline surveillance of registered dogs are carried out to monitor the progress of the control programme (Macpherson et al., 1986b). Surveillance of the disease in livestock in the control area is, however, hampered by lack of abattoirs, and the nomadic life style of the people makes it impracticable to construct any in the region.

Chapter 2

LITERATURE REVIEW

## 2.1  BRIEF HISTORY OF *ECHINOCOCCUS*

The existence and medical importance of unilocular hydatid disease has been known since the earliest times. Hippocrates (B.C. 460 - 379), in his aphorism section VII, makes a reference to the disease thus, "when the liver is filled with water and bursts into the epiploon, in this case the belly is filled with water and the patient dies" (Leuckart, 1886; Dew, 1928). References to hydatids are also found in the works of Aretaeus (A.D. 7-79), Galen (A.D. 139-200), and Rhazes (A.D. 860-932), who regarded them as manifestations of degenerated glands, collections of pus or mucus, and even distended lymph nodes (cited by Thomas, 1894). Redi, in 1684 (Castellani and Chalmers, 1919), was the first to recognize that hydatid cysts were of animal origin, but it was Tyson (1650 - 1708, cited by Dew, 1928) who studied and gave a detailed account of the parasitic nature of the hydatids. Later in 1782, Goeze (Kuchenmeister, 1857; Leuckart, 1886) gave the morphological description of the hydatid cyst and identified protoscoleces as *Taenia visceralis socialis granulosa*. He showed that hydatid cysts were different from other well known cysticerci of animals. In 1786, Batsch independently described and identified the hydatid cysts in sheep as *Hydatigena granulosa* but in 1800, Zedder (cited by Kuchenmeister, 1857) placed it under the genus *Polycephalus*.

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A year later, Rudolphi (1801, cited by Leuckart, 1886) in his work of anatomical appearance of hydatid cysts, separated it from the genus *Polycephalus* and erected a new genus wherein it was designated *Echinococcus granulosus*.

The adult *E. granulosus* worms were for the first time observed in a naturally infected dog by Hartmannus in 1694, (Braun, 1894) and although Pallas, in 1767 (cited by Castellani and Chalmer, 1919), had demonstrated the resemblance of protoscoleces to the adult tapeworm, the theory of spontaneous generation was still upheld by reknowned workers like Rudolphi (1808). Kuchenmeister (1851), by feeding dogs with various cysticerci found in sheep, proved that it was these cystic forms of parasites that gave rise to "taenia" in the intestinal canals of dogs. This paved the way for Sielbold (1853) who showed for the first time that hydatid cysts fed to dogs gave rise to a three segmented adult cestode with two rows of hooks in the scolex. The life cycle of the hydatid parasite was finally completed by Haubner (1855, cited by Kuchenmeister, 1857) when he demonstrated the development of hydatid cysts in a domestic pig orally fed with eggs of *E. granulosus*.

In 1863, Leuckart gave a detailed description of the multilocular hydatid cyst, differentiating it from the unilocular form caused by *E. granulosus* and designated it a new species, *E. multilocularis* Leuckart 1863 (Leuckart, 1886). Meanwhile, Deising, in 1863, described worms collected by Natterer from a puma (*Felis concolar*) in Brazil, as *Taenia crassicollis* which were later designated *Taenia*

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*nana* by Van Beneden in 1850 (Leuckart, 1886) and lastly *E. oligarthrus* Diesing 1863 by Luhe (1910).

Since then 13 more species in the genus *Echinococcus* have been described (Reviewed by Kumaratilake and Thompson, 1982) and speciation within this genus has been surrounded by a lot of controversy which has formed the subject of a number of reviews over the years (Cameron, 1926; Rausch, 1953; Vogel, 1957; Rausch and Nelson, 1963; Sweatman and Williams, 1963a; Smyth and Smyth, 1964; Rausch, 1967a; Rausch and Bernstein, 1972; D'Alessandro *et al.*, 1979; Krotov, 1979; Kumaratilake and Thompson, 1982; Thompson and Lymbery, 1988). Of the thirteen species, *E. granulosus*, *E. multilocularis*, *E. vogeli*, and *E. oligarthrus* are now generally accepted as the only taxonomically valid species within the genus *Echinococcus* (WHO, 1982).

## 2.2 GLOBAL DISTRIBUTION OF *E. GRANULOSUS* WITH SPECIAL EMPHASIS TO AFRICA

Two biological forms of *E. granulosus* have been recognized on the basis of host specificity in the larval stage; the Northern form and the European form (Rausch, 1967b; 1986; Wilson *et al.*, 1968). The distribution of the Northern form is limited by the hosts (wolf, elk, reindeer, mule-deer and red-deer) to the tundra and taiga zones. The two forms overlap on the southern limits of the boreal forest but in north America, they remain geographically separate. The European form (herein after referred to only as *E.*

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*granulosus*) whose cycle involves mostly the domestic dog and domestic ungulates as final and intermediate hosts respectively, is widespread.

The global distribution of *E. granulosus* has been on the increase since the turn of the century. A review by Dew in 1928 indicated a very limited distribution covering Australia, Argentina, New Zealand, north and southern tips of Africa, Iceland, central Europe and the Middle East. The parasite was considered to have been very rare or non-existent in the rest of the world. Forty years later, the parasite had been reported in many of hitherto free countries (Schantz and Schwabe, 1969; Matossian, *et al.*, 1977) resulting in a world wide distribution of the parasite. This probably reflects better diagnosis, research and reporting on the disease, rather than a real increase on the disease incidence and distribution. China is a good case in point, of a country where for a long time the disease was thought to be non-existent (Dew, 1928) but recently shown to harbour high infection rates of the parasite both in man and animals (Kan, 1966; Pu-Sheng and Liang-Ru, 1985).

## 2.2.1  Europe and the Americas

Virtually every country in Europe has reported the presence of the parasite and hydatidosis is considered to be of endemic nature in the Soviet Union (Asadov, 1960), Bulgaria, southern France, Italy, Greece, Portugal, Rumania, Sicily, Spain and Yugoslavia (Gemmell, `1960; Wittenberg, 1964;

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atossian, *et al.*, 1977). Equine hydatidosis in Britain has also been reported to be on the increase (Thompson and Smyth, 1975). Identification of transmission foci of *E. granulosus* infections in California, reported by Sawyer *et al.*, (1969) indicated for the first time that classical dog-sheep transmission cycle occurred in the United States, and further studies revealed the widespread nature of the disease in that country (Schantz and Schwabe, 1969). Unilocular hydatidosis is of low and sporadic occurrence in central American countries (Williams *et al.*, 1971). This is not true of the South American countries, as some of the highest prevalences of the disease are found in this region of the world. Hydatidosis is hyperendemic in Argentina, southern Brazil, Chile, Uruguay and Peru (Williams *et al.*, 1971; Schantz, 1972; Matossian *et al.*, 1977).

## 2.2.2 Asia

Hydatidosis is endemic in most of the Asian countries (Matossian *et al.*, 1977), including Bangladesh (Shamsul Islam, 1979) and China (Kan, 1966; Pu-Sheng and Liang-Ru, 1985), where the disease was previously thought to be rare. In Saudi Arabia, Japan, Indonesia, Malaysia, Sabah and Sarawak, only occasional cases have been observed and hydatidosis is not considered to be a major health problem (Matossian *et al.*, 1977). The disease is very rare in the Philippines (Arambulo, 1974) and has not been reported from Oman (Matossian *et al.*, 1977).

### 2.2.3 Australia and New Zealand

High prevalences of hydatidosis were previously reported from the sheep-raising areas of Australia and New Zealand (Dew, 1928). In 1891, the surgical cases in public hospitals indicated a hydatid infection rate of 6.9 per 100,000 persons per year, and infection prevalences of 42.4% and 37.3% were reported in sheep and dogs respectively in 1958 in New Zealand (Schantz and Schwabe, 1969). By 1970, however, the control programmes in New Zealand and Tasmania had drastically reduced the infection levels in the domestic dogs in those countries to about 1% (Arundel, 1972).

### 2.2.4 Africa

At the turn of the century, hydatid disease was thought to be limited to the northern and southern tips of Africa where the infection rate among sheep was estimated to be more than 20% (Dew, 1928). However, with the development of health services and research coupled with better systems of detection and reporting of the disease, hydatidosis is now established to be widespread in this continent. Echinococcosis has not been recorded in Mauritius and information is lacking concerning its distribution in Burundi, Canary Islands, Gabon and Gambia (FAO.WHO.OIE, 1981). The occurrence and prevalence of this disease reported in dogs, man and domestic livestock from Africa, is as presented in Table 2.1.

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**Table 2.1**

## Occurrence and prevalence (%) of *Echinococcus* in man and domestic animals in Africa

| Country | Man | Dog | Camel | Cattle | Sheep | Goat | Others | References |
|---|---|---|---|---|---|---|---|---|
| | | | | Intermediate hosts | | | | References |
| Algeria | | 4-20 | | | | | | Senevet, 1951. |
| | | | | 30.5 | 21.8 | | | Jore d'arces, 1953. |
| | | 10-40 | | | | | | Pampiglione 1965a. |
| | +++ | | 42.1 | 5.4 | 14.3 | 21.2 | pg hs | Larbaui et al., 1980. |
| Angola | | | R | R | | | | FAO.WHO.OIE, 1981. |
| Benin | + | | | | | | | Fayomi et al., 1987. |
| Botswana | | | R | R | | | | FAO.WHO.OIE, 1981. |
| Burkina Fasso | | | | R | R | | | FAO.WHO.OIE, 1981. |
| Cameroon | | | R | | | | | FAO.WHO.OIE, 1981. |
| Cape Verde | | | R | R | R | | pg | FAO.WHO.OIE, 1981. |
| Central Africa | | | 37.5 | 1.1 | 0.2 | | | |
| | | | | (7465) | (5533) | | | Graber et al., 1969. |
| Chad | C | | | | | | | Fourre and Imbert, 1965. |
| | | 3.4 | | | | | | |
| | | (117) | | | | | | Troncy and Graber, 1969. |
| | 3C | | | | | | | Sirol and Lejevre, 1971. |
| Congo | | | | R | | | | FAO.WHO.OIE, 1981. |
| Cote d'Ivoire | | | | 2.3 | | | | |
| | | | | (520) | | | | Mishra and N'Depo, 1978. |
| | 4C | | | | | | | Schmidt et al., 1982. |
| Djibouti | C | | | | | | | Disy, 1975. |
| | | | | | R | | | FAO.WHO.OIE, 1981. |
| Egypt | | | | | | | | |
| (upper Egypt) | 2-10 | | | | | | | Abdel Azim, 1938. |
| | | | 31 | 10 | 1.5 | | pg | |
| | | | (200) | (200) | (200) | | | El-Kordy, 1946. |
| | | | 31 | 0.3 | 0.3 | | | Halawani, 1956. |
| (Abbasieh) | | 7.1 | | | | | | El-Garhy and selim, 1958 |
| | | (28) | | | | | | |
| | | | 30 | 10 | 5 | | | Abdou, 1965. |
| (Cairo) | | 3.9 | 32.6 | | | | | |
| | | (570) | (288) | | | | | Moch et al., 1974. |
| | | | 1.3 | 0.1 | 0.2 | | pg | |
| | | | (2000) | (4000) | (5000) | | | Sedik et al., 1977. |
| (Mansoura) | | 1.2 | | | | | | |
| | | (85) | | | | | | Abo-Shady, 1980. |
| | | | 8 | 0.3 | 0.3 | | pg | |
| | | | (1811) | (1114) | (2200) | | | Hamdy et al., 1980. |
| (Cairo) | | | 31.6 | | | | | |
| | | | (155) | | | | | Abdel-Gawad et al., 1981. |
| (Dakahlia) | | 1.4 | | | | | | |
| | | (289) | | | | | | Abo-Shady et al., 1982. |
| (Dakahlia) | ** | 1.6 | 8.8 | 0.2 | 0.4 | | | |
| | | (510) | (204) | (991) | (236) | | | Hegazi et al., 1986. |

| Country | Man | Dog | Camel | Cattle | Sheep | Goat | Others | References |
|---|---|---|---|---|---|---|---|---|
| | | | | | Intermediate hosts | | | |
| Equitorial | | | | | | | | |
| Guinea | | | | R | | | | FAO.WHO.OIE, 1981. |
| Ethiopia | | R | | R | R | | | Daynes and Graber, 1974. |
| (south) | +++ | | | | | | | Fuller and Fuller, 1981; |
| | 42C | | | | | | | Lindtjourn et al., 1982; |
| (south) | + | | | | | | | (Macpherson personal communication) |
| Ghana | C | | | | | | | Schmidt et al., 1978. |
| | | | | | R | | | FAO.WHO.OIE, 1981. |
| Guinea | C | | | | | | | Schmidt et al., 1982. |
| | 2C | | | R | R | | | Diallo and Ziarek, 1982. |
| Guinea Bissau | | | | R | | | | FAO.WHO.OIE, 1981. |
| Kenya | | | | See table 1.2 | | | | |
| Lesotho | | | | | R | R | | Fitzsimmons, 1971. |
| | C | | | | | | | Rubin, 1973. |
| Liberia | | | | R | R | | | FAO.WHO.OIE, 1981. |
| Libya | + | | 29.1 (3134) | 13.9 (18572) | 33.4 (147212) | 18.1 (5019) | | Dar and Taguri, 1978. |
| | +++ | | | | | | | Gebreel et al.,1983. |
| | | 27.2 (151) | | | | | | El-Gusbi, 1985. |
| | | | | | 7.9 (402) | | | El-Gusbi et al.., 1985. |
| | | 11 (27) | | | | | | Packer and Ali, 1986. |
| Madagascar | | | | 5 | R | R | | Randriambelona. 1931. |
| | 4C | | | | | | | Haddam et al., 1969. |
| | 3C | | | | | | | Brygoo et al., 1971. |
| | 6C | | | | | | | Ribot and Coulanges, 1982. |
| Malawi | | | | R | | | | FAO.WHO.OIE, 1981. |
| Mali | C | | | | | | | Schmidt et al., 1982. |
| | C | | | | | | | Dembele and Sangore, 1974. |
| | | | | R | R | | | FAO.WHO.OIE, 1981. |
| Mauritania | | | | R | R | | | FAO.WHO.OIE, 1981. |
| Morocco | | | 25 | 43 | 23 | 12 | | Barotte et Velu. 1925. |
| | | | 44 | | | | | Faure. 1949. |
| | ++++ | | | | | | | Chenebault, 1950. |
| | | | 6-19 | | | | | Senevet, 1951. |
| | | 20 | | | | | | Sirol and Lejevre, 1971. |
| | | | | 43 | 2.3-80 | | | Pandey, 1980. |
| | | | | 23.3 (2581) | | | | Pandey, et al., 1985. |
| | | | 80 (35) | | 38.5 (2246) | 7.7 (510) | | Pandey et al., 1986. |
| Mozambique | ++ | | | 4.5 | 2.1 | | pg | Castro-Amoro. 1960. |
| | | 0 (643) | | 2.3 (13390) | | | | Ferreira, 1980. |

Table 2.1   (contd.)

| Country | Man | Dog | Camel | Cattle | Sheep | Goat | Others | References |
|---|---|---|---|---|---|---|---|---|
| | | | | Intermediate hosts | | | | |
| Namibia | | | | R | R | R | pg | Verster, 1962. |
| Niger | | | | R | R | | | FAO.WHO.OIE, 1981. |
| | 2C | 0 | 22 | | | | | Develoux et al., 1985b. |
| | | (76) | | | | | | |
| | 5C | | | | | | | Develoux et al., 1985a. |
| Nigeria | | | | | | | | |
| (north) | | | 55.5 | 9.9 | 7.1 | 13.6 | | Dada, 1980a. |
| | | 2.4 | | | | | | |
| | | (549) | | | | | | Dada, 1980b. |
| (all regions) | | | 0.1 | 0.06 | 0.01 | 0.03 | pg | |
| | | | (31782) | (1439437) | (308984) | (821625) | | Dada, 1980c. |
| (central) | | | 57.5 | 16 | 20.9 | 22.8 | | |
| | | | (2539) | (3555) | (1177) | (1195) | | Dada and Belino, 1979. |
| (Ibadan) | | 48 | | | | | | |
| | | (102) | | | | | | Ayanwale et al., 1982. |
| | 3C | | | | | | | Schmidt, et al., 1982. |
| Re-Union | | | | | R | | | FAO.WHO.OIE, 1981. |
| Rwanda | | | | R | R | | | FAO.WHO.OIE, 1981. |
| Senegal | 2C | | | | | | | Schmidt et al., 1982. |
| | C | | | | | | | Diop et al., 1973. |
| | | | | R | R | | | FAO.WHO.OIE, 1981. |
| | C | | | | | | | Maistre et al., 1984. |
| Sierra Leone | | | | R | R | | | FAO.WHO.OIE, 1981. |
| | | R | | | | | | Hassan, 1982. |
| Somali | + | | R | R | | R | | Kagan and Cahill, 1968. |
| (exported | | | | 27.6 | | | | Behbehani and Hassounah, |
| animals | | | | (765) | | | | 1976. |
| to | | | 10.8 | 40.2 | | | | Hossaunah and Behbehani, |
| Kuwait) | | | (37) | (881) | | | | 1976. |
| Sudan | | 20 | 9 | 0.7 | | | | Sirol and rejevre, 1971. |
| | | | 35.3 | | | | | |
| | | | (51) | | | | | Saad, 1982. |
| | | 51 | | | | | | |
| | | (49) | | | | | | Saad and Magzoub, 1986. |
| | ** | | | | | | | (Macpherson personal |
| | | | | | | | | communication) |
| South Africa | | | | | | | | |
| (Pretoria) | | 20 | | | | | | |
| | | (25) | | | | | | Ortlepp, 1934. |
| | | | | 0.7 | 1.8 | | | |
| | | | | (4.8m) | (12.9m) | | pg | Verster, 1962. |
| | | | | 1.1 | 0.9* | | | |
| | | | | (1.7m) | (5.6m) | | pg | Verster and collins, 1966. |
| (all regions) | | 0.9 | | | | | | |
| | | (1063) | | | | | | Verster, 1979. |
| | >60C | | | | | | | Macpherson, 1981. |
| Swaziland | | | | 10.8 | | | | |
| | | | | (5886) | | | | Mitchell, 1977. |

Table 2.1 (contd.)

| Country | Man | Dog | Camel | Cattle | Sheep | Goat | Others | References |
|---|---|---|---|---|---|---|---|---|
| Tanzania | | | | R | R | | | Department of veterinary |
| Services, Tanganyika, 1924. | | | | | | | | |
| | C | | | | | | | Foley, 1944 |
| | | | | R | | | | Petrovic, 1975. |
| | | | | 4.3 | | | | |
| | | | | (12408) | | | | Masaba et al., 1977. |
| | C | | | | | | | Sangwe, 1982. |
| Togo | | | | R | R | | | FAO.WHO.OIE, 1981. |
| Tunisia | | 42.8 | 30 | | | | | Deve, 1923. |
| | | | 30 | | | | | Cousi, 1951. |
| | | | | 8-86 | 7 | | pg | Menchari, 1965. |
| | | | | 30 | | | | Sirol and Lejevre, 1971. |
| | ++++ | | | | 1-30 | | | Ben Rachid et al., 1984. |
| | ++++ | 22 | | 24.3 | 5.2 | 0 | | |
| | | | | (1359) | (3903) | (2135) | | Jaiem, 1984. |
| | ++++ | | | | | | | Mlika et al., 1984. |
| | ++++ | 22 | | 17* | | | | Bchir et al., 1985. |
| | ++++ | | | | | | | Gharbi et al., 1985. |
| Uganda | C | | | | | | | Snell and Mukasa, 1948. |
| | | R | | | | | | Ministry of Animal Industry Game and Fisheries, Uganda, 1967. |
| | | | | 6.6 | | | | Mitchell, 1968. |
| | | | | 13 | | | | |
| | | | | (11319) | | | | Owor and Bitakaramire, 1975. |
| Western Sahara | | | R | | | | | FAO.WHO.OIE, 1981. |
| | C | | | | | | | Royer, 1950. |
| | | | | 0.1 | | | | Musonera et al., 1979. |
| | C | | | | | | | Lalloo et al., 1985. |
| Zambia | C | | | | | | | Patel, 1969. |
| | | | | 3.5 | | | | Ministry of Agriculture and Water Development, Lusaka, 1981 |
| Zimbambwe | C | | | | | | | Holmgren et al., 1971. |
| | | | | 0.72 | | | | Chambers, 1978 |

+ incidence <1/100,000  
++ incidence between 1&5/100,000  
+++ incidence between 6&10/100,000  
++++ incidence >10/100,000  

* goats included  
(9) number of animals examined  
pg infection in pigs  
hs infection in horses  

C case report  
R reported to occur

datid disease in Africa is propagated primarily by a
mestic cycle between the domestic dog and livestock.
wever, there is evidence of a wildlife cycle among the wild
rnivores and herbivores, particularly the lion and warthog,
ffalo and zebra (Verster, 1962; Verster and Collins, 1966;
oncy and Graber, 1969; Eugster, 1978; Dinnik and Sachs,
72; Young, 1975; Graber and Thal, 1980; Ferreira, 1980;
cpherson *et al.*, 1983; Macpherson, 1986) in Central, East
d South Africa.

2.4.1  North Africa (Morocco, Algeria, Tunisia, Libya)

this region hydatidosis is limited to the agriculturally
veloped Mediterranean coastal areas where most of the human
d livestock populations are concentrated (Ben Rachid *et
l.*, 1984).  Among the main intermediate hosts (sheep, cattle
d camel), high prevalence rates (10% - 90%) have been
ported (Barotte and Velu, 1925; Faure, 1949; Jore d'Arces,
53; Larbaui *et al.*, 1980; Jaiem, 1984; Dar and Taguri,
78).  Other intermediate hosts reported include; goats,
gs and horses (Table 2.1).

hinococcosis prevalence in dogs is high in all the four
untries: Algeria, 10 - 40% (Pampiglione, 1965a); Morocco,
% (Sirol and Lejevre, 1971); Tunisia, 22 - 42.8% (Deve,
23; Jaiem, 1984; Bchir *et al.*, 1985) and Libya, 11 - 27.18%
l-Gusbi, 1985; Packer, 1986).  In Libya where infection
tensity studies were carried out in dogs, heavy infections
1,000 worms) were found in 42% of the infected dogs (El-
sbi, 1985). Despite the high influence of Islam in this

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region, whose laws discourage the association of people with dogs, a large number of dogs are kept and tolerated in houses and at religious or marriage feasts (Dew, 1928; Ben Rachid et al., 1984; El-Gusbi, 1985). In Tunisia, Ben Rachid et al. (1984) reported a dog:man ratio of 1:6 which included pet, hunting and guard dogs. There are also many dogs which scavenge in the cities and around the improperly controlled slaughter places (Dew, 1928; Jore d'Arces, 1953; Pampiglione, 1965b; Ben Rachid et al., 1984; El-Gubsi, 1985). Although infections in jackals have been reported (Jore d'acres, 1953) their role in the disease transmission is thought to be minimal (Ben Rachid et al., 1984).

The close human association with dogs seems to have given rise to the high human disease incidence in this area particularly in children. Gebreel et al. (1983) reported an infection rate of 10% among children and young adults near Benghazi in Libya, while Larbaui et al. (1980) gave an average incidence rate of 9/100,000 in Algeria. In Tunisia different workers have reported hydatid incidence rates of 10-30/10,000 people (Jaiem, 1984; Mlika et al., 1984; Ben Rachid et al., 1984; Bchir et al., 1985; Gharbi et al., 1985). Chenebault (1950) estimated the annual incidence of human hydatidosis in Morocco to be 50/100,000 people, but since then very little of human prevalence studies have been undertaken in that country.

### 2.2.4.2 East Africa (Sudan, Ethiopia, Kenya, Uganda, Tanzania)

A recent mass ultrasound scanning prevalence survey undertaken among a number of pastoralist peoples of East Africa confirmed the hyperendemic state of the disease in this area. High prevalence rates were recorded among the people living in the region between latitude $3^O$ - $8^O$ north and $38^O$ - $37^O$ east, including the Turkana (2.1-5.6%) of northwestern Kenya, the Toposa (3.2%) of southern Sudan, the Nyangatom (2.2%), Hamar (0.5%) and Boran (1.8%) of southwestern Ethiopia (Macpherson, personal communication). Earlier reports had already shown this area to be a high prevalence focus of human hydatidosis (Schwabe, 1969; O'Leary, 1976). From hospital records, the disease incidence in Turkana district of Kenya was estimated to be the highest recorded incidence (40-96/100,000) of human hydatid disease in the world (Schwabe, 1969; O'Leary, 1976; French and Nelson, 1982). The disease was also reported to be high among Dassanetch, Nyangatom, Hamar (Fuller and Fuller, 1981) and Boran (Lindtjourn *et al.*, 1982) of southeastern Ethiopia, the Karamajong and Acholi of northeastern Uganda (Owor and Bitakiramire, 1975) and the Toposa and Lotuko of southern Sudan (Eisa *et al.*, 1962). Very little of the human disease has been recorded outside of this focus except among the Maasai of Kenya and Tanzania in whom Eugster (1978) found an incidence of the disease to be very low (1-2/100,000) compared to the Turkana. This was confirmed by the recent ultrasound survey which showed a low prevalence rate of 1.0% in the Tanzania Maasai (Macpherson, personal communication).

Infection rates ranging from 27% to 70% have been recorded in the domestic dog, the main definitive host in this region (Eisa *et al.*, 1962; Nelson and Rausch; 1963; Eugster, 1978; Macpherson *et al.*, 1985). The prevalence rate of hydatid disease among the main domestic intermediate hosts (camel, cattle, sheep, goat) is generally high (Eisa *et al.*, 1962; Eugster, 1978; Owor and Bitakiramire, 1975) except in Turkana where prevalence rates of less than one percent have been recorded in goats and sheep (Macpherson, 1981). The distribution of the disease in this region and in particular Kenya, will be covered more fully later in the chapter.

## 2.2.4.3    Egypt

In Egypt, the camel seems to be the most important intermediate host of *E. granulosus* where, on average, a prevalence rate of 22% (1.25% - 32%) has been reported (El-Kordy, 1946; Halawani, 1956; Abdou, 1965; Moch *et al.*, 1974; Sedik *et al.*, 1977; Hamdy *et al.*, 1980; Abdel-Gawad *et al.*, 1981; Hegazi *et al.*, 1986). Infection rates among other intermediate hosts are much lower (Table 2.1). Echinococcosis prevalence in the domestic dog, which is the only reported definitive host in Egypt, is also low, ranging between 1.2% and 10% (Abdel Azim, 1938; El Garhy and Selim, 1958; Moch *et al.*, 1974; Abo-Shady, 1980; Abo-Shady *et al.*, 1982; Hegazi *et al.*, 1986).

Despite the endemic nature of the disease in Egypt, human

Infections are comparatively less common than in other north African countries (Madden, 1909; Cahill *et al.*, 1965; Matossian *et al.*, 1977)

## 2.2.4.4    Nigeria

Hydatid disease is prevalent across the northern states of Nigeria including Sokoto, Kano and Kaduna, where 55% of camels, 7% of sheep, 13.6% of goats and 10% of cattle have been found infected (Dada, 1980a).  Of 549 dogs examined in this region, 13 (2.4%) were infected with the parasite (Dada, 1980b).  Very little  disease is found in the south of the country, hence the reported low national prevalence rate of 1.34% in camels, 0.1% in cattle, 0.09% in sheep, 0.04% in goats and 0.12% in pigs (Dada, 1980c).  Human cases of hydatid disease in Nigeria are very rare, and to date only four cases have been reported (Katchy, 1982; Schmidt *et al.*, 1982).

## 2.2.4.5    Somalia

Results of a seroepidemiological study carried out by Kagan and Cahill (1968) with sera from Somalia showed positive reactions for antibodies to hydatids in 31%, 50%, 67%, and 4.2% of camel, goat, cattle and human sera respectively. These results suggest that the disease is rare in humans, but widespread in livestock in the country.  The presence of high infection rates of hydatidosis in camels (10%) and cattle (27-40%) exported to Kuwait from Somalia (Hassounah and

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Behbehani, 1976; Behbehani and Hassounah, 1976) confirm the endemicity of the disease in the domestic intermediate hosts.

### 2.2.4.6    Mozambique

Out of 13,390 cattle examined by Ferreira (1980) in Mozambique, 2.5% were infected with hydatid cysts but none of the 643 dogs examined had the adult parasite.  Earlier, Castro-Amoro (1960) had reported the prevalence of the disease in Cattle (4.5%), sheep (2.1%) and pigs (1.5%), and estimated that there was a disease incidence of 2/100,000 of the human population.

### 2.2.4.7  Madagascar

Randriambelona (1931) reported a hydatidosis incidence rate of 2.7% - 5% in cattle in Madagascar, but in their review, Ribot and Coulanges (1982) suggested that the disease prevalence in that country was quite low.  They estimated the incidence of the disease in about six million animals to be about 12 cases per year and reported that from 1962 to 1982, there were only six human hydatid cases.

### 2.2.4.8    Republic of South Africa

The prevalence of hydatid disease in South Africa is low and, although it varies in different regions, it is fairly distributed across the whole country.  The highest prevalence in domestic livestock is found in the south-eastern region where 13.8%, 1.5%, 3.2% and 2.8% of cattle, sheep, goats and

pigs respectively are infected (Verster and Collins, 1966). The average prevalence for the whole country, however, is less than 2% in all the intermediate hosts (Verster, 1962; Verster and Collins, 1966). Ortlepp (1934) found a rather high incidence of echinococcosis in dogs (5/25) around Pretoria but later, Verster (1979) examined 1063 dogs from all the regions and showed that the countrywide prevalence was low (0.9%). Although infections in wild carnivores (Table 2.1) have been demonstrated, the domestic dog has been shown to be the main definitive host of the disease in South Africa (Verster and Collins, 1966). Human hydatidosis is uncommon in that country and, from the available information, only 43 cases have been reported to date since 1937 (Table .1), which is less than two cases per year.

## 2.2.4.9 Swaziland

The only statistical data on hydatid disease available from Swaziland (Mitchell, 1977) suggests that the disease is endemic in animals. Mitchell (1977) reported 10.82% and 0.27% hydatid infections in lungs and livers, respectively from 5886 cattle obtained from different regions of that country.

## 2.2.4.10 Other countries in Africa

Hydatidosis/echinococcosis in the rest of the continent has either not been investigated or the occurrence is very low (Table 2.1).

## 2.3 SPECIATION AND STRAIN VARIATION OF *E. GRANULOSUS* IN AFRICA

From the day Leuckart (1886) differentiated the multilocular form of hydatids from the unilocular form caused by *E. granulosus* and designated it a new species, *E. multilocularis* Leuckart 1863, the speciation of the genus *Echinocococcus* has been a matter of controversy. The speciation was immediately refuted by a number of workers (Davaine, Eschricht, Creplin, von Siebold, Diesing (Leuckart 1886)) spearheaded by Deve in this century (1934, 1939, 1940), believing that different forms of hydatid cysts represented different manifestations of the same parasite. Those who upheld the specific status of *E. multilocularis* included Rudolphi (1855, cited by Leuckart, 1886), Kuchenmeister (1857), Leuckart (1886), Huber (1881), Stirling and Verco (1907), Posselt (1936) and Vogel (1957).

Although Vogel (1957) confirmed the taxonomic status of *E. multilocularis* by completing the life cycle in the laboratory, the complexity of speciation within *Echinococcus* continued to increase as more species and subspecies of the genus were described. Sixteen species and thirteen subspecies have already been described from different parts of the world as have recently been reviewed by Kumaratilake and Thompson (1982).

Three of the species; *Echinococcus lycaontis* Ortlepp, 1934, *Echinococcus felidis* Ortlepp, 1937 and *Echinococcus ortleppi* Lopez-Neyra and Soler planas, 1943, were described from

Africa. Their taxonomic status was upheld by Rausch in 1953, but invalidated ten years later by Rausch and Nelson (1963) who found that the presumed differences fell within the normal morphological variation for *E. granulosus*. In addition, they argued that the then available knowledge on the host distribution for *E. felidis* was inadequate to justify specific status.

A major review of the South African *Echinococcus* species was undertaken by Verster (1965), comparing them to the type species from Germany. This was prompted by an unusually high hydatid cyst fertility in cattle and varied susceptibility of wild carnivores and the domestic cat to infestation with the adult worm. On morphological basis the invalidated species; *E. lycaontis*, *E. felidis* and *E. ortleppi* (Rausch and Nelson, 1963), were redesignated; *Echinococcus granulosus lycaontis*, (Ortlepp, 1934) *Echinococcus granulosus felidis* (Ortlepp, 1937) and *Echinococcus granulosus ortleppi* (Lopez-Neyra and Soler Planas, 1943) respectively (Verster, 1965). A "strain" of *E. granulosus* from South Africa which occurs between the domestic livestock and domestic dogs and jackals was also found to differ from the type specimen and to differentiate the two, the later was redesignated *Echinococcus granulosus granulosus* and the former *Echinococcus granulosus africanus* (Verster, 1965).

This was not the end of the matter, for in 1967a, Rausch re-examined the South African subspecies described by Verster (1965), and invalidated them on the grounds that there was no

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evidence of ecological isolation or host specificity.
Accumulating reports of wildlife infections however, seem to
suggest the presence of an *Echinococcus* wildlife cycles in
various parts of Africa, ecologically separate from the
domestic cycle. *Echinococcus* infected lions and their prey,
for example, warthog in Central Africa (Graber and Thal,
1980) and East Africa (Dinnik and Sachs, 1972; Eugster, 1978)
and zebra in South Africa (Young, 1975) harbouring hydatid
cysts, have been found in areas where domestic dogs and
livestock are excluded. Macpherson (1986) has therefore
rightly argued that the invalidation of the speciation *E. g.
felidis* by Rausch (1967a) may no longer apply.

Rausch (1967a) insistence on the application of ecological
segregation as a criterion in the designation of subspecies,
was based on concepts derived from Mayr's (1963) definition
of species as interbreeding populations of individuals. This
definition does not seem to take into consideration organisms
that are potentially self fertilizing and has been regarded
inappropriate to cestodes (Jones *et al.*, 1963). On the same
basis, Beveridge (1974) found unacceptable the application of
concepts drawn from this definition in the subspeciation of
*Echinococcus*. Smyth and Smyth (1964) had already pointed out
the complexity involved in attempting to systematically
categorize the genus *Echinococcus* into well defined species
or subspecies in view of its mode of reproduction which
favours expression of mutants which in turn might lead to
establishment of variants. They argued that the
hermaphroditism of the adult parasite, coupled with the
polyembryony of the larval stage gives *Echinococcus* the

potential to rapidly speciate without any need for geographical or ecological isolation.

Currently it is generally accepted that intraspecific variants exist within the genus *Echinococcus* (WHO, 1982; Kumaratilake and Thompson, 1982; Thompson and Lymbery, 1988). However, only four species; *E. granulosus*, *E. multilocularis*, *E. oligarthrus*, and *E. vogeli* are taxonomically accepted as valid and to avoid further taxonomic confusion the intraspecific variants are now designated as strains until their biological status is well established (Rausch and Bernstein, 1972; WHO, 1982; Kumaratilake and Thompson, 1982; Thompson, 1986).

Of the four taxonomically valid species, *E. granulosus* is the only species in the genus *Echinococcus* reported from Africa. Two separate reports of *E. multilocularis* in cattle from Mozambique (Ferreira, 1980) and in two Tunisian patients (Zitouna *et al.*, 1985) have been published but both appear to be mistaken forms of multicystic infections or multilocular cysts caused by *E. granulosus*. The metacestode of *E. multilocularis* is relatively host specific and infections are confined to rodents (Rausch, 1967b; Fay, 1973; WHO, 1982). Ferreira (1980) used the rate of growth of the parasite in dogs as an identification criterion but completely disregarded the various morphological features of the adult worm.

## 1.4 CONTROL OF HYDATID DISEASE

An accurate and detailed description of the life cycle of *Echinococcus veterinorum* (*E. granulosus*) was first given by Siebold (1853). The description made it theoretically apparent that control of unilocular hydatidosis was a simple matter involving interruption of transmission by preventing dogs from eating raw viscera from infected intermediate hosts. Ten years later, the first hydatid control programme began in Iceland where one in every six persons were estimated to harbour a hydatid cyst (Schantz and Schwabe, 1969). Other hydatid control programmes have since been started in Argentina, Bulgaria, Cyprus, the Falkland Islands, Greece, Peru, Spain, Sardinia, Yugoslavia, Uruguay, USSR, Tasmania, New Zealand (Gemmell, 1979; Gemmell and Lawson, 1986), and recently Kenya (Macpherson *et al.*, 1984a). Control measures against the disease are also being instituted in Tunisia (Zarrouk, 1986).

Although eradication of hydatid disease is theoretically simple, it took a century for the disease to be eradicated from Iceland, and it is now clearly understood that socio-cultural, economic and technical factors make the implementation of control measures difficult. The success of the hydatid eradication campaign in Iceland is mainly attributed to the then nearly 100% literacy rate (Gemmell, 1978). A pamphlet on the nature, cause and prevention of the disease, which was published and distributed to every family in Iceland, was widely read, especially during the long

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winter months (Schantz and Schwabe, 1969).

It is now appreciated that control of hydatid disease is a problem of people rather than merely one of dogs and domestic animals. Epidemiological research over the past thirty years has enhanced knowledge of the dynamics of transmission and the methods required for its control, which have formed the subject of a number of reviews (Gemmell, 1978; 1979; Lawson and Gemmell, 1983; Gemmell and Lawson, 1986). Two main hydatid control measures are now recognized, which include the prevention of dogs gaining access to raw offal and reduction of the parasite biomass, either by reducing the dog population or by mass dog treatment (Gemmell, 1979). However, emphasis on each control measure and methods adopted in the implementation by hydatid control authorities in different countries have tended to differ with the varying facilities, financial constraints, literacy level and other conditions that exist in each country (Gemmell, 1979). Both New Zealand and Tasmania hydatid control programmes gave special emphasis to community involvement in the prevention of dogs gaining access to raw offal and encouraged the building of effective offal disposal systems (Schantz and Schwabe, 1969). In Uruguay and Cyprus on the other hand, it was found necessary to reduce the dog population to achieve control of the disease (Polydorou, 1980; Gemmell and Lawson, 1986). Similar success was achieved in Chile and the Falkland Islands, but using six weekly mass dog treatment with the newly introduced, highly effective drug, praziquantel (Gemmell and Lawson, 1986).

control measures, including surveillance techniques adopted
by different hydatid control authorities in different
countries, have extensively been reviewed by Gemmell (1979)
and Gemmell and Lawson (1986). The available hydatid disease
surveillance methods are reviewed later in this chapter
(section, 2.5.8).


## 2.5 EPIDEMIOLOGY OF *E. GRANULOSUS* IN KENYA

Hydatidosis is a cyclozoonosis of major economic and public
health importance in Kenya. The disease is endemic in the
Turkana district and Maasailand where it affects both man and
domestic animals. Infected wild animals have also been
reported particularly in Maasailand where a wildlife cycle is
suspected to operate (Macpherson *et al.*, 1983)


### 2.5.1 Hydatidosis in domestic livestock

The occurrence of hydatidosis in Kenya was reported first in
camels (Leese, 1915) and later in sheep (Walker, 1925),
cattle (Daubney, 1926) and pigs (Hudson, 1934), but
statistical data on the disease was not available until
twenty years later following the improvement of meat
inspection standards in the country. Three years after the
construction of a meat export abattoir (Kenya Meat
Commission) at Athi River in 1953, Ginsberg (1956) reported a
high prevalence of hydatidosis in cattle (17-46%), sheep (17-

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13%) and goats (15-18%). This was confirmed later by other workers (Appendix 2.1) (Froyd, 1960a,b; Mango, 1971; Ng'ang'a, 1974, Eugster, 1978; Macpherson, 1981; Cheruiyot, 1983).

Until 1981, the prevalence of hydatid disease in domestic livestock was generally accepted to be high and widespread in Kenya. However, a study conducted in Turkana district reported low prevalence of the disease in cattle (10%) and more so in sheep (1.6%) and goats (0.8%) (Macpherson, 1981). It is now established that Maasailand and to a lesser extent Turkana district are the two regions in Kenya where livestock hydatidosis is endemic (Eugster, 1978; Macpherson, 1981; Macpherson, 1985)

In Kenya, cattle have been shown to be a poor host of *E. granulosus* as most of the cysts are found to be either sterile (70%) or calcified (20%) (Eugster, 1978, Macpherson, 1985). Larvae from the fertile cysts are usually in poor conditions and seem to be of low infectivity to dogs (Macpherson *et al.*, 1985). The low fertility rate (<15%) in cattle hydatid cysts, is a phenomenon which has been observed in other countries including; Egypt (El-Kordy, 1946), Italy (Biocca and Massi, 1952), Argentina (Mendy, 1975), Great Britain (Thompson, 1977), Nigeria (Dada, 1980a), Iraq (Mahmoud and Al-Janabi, 1981), Chile (Gonzalez *et al.*, 1981), Bangladesh (Islam, 1982) and Australia (Durie and Riek, 1952; Gemmell, 1960). However, in Belgium (De Rycke, 1968), South Africa (Verster, 1962), Sri Lanka (Dissanaike, 1957), Central Sudan (El-Badawi *et al*, 1979) and Switzerland (Thompson *et*

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al., 1984), fertility rates ranging from 66%-97% have been reported in cattle hydatid cysts. Available evidence seems to suggest the presence of a different strain of *E. granulosus* in these countries where cattle play a principle role as an intermediate host (Verster, 1965; Thompson *et al.*, 1984).

Camels in Kenya are found in the dry northern region of the country, but camel hydatidosis has only been reported from the Turkana where a high prevalence rate of 80% was recorded (Macpherson, 1981). The other major group of people in Kenya who keep camels, are the Somalis who also live across the border in the Republic of Somalia where camel hydatidosis is prevalent (Hassounah and Behbehani, 1976). Camels in Turkana are mostly kept for milk and blood and are slaughtered at a more advanced age than other slaughter stock. This has been suggested to be the contributing factor leading to the high prevalence rate of the disease in these animals (Hamdy, *et al.*, 1980) as is also observed in Egypt, Morocco and Tunisia (Table, 2.1). Camels therefore appear to play a major role in the perpetuation of *E. granulosus* cycle not only in Turkana but also in the North African countries.

In most parts of the world, sheep has been found to be the main intermediate host for *E. granulosus* (Gemmell, 1960; Schantz and Schwabe, 1969). Hydatid cysts from these animals are usually in good condition with high fertility rates. In Kenya, protoscoleces from sheep and goat cysts have been shown to readily infect experimental dogs (Macpherson *et al.*,

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1985). Due to their size and high turnover rate, sheep and goat form the main part of home slaughter among the Maasai and Turkana people thus providing dogs with greater opportunity of gaining access to infected offal. Therefore, despite the low prevalence of the disease in the smaller stock in Turkana, they appear to be the main intermediate hosts of the domestic cycle for the parasite in Kenya.


.5.2  Echinococcosis in domestic dog (*Canis familiaris*)

The existence of the hydatid parasite has been recognized in Kenya since 1915 when it was for the first time reported from camel (Leese, 1915).  However, the definitive host remained unclear for more than forty years following reports of hydatidosis in intermediate hosts.  Wild carnivores were thought to play a major role in the transmission of the disease (Wray, 1958; Round, 1962) in this country and the opinion was strengthened by a finding of an *E. granulosus* infected jackal (Ginsberg, 1958).  Nelson and Rausch (1963) however, found heavily *E. granulosus* infected dogs around Nairobi and Turkana district, while very few of the wild carnivores examined were infected, and only lightly.  They therefore concluded that in Kenya there was no evidence of an *Echinococcus* wildlife cycle.  Since then three more surveys in dogs have been conducted, but only in localized regions. The results of these surveys are presented in Appendix 2.1.

Out of 165 dogs examined by Eugster (1978) in Kajiado district, 45 (27.3%) were found infected with *E. granulosus* and only 17.6% of them had more than 20 specimens (Eugster,

1978). Macpherson *et al.* (1985) found little change in infection rates in Turkana dogs twenty years after Nelson and Rausch (1963), reporting a prevalence of 39.4% among 695 of the dogs examined, out of which 31.6% had more than 200 specimens. It therefore appears that dogs in Turkana District are more infected in terms of both prevalence and intensity than those from Maasailand. This is puzzling considering that relatively higher hydatid infection levels are found in the domestic intermediate hosts in Maasailand. It is possible however, in areas that experience prolonged droughts, for dogs to acquire high infections from scavenging on the many carcasses that fall victim to the drought (Ngunzi, 1985). The two studies in Turkana (Nelson and Rausch, 1963; Macpherson *et al.*, 1985) were both carried out soon after prolonged drought periods.

A countrywide study on the prevalence of *E. granulosus* in dogs in Kenya is lacking and although few human infection cases are reported from regions other than Turkana and Maasiland, livestock hydatidosis seem to have a generalized distribution with regional variations in prevalence (Macpherson, 1981).

2.5.3 Human hydatidosis

The worldwide awareness of hydatidosis in Kenya is attributed largely to the high prevalence of human hydatidosis, particularly in northwestern part of the country. This was first brought to light by Wray in 1958. More than half of

the 117 hydatid operations he carried out between 1952 and 1955 were on Turkana patients. Schwabe (1969), who visited Kitale district hospital where most of the hydatid patients from Turkana were being referred, estimated the annual hydatid incidence in Turkana as 40 per 100,000 people. O'Leary (1976), the then Medical Officer of Health at Lodwar district hospital, in a five-year review of the hospital records, showed that the incidence was more than 96 per 100,000 per year in Turkana district, the highest known incidence of human hydatidosis. From her work, it was apparent that most of the cases were from the northwestern part of the district. This work was confirmed by mass serological survey (French and Ingera, 1984) and recently by ultrasound scan survey which showed the prevalence of infection in Turkana district as varying from 0.3% along the shores of Lake Turkana to 5.6% in the northwest of the district (Macpherson, personal communication)

In contrast, human hydatidosis is very low in the rest of the country and the only other notable hydatid problem region but to a lesser extent is Maasailand (Fig. 2.1). Eugster (1978) reported that from 1962 to 1976 only 76 hydatid patients from Maasailand were operated in various hospitals in Kenya and Tanzania. Reports of hydatid infections from other tribes in Kenya have included the Suk (Wray, 1958) the Pokot (Clifford, 1968; O'Leary, 1976) the Kikuyu (Okello and Kyobe, 1981) and the Boran (Macpherson, personal communication).
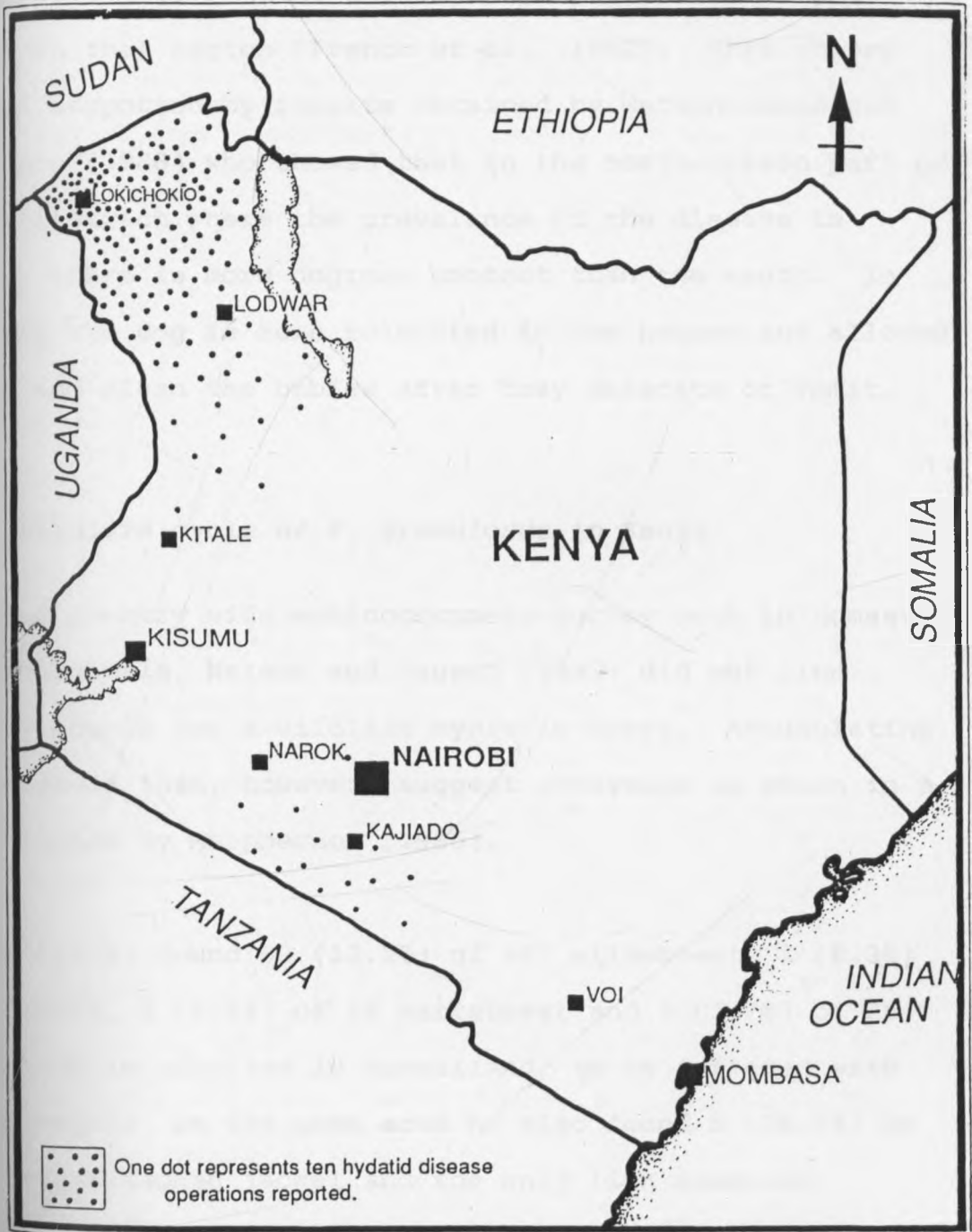
Distribution of human hydatid reported cases operated on
in  Kenya (1957 - 1988)

It has been postulated that human habits and practices among the Turkana people which allow for the close association with the domestic dog, account for the high prevalence of the disease in that region (French *et al.*, 1982). This theory has been supported by results obtained by Watson-Jones and Macpherson (1988) who showed that in the northwestern part of Turkana district where the prevalence of the disease is highest, there is more dog:man contact than the south. In the north the dog is more tolerated in the houses and allowed to lick and clean the babies after they defecate or vomit.

1.5.4  Wildlife cycle of *E. granulosus* in Kenya

Following country wide echinococcosis survey both in domestic and wild animals, Nelson and Rausch (1963) did not find enough evidence for a wildlife cycle in Kenya. Accumulating reports since then, however, suggest otherwise as shown in a recent review by Macpherson (1986).

Eugster (1978) found 69 (12.2%) of 567 wildebeest, 2 (8.3%) of 24 impala, 2 (5.3%) of 38 hartebeest and 2 (7.7%) of 26 Grants gazelle examined in Maasailand, to be infected with hydatid cysts. In the same area he also found 5 (38.5%) of the 13 silverbacked jackal and the only lion examined, infected with the parasite. Another lion examined in the same locality four years later was also found similarly infected (Muchemi, 1982). It therefore appears that a wildlife cycle perpetuated between the lion and wild herbivores exists in Maasailand. The situation is different in Turkana where out of 152 wild herbivores examined, none

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been found infected with hydatid cysts. Although silver-
backed jackal (11/38) and golden-jackal (6/22) have been
found infected with *E. granulosus* in Turkana, it has been
argued that they have no more than a supportive role in the
maintenance of the domestic hydatid cycle (Macpherson *et al.*,
1983).

## 1.5.5 Diagnosis and treatment in the definitive host

Diagnosis of *E. granulosus* in definitive hosts is important
in epidemiological studies or hydatid control programme
surveys. There are two methods currently available for use
in the diagnosis of echinococcosis infections in the
definitive host; autopsy and arecoline purgation. Both
methods rely on the demonstration of the tapeworm from the
infected hosts and are currently used in the Turkana pilot
hydatid control programme (Macpherson *et al.*, 1986b).

Arecoline which is the main alkaloid of areca nuts (*Areca
catechu*) was for a long time used for the elimination of dog
tapeworms (Lentz, 1921). When given to dogs, the drug causes
purgation by increasing gut motility. It also causes the
worms to relax releasing their hold on the intestinal wall
and are thus carried out of the gut and can be demonstrable
in the resultant purge. Dogs vary in their reaction to
arecoline, and it has been shown that the absence of worms in
a purge is not necessarily an indication of an animal being
free of *E. granulosus* infection (WHO, 1982). In addition, it
has been shown that under field conditions 20% of dogs fail

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o purge (Gemmell, 1958, 1968). The use of arecoline
hydrobromide for the diagnosis of echinococcosis in dogs is
also limited by side effects. These include, vomiting,
stimulation of mucus secretions and dehydration which may be
lethal in hot weather (Gregory and McConnell, 1978). The
stress caused by struggling in the process of dosing,
especially in animals not used to handling, may magnify the
side effects leading to the death of the animal.
Nevertheless, with standardization, the arecoline purge has
been found to be useful in determining the prevalence of *E.*
*granulosus* in dogs (Gemmell, 1968, 1973; Schantz, 1973) and
has been used in the Argentina, Cyprus, New Zealand, Tasmania
and Uruguay hydatid control programmes as one of the methods
of surveillance (Gemmell, 1979).

Necropsy is the most reliable method of diagnosing *E.*
*granulosus* infections but the method is limited to stray and
unwanted dogs. In Kenya, it has been used in four *E.*
*granulosus* surveys (Nelson and Rausch, 1963; Eugster, 1968;
Macpherson *et al.*, 1983; Ngunzi, 1985). Other available
methods include, anal swab and use of transparent tape (Craig
*et al.*, 1988) and serology (Jenkins and Rickard, 1985). The
former two methods rely on the demonstration of taeniid eggs
on the body of infected dogs. Although it has been known for
a long time that taeniid eggs stick to dog hair (Nosik, 1952;
Mattoff and Kolev, 1964), the demonstration of this has been
of little diagnostic value as no applicable taeniid egg
differentiation test was available. The recent development
of such a test (Craig *et al.*, 1986a) has now made way for the
utilization of these methods as has recently been

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demonstrated in Turkana (Craig *et al.*, 1988). The test is obviously limited to dogs with gravid infections of the parasite.

Serodiagnosis of *E. granulosus* infection in dogs is a field that has not been developed and yet has great diagnostic potential. Babos and Nemeth (1962) were able to detect *E. granulosus* in faeces of infected dogs prior to worm patency using precipitation reactions with hyperimmune rabbit serum. Later, Jenkins and Rickard (1985) using ELISA (enzyme linked immunosorbent assay), found antibodies to protoscolex antigens in sera of dogs 32 days after infection with 100,000 *E. granulosus* protoscoleces. This test is currently being tested in Kenya to evaluate its usefulness in the diagnosis of *E. granulosus* under field conditions. A specific serological test in dogs would be particularly valuable as it would reduce the risks to infection when handling infected dogs as compared to arecoline purgation. Autopsy and use of the arecoline for the diagnosis of echinococcosis infections, have an advantage over the other methods in that they are simple, requiring little or no laboratory back-up and have an educational value as the infecting worms can be demonstrated to onlooking owners.

The field of dog tapeworm chemotherapy has seen an array of drugs of varying cestocidal activity, over the last twenty-five years (reviews by Gemmell and Johnstone 1981; WHO, 1982). Praziquantel is the only compound that has been found to be 100% effective at a dose of 10mg/kg body weight (Thakur

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et al., 1978). Praziquantel is not ovicidal but it is effective against both immature and mature stages of *E. granulosus* when administered by the intramuscular or·oral routes (Andersen, *et al.*, 1978; 1979). The drug is safe to use with a wide safety margin and there are no known side effects (Murmann *et al.*, 1976). In Kenya, treatment against echinococcosis infections in dogs is not routine except in the Turkana pilot hydatid control area, where mass treatment of dogs with Droncit[R] is undertaken (Macpherson, *et al.*, 1986b)

2.5.6 Diagnosis and treatment in intermediate hosts

Hydatid disease in livestock is only diagnosed at routine meat inspection. Serological diagnosis has been hampered by false positive reactions associated with parasitism by related cestodes (Yong *et al.*, 1978; Schantz, 1973; Lightowlers *et al.*, 1984) and to a lesser extent by false negative reactions due to poor antibody responses (Lightowlers *et al.*, 1984) or variation in antibody responses to different *E. granulosus* "strains" (Rickard and Lightowlers, 1986). Investigations carried out in Kenya for the possible uses of serodiagnosis in livestock hydatidosis, have also met with similar setbacks (Kagiko *et al.*, 1986; Gathura *et al.*, 1987).

A specific diagnostic test for hydatid disease in livestock would be of great value to hydatid control programmes. Infected animals identified on antemortem examination could then be slaughtered under strict supervision. In areas where

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organized slaughter is absent, the test could be used to selectively remove hydatid infected animals.

## 2.5.7 Diagnosis and treatment in humans

Diagnosis of unilocular hydatid disease in man is usually based on case history and clinical symptoms which are non-specific, associated with pressure effects of the growing cyst. Diagnostic aids available to the clinician include; radiology, scintigraphy, computerized axial tomography, ultrasonography and a number of immunodiagnostic techniques.

For a long time, medical diagnostic facilities in Turkana district were scarce and hydatid diagnosis relied heavily on clinical diagnosis alone. As the region has such a high prevalence of the disease, it is possible that every space occupying lesion diagnosed was a hydatid cyst unless proved otherwise. But it may be noted here that, Turkana people do recognize the disease, especially when the pressure symptoms are fully manifest and it is at this stage that self-diagnosed patients seek help at the medical centres.

The installation of an x-ray at Lodwar hospital in Turkana improved the diagnosis of the disease, especially of extra-abdominal cysts. During the preparation of a control programme in Turkana it became obvious that there was need for a more specific diagnostic aid that could be used for large scale epidemiological surveys. Different serological tests investigated using sera from surgically proven Turkana

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hydatid patients have resulted in false seronegativity rates of between 44-55% (Chemtai *et al.*, 1981a,b; Okelo and Chemtai, 1981; Okelo and Kyobe, 1981; French and Ingera, 1984; Craig *et al.*, 1986b). This is puzzling as observations in other endemic hydatid regions including; Europe (Bradstreet, 1969), the Middle East (Matossian and Araj, 1975), South America (Varela-Diaz *et al.*, 1975) and Australia (Rickard, 1984) have resulted in fairly accurate correlation between seropositive rate using indirect haemagglutination test (IHA) and complement fixation test (CFT) infections. A thermo-stable hydatid antigen has recently shown promising results, with specificity and sensitivity of more than 80%, in the diagnosis of hydatid patients in Kenya (Njeru, personal communication). This will be of great advantage in the treatment of human hydatidosis in Kenya.

The use of ultrasonography in the diagnosis of hydatid cysts in Turkana was introduced in 1984 and it soon proved much superior to serological diagnosis (Macpherson *et al.*, 1987) especially for repeated annual mass surveys. Annual mass ultrasound scanning surveys are now used in preference to seroepidemiological surveys in the Turkana hydatid control programme. The advantages of the scanner over clinical and serological diagnosis for such surveys are numerous including, being non-invasive, rapid, less expensive, provides instant results and is more acceptable and educationally valuable to the people (Macpherson *et al.*, 1987). Despite its limited ability for use in diagnosing extra-abdominal cysts, the prevalence data obtained with this technique has been found useful in the surveillance of the

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disease in the control programme as it also includes the 0-5 age, as its a non-invasive method (Macpherson et al., 1987).

Surgery has for a long time been the method of choice for the treatment of patients with hydatid disease in Kenya. However, postoperative recurrence rates of 20% and 22% have been reported from Turkana (O'Leary et al., 1979; Macpherson et al., 1987). Prognosis following surgery is usually poor, especially in multiple cyst cases or where cysts are difficult to extract whole. The need for chemotherapy is therefore great in this area and drug trials have concentrated on mainly praziquantel and the benzimidazole derivatives including, mebendazole, albendazole and flubendazole. This group of drugs have shown significant efficacy against unilocular hydatidosis (Eckert, 1986). From 1976 to 1981, mebendazole was used for the treatment of human hydatidosis in Kenya but gave poor results (Okello and Chemtai, 1981; French, 1984). Additionally there were reports of severe glomerulonephritis in some of the patients treated (French, 1981; Kung'u, 1982). Albendazole is currently being tried in Kenya and is proving to be a promising drug in this field (Okello, 1986). Favourable responses with this drug have also been reported from other parts of the world which has so far demonstrated no serious side effects (Eckert, 1986).

In vivo experiments with praziquantel have yielded promising results in the treatment of hydatid cysts in mice (Richards et al., 1988). This results are supported by in vitro

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studies with protoscoleces of *E. granulosus* as has been shown
in other countries (Morris *et al.*, 1986) and recently, in
Kenya (Lubano, personal communications).

## 1.5.8 Control of hydatid disease in Kenya

Hydatid disease in Kenya is found mainly among nomadic
communities who have a different life style to that
prevailing in other countries, where control programmes have
successfully been initiated among settled communities.

Following a number of reports on the high incidence of human
hydatid infections among the Turkana people of Kenya (Wray,
1958; Schwabe, 1969; Rottcher, 1973; Irvin, 1974; O'Leary,
1976), a Hydatid Research Unit was set up in Turkana.  The
unit was to find out more about the disease in Turkana, and
eventually institute a control programme.  The unit was
established by the African Medical and Research Foundation
(AMREF) in 1976 (AMREF, 1977).  Between 1977 and 1982,
considerable amount of information was accumulated and formed
the subject of many publications on the human distribution of
the disease (French *et al.*, 1982) and hypothesis to account
for the remarkably high prevalence of the disease in man
(French and Nelson, 1982), and the role of man (Macpherson,
1983), wildlife (Macpherson, *et al.*, 1983), dogs (Macpherson
*et al.*, 1985) and livestock (Macpherson, 1981) in maintaining
the life cycle of the parasite in Turkana.  Additionally the
biology of the parasite (Macpherson, 1981; McManus, 1981;
Macpherson and McManus, 1982; Macpherson and Smyth, 1985) and
the possible mode of its transmission in Turkana (Stevenson

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and Macpherson, 1982) were also studied.   With this information, options for control strategies were critically evaluated (Macpherson *et al.*, 1984b) and in 1983 a hydatid control programme was started by AMREF in conjunction with the Kenya Ministry of Health in the northwest of Turkana (Macpherson *et al.*, 1984a).   This was the first attempt of controlling hydatid disease in a nomadic community.   The control measures adopted in this programme were described earlier in the first chapter.

Chapter 3

A STUDY OF STRAIN VARIATION WITHIN *E. GRANULOSUS* IN KENYA

## 3.1 INTRODUCTION

It has become increasingly evident in recent years that the knowledge of the existence and extent of strain variation within *E. granulosus* is important for the effective control of hydatid disease (Schwabe, 1968; Thompson, 1979; WHO, 1982; Thompson, 1986). This follows the recognition that different strains of *Echinococcus* may exhibit differences in their infectivity to man (Nelson, 1972; Thompson and Smyth, 1976; McManus, Smyth and Macpherson, 1985), rate of maturation (Kumaratilake *et al.*, 1983; Thompson *et al.*, 1984) or response to particular drug treatments (Saimot *et al.*, 1981; Schantz *et al.*, 1982; Kummerer and Schantz, 1984).

Studies on characterization of *E. granulosus* parasite in Kenya were started in 1978 and have since formed the subject of a number of publications (McManus, 1981; Macpherson; 1981, Macpherson and McManus, 1982; McManus and Macpherson, 1984; Macpherson and Smyth, 1985;

Macpherson *et al.*, 1986a). However, the results of these studies have not been fully conclusive and further studies have been suggested.

### .1.1 Morphological approach

Morphology has been a major criterion used in the taxonomy and speciation of *Echinococcus*, possibly due to its low cost, and the fact that it does not require sophisticated equipment. It also has the advantage in that it does not depend on the availability of fresh material. Different authors have pointed out the variability of most of the morphological characteristics of the parasite depending on the age, relaxation and fixation procedure (Verster, 1965; Thompson, 1977), the definitive host species (Rausch, 1953; Vogel, 1957; Lubinsky, 1960a,b, 1962; Sweatman and Williams, 1963a; Verster, 1965; Dailey and Sweatman, 1965; Smyth and Smyth, 1968; Schantz *et al.*, 1975; 1976), and its nutritional, hormonal, health and immunological status (Gill and Rao, 1967; Rausch 1967a). In addition, hook morphology, especially the number (Lubinsky, 1960b; Sweatman and Williams, 1963a; Verster, 1965) and shape of the rostellar hooks (Sweatman and Williams, 1963a), has occasionally been found to vary in parasites from different intermediate hosts.

To minimize the host influence on the morphology of the parasite, Rausch (1967a) advocated the use of littermate experimental definitive hosts to obtain material for comparative studies. It has also been stressed that the material used should be of the same infection age and processed in a standardized manner (Verster, 1965; Thompson, 1977; Kumaratilake, 1982). Kumaratilake (1982) has further emphasized the importance of using a combination of

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morphological characteristics as a measure of eliminating individual variation.

E. granulosus in Kenya was first identified by Nelson and Rausch (1963) on a morphological basis only. The morphological features studied included; strobilar length, number of segments, position of the genital pore, number and distribution of testes, shape of the gravid uterus and the size of the rostellar hooks. Later, Macpherson (1981) further examined the morphology of experimentally and naturally obtained E. granulosus worms from dogs and jackals, and the morphology of larval hooks from different intermediate host species, in an attempt to elucidate the speciation pattern of the parasite in Kenya. He concluded that E. granulosus in Kenya was morphologically homogeneous.

In the first part of the present chapter a more detailed study of the morphological features of the adult E. granulosus is undertaken in order to more comprehensively characterize the parasite in Kenya. In addition, the Kenyan parasite is morphologically compared to E. granulosus worms of the same infection age from Australia, Britain, Canada, South Africa and Switzerland. The results are discussed in the light of the rest of the chapter which includes the developmental and DNA studies of the Kenyan parasite.

## .1.2 Developmental approach

Vogel (1957) demonstrated differences in the developmental rates of E. multilocularis and E. granulosus in dogs. Since

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hen a number of developmental studies, both *in vitro* and *in vivo*, have been carried out and utilized in taxonomy of *chinococcus* at the species and subspecies levels (Webster and Cameron, 1961; Williams and Sweatman, 1963; Thompson, 976; 1977; Smyth, 1977; Smyth and Davies, 1979; Macpherson, 981; Kumaratilake, 1982; Thompson *et al.*, 1984).

*In vitro* developmental studies of *E. granulosus* of cattle, human, sheep, goat and camel origins in Kenya showed no significant differences in growth and segmentation (Macpherson and Smyth, 1985). However, viable protoscoleces of cattle origin have been shown to be of very poor infectivity to dogs when contrasted to those of human, sheep, goat or camel origin in Kenya (Macpherson *et al.*, 1985), although this was not reflected in the experimental infections in baboons where the parasite material of cattle origin was shown to be infective (Macpherson *et al.*, 1986a).

The second part of the present chapter compares the development of *E. granulosus* at day 14, 17, 21, 26, 35, 42 and up to the shedding of segments from dogs infected with protoscoleces of different intermediate host origin including camel, cattle, human, sheep and goat material. This forms part of the study examining the *E. granulosus* strain pattern in Kenya but at the same time seeks to determine the prepatent period of the parasite.

## 1.3 Biochemical approach

In the last ten years speciation studies of *Echinococcus* have

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en a shift in emphasis from morphological and developmental
riteria in favour of a biochemical approach. This criterion
dependent on the least adaptive characters of the
rasite. Most of the work done in this area has been based
the differentiation of genomically coded and carbohydrate
tabolic products (Le Riche and Sewell, 1978; McManus and
yth, 1978; 1979; Kumaratilake et al., 1979; Macpherson and
Manus, 1982; McManus and Smyth, 1982; Kumaratilake and
ompson, 1984).

a biochemical analysis of the composition and the
tabolic products of larvae and adult *E. granulosus* from
nya, McManus (1981) found some similarities between the
rasite material of sheep and human origin, but distinct
fferences between the parasite material of cattle, camel
d goat origin both from each other and from that of sheep
d human origin. Further examination of the Kenyan parasite
sing isoelectric focusing and isoenzyme analysis (Macpherson
d McManus, 1982) confirmed the similarities between the
eep and human forms of the parasite which were found to be
fferent from the parasite of camel origin. Inconsistent
esults were obtained with parasite material of cattle origin
ich closely resembled the sheep and human forms of the
rasite. Enzyme analysis of *E. granulosus* of goat origin
evealed two types of patterns, one similar to the camel form
f the parasite while the other resembled the sheep and human
rm of the parasite. Although these results revealed the
omplex nature of *E. granulosus* strains in Kenya, no
uivocal conclusions could be drawn.

another biochemical method, DNA sequence analysis, has been used with much success in other countries to differentiate closely related organisms, including *Echinococcus* strains (Lansman *et al.*, 1981; Curran *et al.*, 1985; McManus *et al.*, 1985). This method offers a more sensitive strain characterization technique than has been used before in Kenya, as it studies the genomic "library" rather than the expressed products.

In a third and final part of the present chapter, the study aims at characterizing the hydatid parasite strains in Kenya, using specific sequences among the parasite DNA fragments separated by gel electrophoresis.

## 2.2 MATERIALS AND METHODS

### 2.2.1 Source of parasite material

Protoscoleces were obtained from hydatid cysts removed from naturally infected sheep, goats, and cattle slaughtered at KMC (Athi River), Ongata Rongai and Dagoretti abattoirs near Nairobi. Cysts were also obtained from camels, goats and cattle slaughtered at Lodwar abattoir in Turkana. Two hydatid cysts were obtained at surgery on a Maasai patient.

The viability of the protoscoleces was assessed using the eosin exclusion dye test (Smyth and Barrett, 1980). Only material having a viability of more than 75% were used.

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toscoleces from the same intermediate host species were
oled.   Storage of the parasite material was at 4°C but
ability was reassessed before use.   Protoscoleces from
dividual hosts, intended for DNA studies, were rinsed three
mes in sterile phosphate buffered saline and preserved
parately in 70% ethanol.


## 2.2  Experimental infections in dogs

xteen litters of two to eight, totaling to 61 puppies of
out two months old, were purchased from Kawangware in
irobi and around Lodwar in Turkana.   The Nairobi puppies
mprised of a mixture of breeds but the puppies from Lodwar
re composed solely of the Turkana breed of dog.   The
ppies were dosed with Canex[R] (Pyrantel pamoate, Pfizer,
K.) and Droncit[R] (Praziquantel, Bayer, West Germany) to
iminate any naturally acquired helminth infections.   The
gs were reared in kennels in Nairobi or Lokichokio and
intained on a ration of cooked meat and dog meal (Unga
d., Nairobi) supplemented with milk and water *ad libitum*.

ch dog was starved for 18 hours prior to being fed a 0.2ml
iquot of pooled protoscoleces sediment (approx. 160,000
rvae) packed in a gelatin capsule.   Tables 3.1 and 3.2 give
ummary of the number of dogs infected, including the
ration of infection.

## 2.3 Collection of worms

The puppies were sacrificed at various days post infection (Table 3.2) by an intravenous injection of Euthatal after being starved for eighteen hours. The small intestines were removed and opened longitudinally and then incubated separately for one hour in warm saline (37°C). The detached E. granulosus worms were then relaxed for two hours in water and the level of infection estimated as either light (1-200), medium (201-1000) or heavy (>1000).

## 2.4 Preparation of worms for morphological examination

Whole specimens of the experimentally obtained worms were stained with lactic acid carmine or semichon's acetic carmine and then dehydrated in increasing concentrations of ethanol. The worms were then cleared in xylene and mounted in DPX. Rostellar hooks from the 35dpi (days post infection) worms were prepared for study by detaching scolices from strobilae, clearing in Berlese and mounting under gentle pressure to make the hooks lie flat (Fig. 3.1). The rostellar hook measurements were as shown in Figure 3.2.

Stained and mounted 35dpi E. granulosus worms from various parts of the world were kindly made available for study by Professor R.C.A. Thompson of Murdoch University, Western Australia. The worms studied were of sheep/dog and macropod/dingo (Australia), horse/dog (Britain), moose/dog (Canada), cattle/dog (Switzerland) and cattle and human/dog (South Africa).

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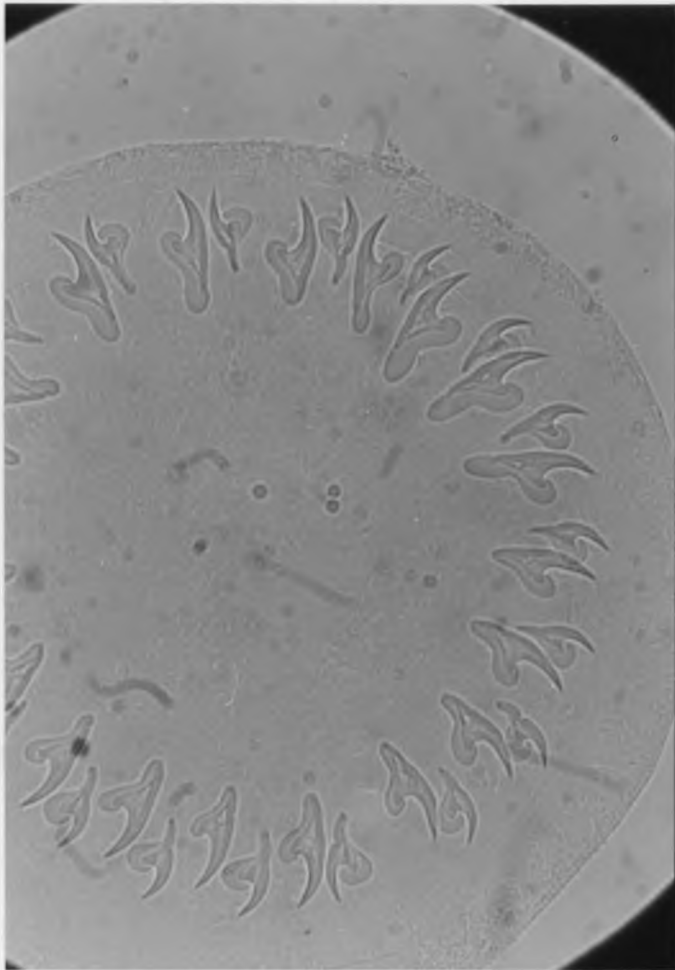
*E. granulosus* rostellar hooks orientated for measurement (note the alternating pattern of large and small hooks)
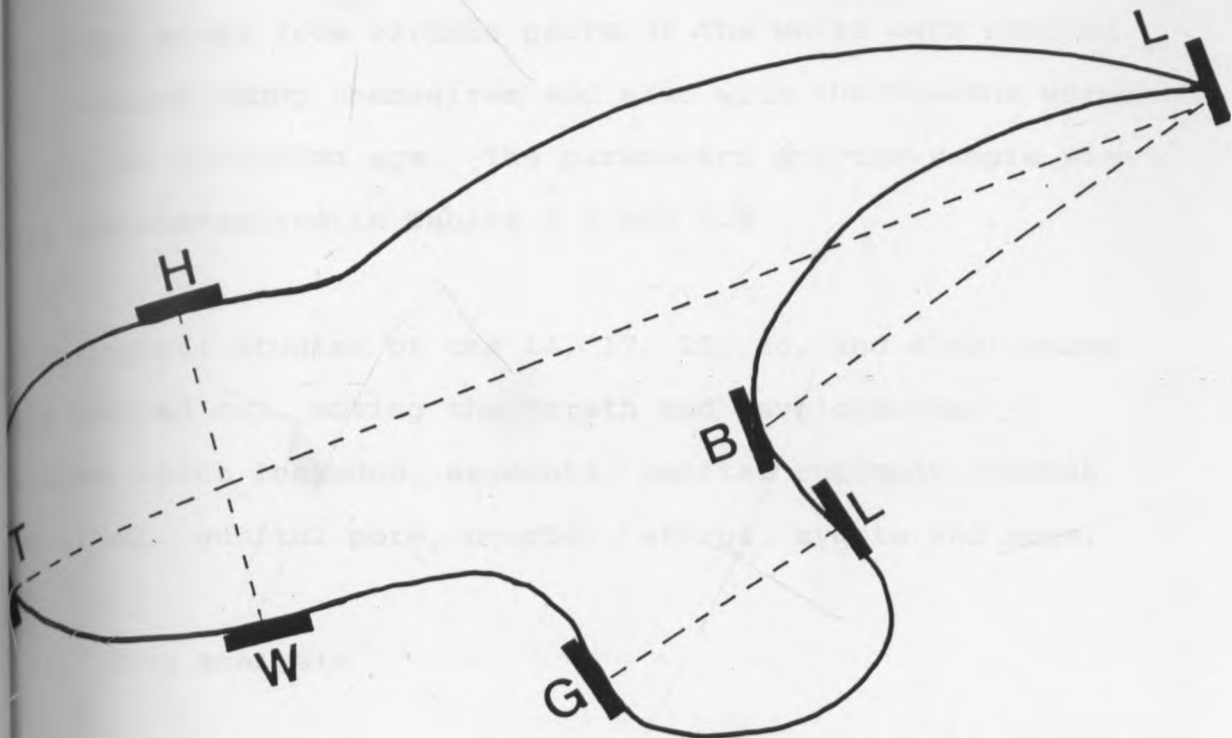
TL - Total length;   BL - Blade length;
HW - Handle width;   GL - Guard length

FIGURE 3.2

A diagram of *E. granulosus* large rostellar hook
showing method of measuring the hooks

## 2.5 Morphological examination of the worms

[De]tailed morphological studies were carried out on the
[st]robilar and the rostellar hooks of the 35dpi worms of the
[di]fferent intermediate host origin from Nairobi and Turkana.
[Th]e 35dpi worms from various parts of the world were studied
[an]d compared among themselves and also with the Turkana worms
[of] similar infection age.  The parameters and the sample size
[us]ed are summarized in Tables 3.3 and 3.4.

[Mo]rphological studies of the 14, 17, 21, 26, and 42dpi worms
[we]re carried out, noting the growth and developmental
[fe]atures which included, segments, genital rudiment, testes,
[ci]rrus sac, genital pore, ovaries, uterus, zygote and eggs.

## 2.6 Data analysis

[Th]e segmentation and maturation data were analysed for
[sp]ecies and host differences using two way tables for each
[li]ttermate set and period post infection.  For each table a
[ch]i-squared statistic was calculated and significance of
[di]fference in the results obtained determined.  Overall
[co]nclusions were drawn by considering the results from all
[st]ages.

## 2.7   *E. granulosus* DNA extraction

[Pr]otoscoleces of cattle, camel, sheep and goat origin
[pr]eserved in 70% ethanol as described earlier, were used for
[DNA] extraction as described by McManus *et al.,* (1985).  They
[we]re rinsed thrice in distilled water, leaving them to stand

about two minutes between each rinse, to allow for
complete rehydration.  The protoscoleces were then sedimented
centrifugation in an Eppendorf-centrifuge for 30 seconds
d the water decanted.  About 0.15ml of packed protoscoleces
re gently resuspended in 1ml of extraction buffer (50mM
is, pH. 8.0; 50mM EDTA; 100mM NaCl.).  Then 50ul of 10% SDS
dium dodecyl sulphate) was added for lysing the
otoscoleces and an equivalent amount of 2% proteinase K, to
gest the proteins.  This was mixed gently and incubated in
50°C water-bath for three hours.  The extraction of the DNA
s then carried out using buffered equilibrated phenol
llowed by phenol-chloroform and chloroform extractions.
e total DNA was then precipitated in 3M sodium acetate
:10v/v) and cold absolute ethanol (1:2.5v/v). The DNA was
en looped out using a sterile sealed pasteur pipette and
shed for 10 minutes in 70% ethanol, after which it was
ssolved overnight in a suitable amount of Tris-EDTA (10mM
is-Cl pH. 8.0; 1mM EDTA).  The concentration of the DNA was
timated by taking the optic density (O.D.) values at 260 &
0 nm.

2.8  **Digestion and separation of the DNA fragments by gel**
     **electrophoresis**

gestion of the DNA (5ug) with restriction enzymes was
rried out to completion using the conditions recommended by
a manufacturer (Boehringer Mannheim Laboratories).  The DNA
agments were then separated by horizontal electrphoresis
5v constant voltage, 17hrs) on 0.8% (w/v) agarose slab gels

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bromolphenol-blue was used as a tracking dye. Different

striction enzymes which included: Taq I, Hinc II, Rsa I,

96 I, Sau 3A I, Hae III, Ava I, Hinf I, Alu I, SFI I, Not

Bam I and EcoR I, were tested. The gels were then

mersed in ethidium bromide solution for one hour(1μg/ml) to

ain the DNA material. The gels were then viewed under a

. light. Only Sau 96 I gave appreciably distinct bands

ich could be observed on the gel and Sau 96 I was therefore

ed for comparison studies of the different isolates.

3 RESULTS

3.1 Experimental infections in dogs

e results of the experimental infections in dogs are

marized in Tables 3.1 and 3.2. Of the 61 experimental

s, three died of suspected parvo-virus infections. Two of

puppies were found negative for *E. granulosus* on autopsy,

le 47 of them (81%) harboured medium or heavy infections

the parasite. Only two of the twelve puppies infected

th worms of cattle/dog origin had heavy infections compared

h more than half of the puppies dosed with protoscoleces

camel (10/12), goat (10/17) and sheep (6/15) origin.

3.2 Developmental rate

les 3.1 and 3.2 give a summary of the rate of development

proglottids and maturation of worms in dogs from Nairobi

Turkana

# TABLE 3.1

Rates of segmentation of *E. granulosus* worms in dogs fed with protoscoleces of different intermediate host origin

| Dog litter number | Hydatid cyst origin | Experimental dogs Origin/No./sex | | | Number of worms examined | Number of worms per dog | % of total number of worms with 0, 1, 2 or 3 segments Zero | One | Two | Three | Statistics (p<0.01) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | cattle N | N | 1 | M | 92 | low | 49 | 51 | | | a |
| | goat N | N | 1 | F | 97 | high | 9 | 91 | | | b |
| | sheep N | N | 1 | F | 95 | high | 68 | 32 | | | c |
| 2 | cattle N | T | 1 | M | 61 | high | 41 | 59 | | | a |
| | sheep N | T | 1 | M | 33 | high | 48 | 52 | | | a |
| | goat N | T | 1 | F | £ | | | | | | |
| 3 | goat N | N | 1 | M | 129 | high | 14 | 55 | 31 | | a |
| | sheep N | N | 1 | F | 83 | medium | | 35 | 65 | | b |
| | cattle N | N | 1 | M | £ | | | | | | |
| 4 | cattle N | N | 1 | M | 119 | medium | 2 | 61 | 37 | | a |
| | goat N | N | 1 | F | 67 | high | 3 | 84 | 13 | | b |
| | sheep N | N | 1 | F | 129 | medium | 11 | 64 | 25 | | a |
| 5 | camel T | T | 1 | M | 265 | high | | 1 | 92 | 7 | a |
| | camel T | T | 1 | F | 181 | high | | 3 | 92 | 5 | a |
| | goat T | T | 1 | M | 373 | high | | 22 | 78 | | b |
| 6 | sheep N | T | 1 | M | 89 | medium | | 15 | 83 | 2 | a |
| | camel T | T | 1 | F | 244 | high | | 2 | 95 | 3 | b |
| | goat T | T | 1 | M | 238 | high | | 16 | 84 | | a |
| 7 | cattle N | N | 1 | F | 55 | low | | 22 | 65 | 13 | a |
| | goat N | N | 1 | M | 220 | high | | 13 | 87 | | b |
| | sheep N | N | 1 | F | 164 | high | | 20 | 77 | 3 | a |
| 8 | sheep N | T | 1 | M | 122 | medium | | 1 | 51 | 48 | a |
| | camel T | T | 1 | M | 156 | high | | | 23 | 77 | b |
| | camel T | T | 1 | M | 90 | high | | | 59 | 41 | a |
| | goat T | T | 1 | F | 97 | high | | | 97 | 3 | c |
| 9 | human N | N | 1 | M | 58 | medium | | | 32 | 68 | |
| | human N | N | 2 | F | £ | | | | | | |
| 10 | cattle N | N | 3 | M | 76 | medium | | | 86 | 14 | a |
| | goat N | N | 3 | F | 195 | medium | | | 1 | 99 | b |
| | sheep N | N | 2 | F | 197 | high | | | 10 | 90 | b |
| 11 | cattle T | T | 1 | M | 100 | high | | | 2 | 98 | a |
| | camel T | T | 1 | M | 122 | high | | | 2 | 98 | a |
| | goat T | T | 1 | M | 102 | high | | | | 100 | a |

DPI   days post infection
M     Male
F     Female
\<200 worms
201-1000 worms
high   >1001 worms
£     dog died

N     Nairobi (hydatid cysts were from animals slaughtered around Nairobi which originated mostly from Maasailand)
T     Turkana
a,b,c   Statistics - different alphabets denotes statistical difference (note: comparisons were only made among littermates)

# Rates of maturation of *E. granulosus* worms in dogs fed with protoscoleces of different intermediate host origin

| Dog litter number | Hydatid cyst origin | Experimental dogs Origin/No./sex | | | Number of worms examined | Number of worms per dog | % of total number of worms with | | | | | | Shed segments in faeces | Statistics (p<0.01) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | GR | CS/T | MS | Zg | SSE | TSE | | |
| 1 | cattle N | N | 1 | M | 92 | low | 51 | | | | | | | a |
| | goat N | N | 1 | F | 97 | high | 91 | | | | | | | b |
| | sheep N | N | 1 | F | 95 | high | 32 | | | | | | | c |
| 2 | cattle N | T | 1 | M | 61 | high | 59 | | | | | | | a |
| | sheep N | T | 1 | M | 33 | high | 52 | | | | | | | a |
| | goat N | T | 1 | F | £ | | | | | | | | | |
| 3 | goat N | N | 1 | M | 129 | high | 55 | 31 | | | | | | a |
| | sheep N | N | 1 | F | 83 | medium | 35 | 65 | | | | | | b |
| | cattle N | N | 1 | M | £ | | | | | | | | | |
| 4 | cattle N | N | 1 | M | 119 | medium | * | | | | | | | |
| | goat N | N | 1 | F | 67 | high | * | | | | | | | |
| | sheep N | N | 1 | F | 129 | medium | * | | | | | | | |
| 5 | camel T | T | 1 | M | 265 | high | 1 | 63 | 36 | | | | | a |
| | camel T | T | 1 | F | 181 | high | 3 | 92 | 5 | | | | | b |
| | goat T | T | 1 | M | 373 | high | 12 | 78 | 10 | | | | | c |
| 6 | sheep N | T | 1 | M | 89 | medium | * | | | | | | | |
| | camel T | T | 1 | F | 244 | high | 2 | 67 | 31 | | | | | a |
| | goat T | T | 1 | M | 238 | high | 16 | 80 | 4 | | | | | b |
| 7 | cattle N | N | 1 | F | 55 | low | | 78 | 22 | | | | | a |
| | goat N | N | 1 | M | 220 | high | | 40 | 60 | | | | | b |
| | sheep N | N | 1 | F | 164 | high | | 46 | 54 | | | | | b |
| 8 | sheep N | T | 1 | M | 122 | medium | | 8 | 79 | 13 | | | | a |
| | camel T | T | 1 | M | 156 | high | | | 38 | 62 | | | | b |
| | camel T | T | 1 | M | 90 | high | | | 50 | 50 | | | | b |
| | goat T | T | 1 | F | 97 | high | | | 97 | 3 | | | | a |
| 9 | human N | N | 1 | M | 58 | medium | | | 14 | 86 | | | | |
| | human N | N | 2 | F | £ | | | | | | | | | |
| 10 | cattle N | N | 3 | M | 76 | medium | | | | 100 | | | | a |
| | goat N | N | 3 | F | 195 | medium | | | | 76 | 10 | 14 | | b |
| | sheep N | N | 2 | F | 197 | high | | | | 100 | | | | a |
| 11 | cattle T | T | 1 | M | 100 | high | | | | 17 | 32 | 51 | | a |
| | camel T | T | 1 | M | 122 | high | | | | 45 | 40 | 15 | | b |
| | goat T | T | 1 | M | 102 | high | | | | 5 | 28 | 67 | | a |
| 12 | sheep N | N | 1 | M | 48 | low | | | 35 | 75 | | | | a |
| | sheep N | N | 1 | M | 59 | medium | | | | 100 | | | | b |
| | cattle N | N | 1 | M | 68 | medium | | | 3 | 80 | 17 | | | c |
| | goat N | N | 1 | F | 55 | medium | | | 10 | 90 | | | | b |
| | cattle N | N | 1 | M | 48 | medium | | | | 21 | 44 | 35 | | d |
| | goat N | N | 1 | F | 48 | low | | | | 88 | 12 | | | c |
| 13 | camel T | T | 1 | F | 89 | medium | | | | 68 | 22 | 10 | | |
| | goat T | T | 1 | M | - | high | | | | | | | + | |
| | cattle T | T | 1 | F | - | medium | | | | | | | + | |
| | camel T | T | 1 | M | - | high | | | | | | | + | |

| Dog litter number | Hydatid cyst origin | Experimental dogs Origin/No./sex | | | | Number of worms examined | Number of worms per dog | % of total number of worms with | | | | | | Shed segments in faeces | Statistics (p<0.01) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | GR | CS/T | MS | Zg | SSE | TSE | | |
| 14 | camel | T | T | 1 | M | - | high | | | | | | | + | |
| | sheep | N | T | 1 | F | - | high | | | | | | | + | |
| | camel | T | T | 1 | M | - | high | | | | | | | + | |
| | goat | T | T | 1 | M | - | 0 | | | | | | | | |
| 15 | sheep | N | T | 1 | F | - | low | | | | | | | + | |
| | sheep | N | T | 1 | M | - | medium | | | | | | | + | |
| | goat | T | T | 1 | M | - | low | | | | | | | + | |
| | camel | T | T | 1 | F | - | 0 | | | | | | | | |
| 16 | camel | T | T | 1 | F | - | high | | | | | | | + | |
| | sheep | N | T | 1 | F | - | medium | | | | | | | + | |
| | goat | T | T | 1 | M | - | high | | | | | | | + | |
| | camel | T | T | 1 | M | - | medium | | | | | | | + | |

days post infection     N    Nairobi (hydatid cysts were from animals slaughtered around
Male                     Nairobi which originated mostly from Maasailand)
Female           T    Turkana
<200 worms      a,b,c,d Statistics - different alphabets denotes statistical
201-1000 worms          difference (note: comparisons were only made among
>1001 worms             littermates)
gravid segments       *    staining not distinct for morphological study
shed in host faeces     £    dog died

genital rudiment    CS/T   cirrus sac + testes    MS    mature segment
zygote            SSE    soft shelled eggs      TSE    thick shelled eggs

## 3.2.1 Variation in development rates in worms of similar age and origin but from different dogs

Similar rates of segmentation, but inconsistent results for maturation, were observed between sources of protoscoleces within the same intermediate host species. Significant differences (p<0.01) were found between 21dpi camel/dog worms in Turkana dogs and 42dpi sheep/dog and goat/dog worms in Nairobi dogs.

3.2.2  Variation in development rates in worms of different
         origin but of similar age and from littermate dogs

...ere were significant differences (p<0.01) among worms of
...fferent intermediate host origin, both in the rates of
...gmentation and maturation for all days post infection in
...irobi and Turkana dogs, except at 14 dpi and segmentation
... 35 dpi in Turkana dogs.  However, examination of the data
...bles 3.1 and 3.2) does not show any consistent pattern.
... example in the comparison of protoscoleces of goat origin
...those of sheep origin in the Nairobi dogs at 17, 21 and 26
... the worms of goat origin had statistically (p<0.01)
...wer segments than the worms of sheep origin, while at 14
...i 35 dpi the reverse was true.  At 26 dpi, the worms of
...at origin from Turkana dogs were the least developed but
...c picture was reversed at 35 dpi.

...e shedding of gravid segments was observed 38 to 51 dpi in
...perimental Turkana dogs, irrespective of the source of the
...fecting protoscoleces.

...2.3  Comparison in the rates of parasite development
         between Turkana and Nairobi dogs

...granulosus worms from experimentally infected Turkana dogs
...ibited a faster development rate compared to the worms
...m the Nairobi dogs.  This difference was not significant
...14dpi.  However, by 17dpi onwards significant (p<0.01)
...ferences in the percentage of segmentation and maturation
...een worms experimentally obtained from  Turkana and
...robi dogs, were observed.  Eleven of the fourteen Turkana

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imentally infected dogs maintained with *E. granulosus*
ection beyond 35dpi, were observed to shed gravid segments
the faeces at/or earlier than 43 dpi.  However, only in
of six Nairobi dogs autopsied 42dpi had worms developed
ck shelled eggs.  Figure 3.3 shows a comparison of *E.*
nulosus worms of goat origin from Turkana (35dpi) and
robi (42dpi) dogs.


3.3    Morphology

3.3.1  The Kenyan parasite

granulosus worms experimentally obtained from dogs
rchased from Nairobi and Turkana showed much similarity in
ir morphology.


stellar *hooks (Table 3.3)*
all the worms studied, the two rows of rostellar hooks
re arranged in such a way that the large and the small
oks alternated (Fig. 3.1).  Occasionally small extra hooks
d/or a third row of incomplete minute hooks were observed,
tly in worms of camel origin but not of goat origin.  The
al number of rostellar hooks in the worms of cattle/dog,
ep/dog, human/dog and goat/dog origin were not
gnificantly different and showed little individual
riation.  They deferred significantly from those of
el/dog origin, which also displayed a lot of individual
riation.  In general the shape of the rostellar hooks in
l the samples was smooth with minimal variation.

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FIGURE 3.3 A.   *E. granulosus* of goat origin from a Turkana dog (35dpi - with thick shelled eggs)
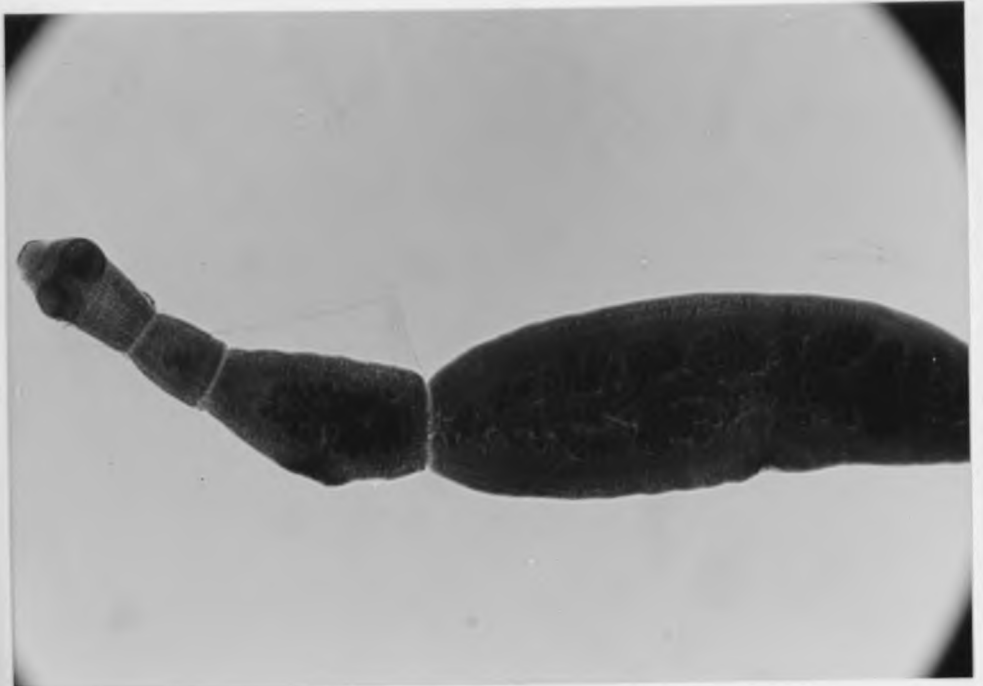


FIGURE 3.3 B.   *E. granulosus* of goat origin from a Nairobi dog (42dpi - with soft shelled eggs)

**TABLE 3.3**

**Measurements of rostellar hooks of *E. granulosus* worms (35dpi) of different intermediate host origin**

### Experimentally obtained from Nairobi dogs

| Protoscoleces from | Length (um) | | | | |
|---|---|---|---|---|---|
| | Human $H^f$ | Bovine B | Sheep S | Goat G | Statistics p<0.05 |
| | n =60 | n >60 | n >90 | n >90 | |
| Total number [*] | 31±1 | 35±1 | 35±2 | 33±1 | $b^a$ $s^a$ $g^a$ |
| **Large hooks** | | | | | |
| total length | 31.6±1.2 | 32.8±1.3 | 36.6±2.0 | 37.1±1.4 | $b^a$ $s^b$ $g^b$ |
| blade length | 12.3±0.6 | 12.1±0.8 | 12.4±0.6 | 12.6±0.2 | $b^a$ $s^a$ $g^a$ |
| handle width | 6.5±0.6 | 5.2±0.7 | 6.0±0.5 | 6.9±0.7 | $b^a$ $s^a$ $g^a$ |
| guard length | 7.0±0.7 | 6.8±0.7 | 7.4±0.8 | 7.8±1.0 | $b^a$ $s^a$ $g^a$ |
| **Small hooks** | | | | | |
| total length | 24.4±3.0 | 24.5±2.7 | 24.3±2.9 | 31.3±2.9 | $b^a$ $s^a$ $g^b$ |

### Experimentally obtained from Turkana dogs

| Protoscoleces from | Length (um) | | | |
|---|---|---|---|---|
| | Bovine B | Camel C | Goat G | Statistics p<0.05 |
| | n = 60 | n = 60 | n = 60 | |
| number [*] | 34±1 | 39±4 | 34±1 | $B^a$ $C^b$ $G^a$ |
| **Large hooks** | | | | |
| total length | 33.1±1.8 | 34.1±2.9 | 37.1±1.0 | $B^a$ $C^a$ $G^b$ |
| blade length | 11.9±0.3 | 14.1±0.8 | 13.2±0.7 | $B^a$ $C^a$ $G^a$ |
| handle width | 6.6±0.7 | 5.9±0.9 | 7.2±0.7 | $B^a$ $C^b$ $G^a$ |
| guard length | 7.2±1.0 | 7.6±1.0 | 8.0±0.7 | $B^a$ $C^b$ $G^c$ |
| **Small hooks** | | | | |
| total length | 23.4±2.3 | 23.4±2.3 | 26.5±2.8 | $B^a$ $C^a$ $G^b$ |

n =30

statistical comparison not undertaken in this case as the infected puppy was not part of the litter.

different superscript denotes that statistical difference was found

...ever, a protuberance which occurred in about 30% of the

...ge rostellar hooks of worms of goat/dog origin (Fig. 3.4),

...occasionally observed on the dorsal surface of the large

...ks in the worms of cattle/dog, human/dog, sheep/dog and

...el/dog origin.  The worms of goat/dog origin were also

...nd to posses significantly (P<0.05) larger hooks in

...ious respects.

*strobilar*

...e than 90% of the worms from each experimentally infected

...had three segments 35dpi, except worms of human/dog and

...tle/dog origin grown in the Nairobi puppies, where 80% and

...of the worms respectively, had three segments.  The ratio

...he last segment to the whole worm or to the anterior

..., was not significantly (p>0.05) different in worms

...ined from the same litter except those of goat/dog origin

...he Nairobi dogs.  The position of the genital pore was

...d to be either slightly anterior or posterior in mature

...ents but mostly posterior in gravid segments and little

...ation was found in all the worms examined.  The

...ltimate segment in the gravid worms was found to be

...riably mature and the ante-penultimate was at the genital

...ment stage of development (Fig. 3.3).

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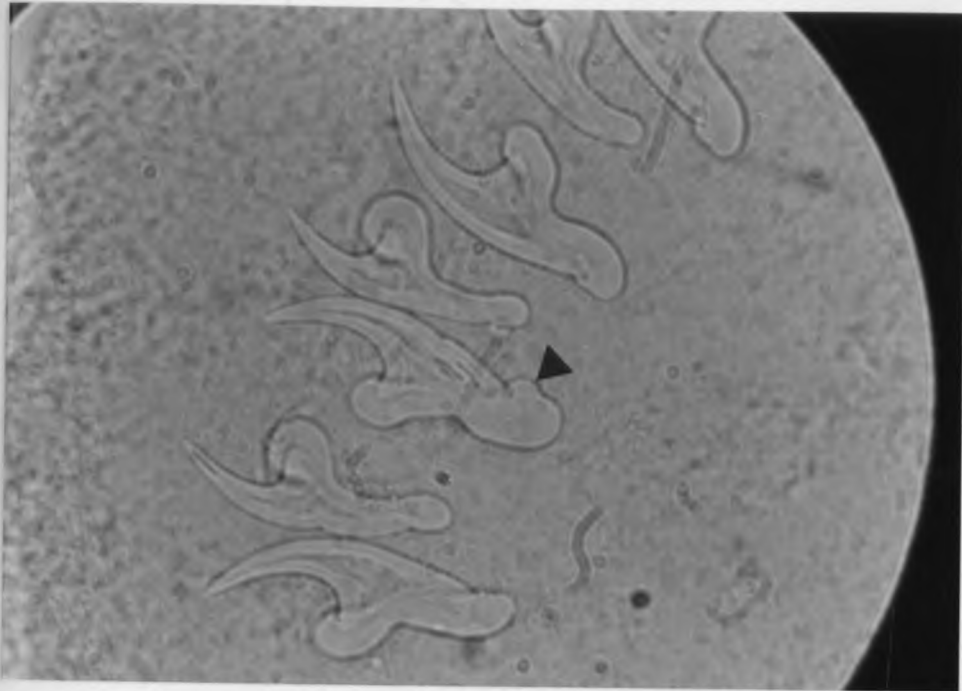
Rostellar hooks of *E. granulosus* of goat origin showing
the protuberance on one of the large hooks

number of testes varied from 35 to 60 in the worms of
le/dog, camel/dog, goat/dog and sheep/dog origin, but the
s of human/dog origin had between 45 and 72 testes.
ver the differences were not significant (p>0.05).   In
the worms, less than 50% of the testes were above the
tal pore except in human/dog parasite (mean 52%).
ally two rows of testes occurred below the vitelline
ds (Fig. 3.5).

*rus sac*

re was very little variation in the shape, angle, length
size of the cirrus sac.   The size, which measured
80u, gave it a spherical shape.   The cirrus was tilted
ghtly anteriorly.

*ale reproductive organs*

re were no significant variations in the female
roductive organs.   The two ovaries were attached on the
sal side and were lung-shaped and lobulated.   The gravid
rus had well developed sacculations and extended to the
ltimate segment.   The reproductive ducts were observed to
hrough a number of coils before and after the seminal
ptacle (Fig. 3.6).

uterine streak

ovary

vitelline glands
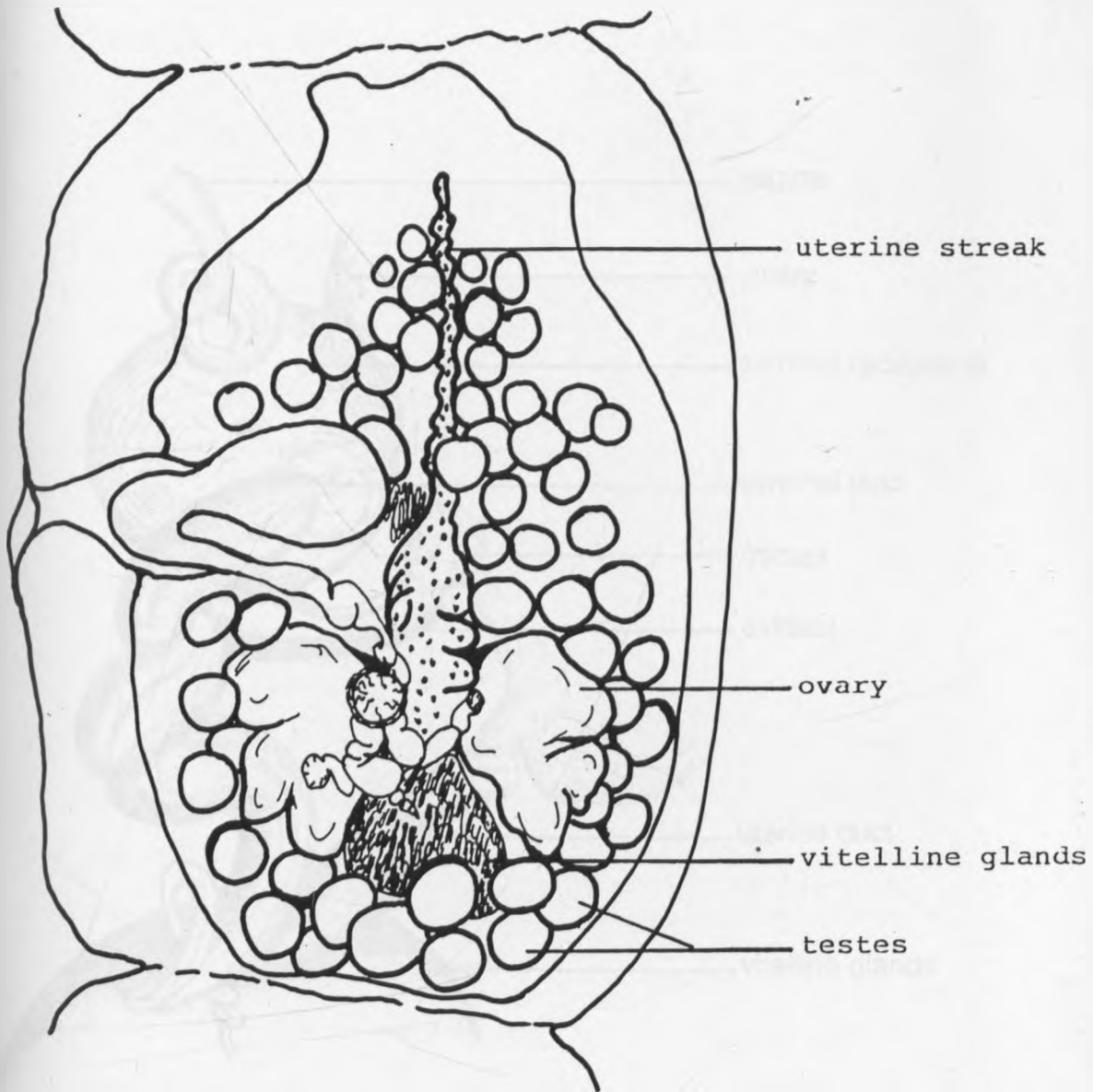
testes

FIGURE 3.5

Diagramatic representation of a mature penultimate
segment of *E. granulosus* from Kenya (camera lucida)
showing the distribution of testes (note the two rows
below the vitelline glands)

vagina

ovary

seminal receptacle

seminal duct

oocapt

oviduct

uterine duct

vitelline glands
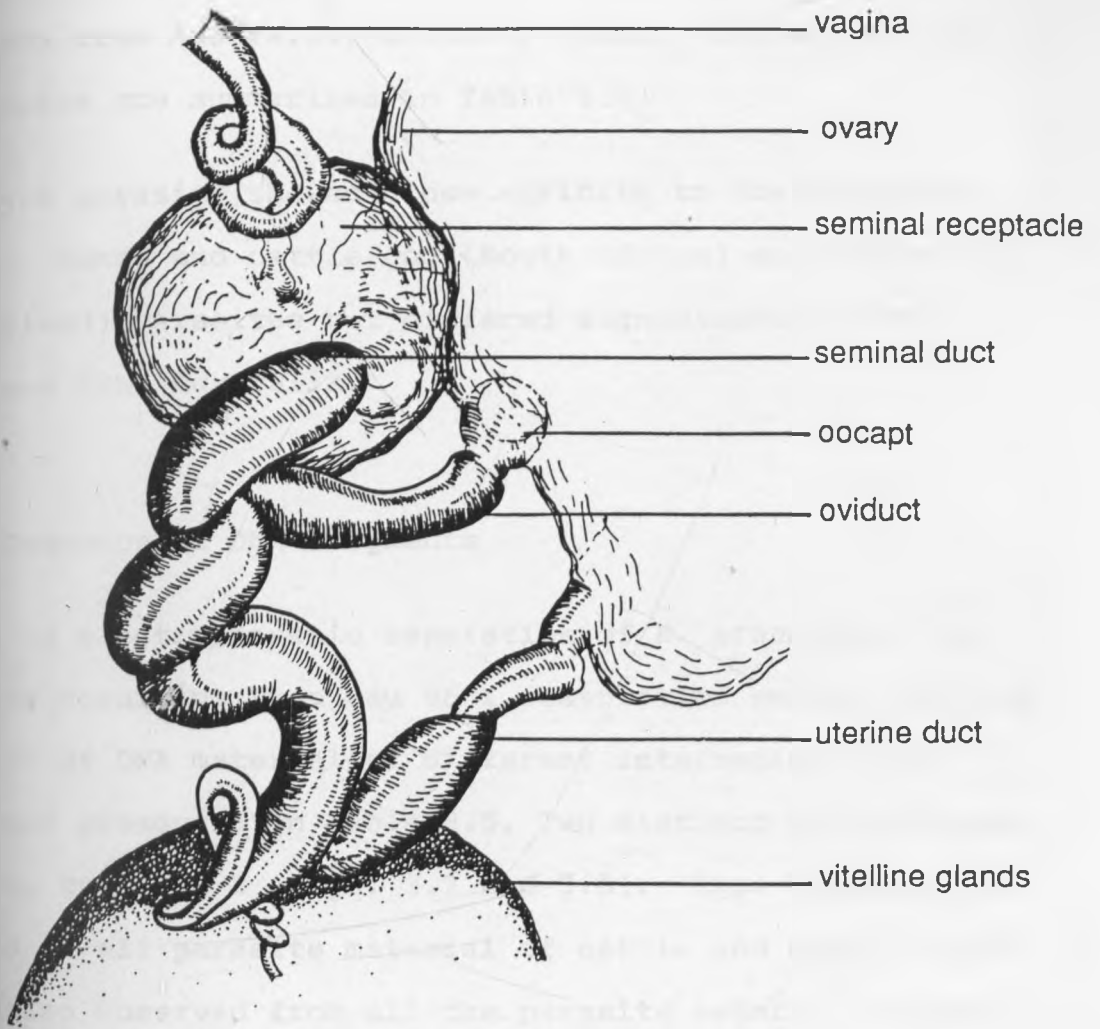
FIGURE 3.6

Female reproductive system of *E. granulosus* from Kenya
(note the coiled ducts)

3.3.2 Comparisons of the Kenyan parasite with

     *E. granulosus* from other countries

morphological comparisons of the Kenyan parasite with *E.*
*nulosus* from Australia, Britain, Canada, Switzerland and
th Africa are summarized in Table 3.4.

Kenyan parasite showed close affinity to the moose/dog
nada), human and cattle/dog (South Africa) and cattle/dog
itzerland) parasites but differed significantly from *E.*
*nulosus* from Australia.


4 Sequence of DNA fragments

lts of electrophoretic seperation of *E. granulosus* DNA
ments resulting from Sau 96 I restriction enzyme complete
stion of DNA material of different intermediate host
n are presented in Table 3.5. Two distinct patterns were
ted, type 1 & 2 (Figs. 3.7 and 3.8). Type 1 pattern was
rved in all parasite material of cattle and sheep origin.
as also observed from all the parasite material of goat
n except from two isolates, from which type 2 pattern
bserved. The observed patterns had no correlation to
e location of the hydatid cysts in the hosts. The two
tes showing type 2 pattern were from a lung and a liver
obtained from a single Maasai goat. The DNA material
ted from the two protoscolex samples of camel origin did
ow any separation on the gel electrophoresis although
striction enzyme digestion process was similar in all
es (Fig. 3.8).

## Morphological comparison of adult *Echinococcus granulosus* (35dpi) from Turkana, Kenya and other countries

| | Kenyan parasite material<br>(goat/dog  sheep/dog)<br>(cattle/dog)<br>(camel/dog)<br>(human/dog) | Parasite material from other countries<br>(1 moose/dog - Canada    5 cattle/dog - Switzerland)<br>(2 horse/dog - Britain     6 macropod/dingo - Australia)<br>(3 sheep/dog - Australia   7 cattle/dog - S. Africa)<br>(4 human/dog - S. Africa) | |
|---|---|---|---|
| | | Those similar to the<br>Kenyan parasite | Those disimilar to the<br>Kenya parasite |
| | Large & small hooks | 1,2,3,4,5 & 7 | 6 |
| | Alternate 1:1 | | alternate 1:2 |
| | mostly have smooth<br>edges | 1,2,4,5 & 7 | 3 & 6  notches<br>and protrubrances |
| | Varied | 3 & 6 | 1,2,4,5 & 7  much large |
| | Varied (30-40) | 1,2,3,4,5 & 7 | 6 (mean 58) |
| | 3 (over 90% of worms) | 4,5 & 7 | 1,2,3 & 6 (range 2-5) |
| | apprx.   1:1 | 2 & 3 | 6<br>1,4,5 & 7 |
| | apprx. mid-point but mostly<br>posterior in last seg. | 1,4,5 & 7 | 2 & 3  posterior<br>6 |
| | penultimate | 1,4,5 & 7 | 2 & 3<br>6 |
| | thick shelled | 1,4,5 & 7 | 2,3 & 6  zygote |
| | 48 (35-60) but<br>human/dog (45-72) | 1,4, 6 & 7<br>(35-56) | 2,3 & 5<br>mean <36 |
| | <50% | 4 | 1,2,3,5,6 & 7 |
| | human/dog 55% | | >50% |
| | 2 rows | 4 (2-4 rows) | 1,2,3,5,6 & 7<br>(1 row or none) |
| | spherical | 1,2,4,5, & 7 | 3 & 6  piriform |
| | 90u (70-100) :<br>85u (70-100) | 1 & 4 | 2,3,5,6 & 7 |
| | tilted slightly<br>anteriorly | 1,3,4,5,6 & 7 | 7 pointed anteriorly |
| | lung shaped<br>& lobulated | 1,4,5 & 7 | 2,3 & 6 |
| | upper side | 1,4,5 & 7 | 2,3 & 6  middle |
| | well developed | 1,4,5 & 7 | 2,3 & 6 poorly developed |
| | coiled before and<br>after seminal receptacle | 6 | 1,2,3,4,5 & 7 not coiled |

Hydatid DNA Sau 96 I restriction enzyme digest gel
electrophoresis fragment patterns

| Hydatid cyst | | Host | | Pattern |
|---|---|---|---|---|
| number | Tissue location | Species | Origin | |
| | liver | cattle | Maasailand | type 1 |
| | lung | cattle[a] | Maasailand | type 1 |
| | liver | cattle[a] | Maasailand | type 1 |
| | lung | cattle | Maasailand | type 1 |
| | lung | sheep | Maasailand | type 1 |
| | lung | sheep | Maasailand | type 1 |
| | lung | sheep | Maasailand | type 1 |
| | liver | sheep | Maasailand | type 1 |
| | liver/lung | goat | Maasailand | type 1 |
| | liver | goat[b] | Maasailand | type 2 |
| | lung | goat[b] | Maasailand | type 2 |
| | lung | goat | Turkanaland | type 1 |
| | liver | goat | Maasailand | type 1 |
| | lung | goat | Turkanaland | type 1 |
| | lung | camel | Turkanaland | ? |
| | lung | camel | Turkanaland | ? |

imilar alphabetical superscript indicates cysts obtained
om the same host animal.

```
      1   2   3   4   5   6   7
```
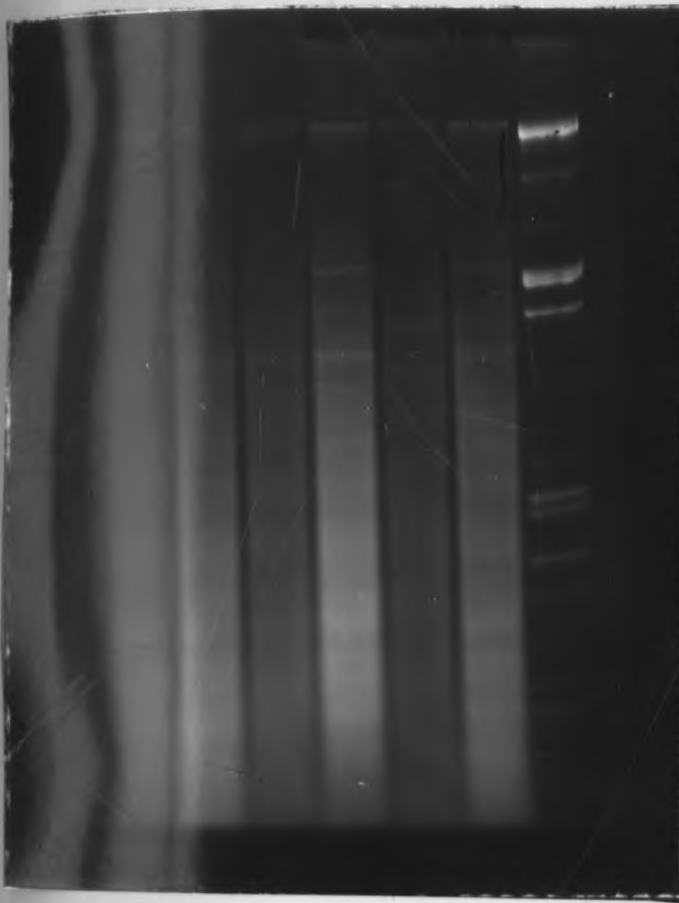
21.2   Kilobase

5.8
5.15
4.27

3.5

2.02
1.9

1.3
0.9

0.5

Molecular markers (labda Hind iii/EcoR 1 double
digest) - 7;   DNA parasite material of sheep origin -
2 & 4; cattle origin - 1 & 3 and goat origin - 5 & 6.

FIGURE   3.7

Electrophoretic profile of Kenyan hydatid parasite
DNA fragments following Sau 96 1 complete digest,
showing two distinct patterns: type 1 - Nos. 1,2,3,4 &
6;   type 2 - No. 5
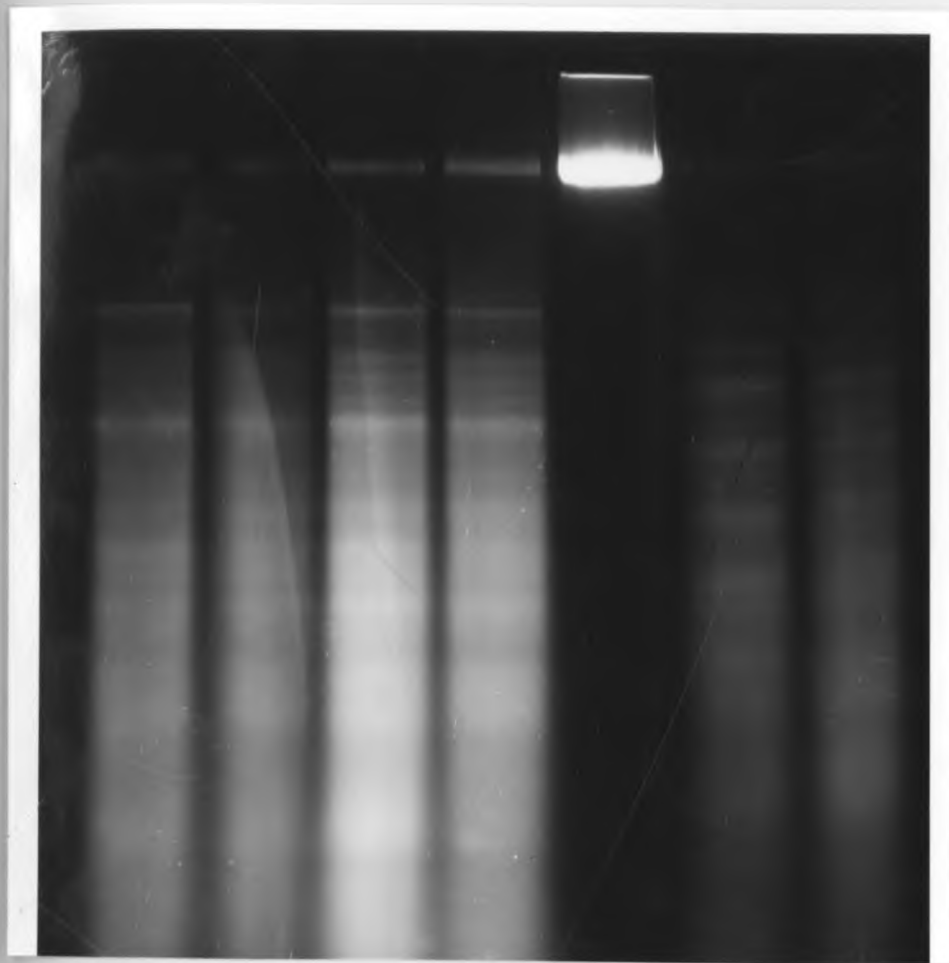
DNA parasite material of cattle origin - 1; sheep
origin - 2 & 3; goat origin - 4,6 & 7 and camel
origin - 5 (showed no seperation)

URE  3.8

Electrophoretic profile of Kenyan hydatid parasite DNA
fragments following Sau 96 1 complete digest, showing
the two patterns: type 1 - Nos. 1,2,3 & 4 and type 2 -
Nos. 6 & 7. No. 5 (camel origin) showed no separation.

## 3.4 DISCUSSION

Susceptibility of puppies purchased in Nairobi and Turkana, to hydatid material from different intermediate hosts showed no overall differences, but displayed individual variations among puppies fed with similar or different material. Such varied susceptibility in definitive host to infection with *E. granulosus* has also been reported by a number of workers (Forster, 1965; Pandey, 1972; Macpherson, 1985). The susceptibility of the puppies to hydatid material of cattle origin was, however, much lower than the rest, an observation also made by Macpherson (1985). This difference is probably host induced factor, as cattle in Kenya have been shown to poor hosts to the hydatid parasite, with most cysts being either calcified or sterile (Eugster, 1978; Macpherson, 1981).

The rate of development of *E. granulosus* has been observed to vary in different individuals of the same host species (Forster, 1965; Gill and Rao, 1967; Rausch, 1967a). The results of the present study were consistent with this observation and no difference could be attributed to the infecting protoscoleces of different host origin. The rate of development of the parasite in Turkana puppies was, however, significantly (p<0.05) faster than in Nairobi puppies. Although statistical comparison was only carried out in worms from 16 puppies, it was also observed that shedding of segments in host faeces started much earlier in Turkana dogs. Whereas this occurred about six weeks post infection in Turkana infected dogs, 42dpi worms from all but

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of six Nairobi infected dogs had immature eggs and only a
worms from the remaining dog had thick shelled eggs.
turally infected dogs with *E. granulosus* in Turkana, have
n found to harbour significantly heavier worm burdens than
s from the south of the country, despite similar infection
ceptibility with the parasite (Macpherson *et al.*, 1985).
therefore seems that the Turkana dogs are better adapted
E. *granulosus* in Kenya, both in terms of the rate of
lopment and size of worm load in each dog.  This may be a
ributing factor to the stability of the parasite in
kana.

ty-five-days post infection worms of different
rmediate host origin in Kenya were found to be
hologically similar, with little variation.
hological differences between worms from Turkana and
bi dogs were attributable to the observed difference in
ate of development.  This was in agreement with
ers' (1965) observations that certain morphological
cteristics in *E. granulosus* are dependent on the degree
velopment of the parasite.

ignificant variations observed in number, size and shape
stellar hooks in the Kenyan parasite material are
ly host induced.  Extra minute rostellar hooks observed
ommonly in camel/dog material have also been reported
toscoleces of the same host origin in Kenya
erson, 1981) and Syria (Dailey and Sweatman, 1965).
currence of minute accessory rostellar hooks has been
bed in other hosts including goat, sheep, cattle and

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horse (Vogel, 1957; Sweatman and Williams, 1963a; Williams
and Sweatman, 1963; Rausch and Nelson, 1963; Verster, 1965;
Pandey, 1972). Variability in the total number of rostellar
hooks has been demonstrated by Lubinsky (1960b), Sweatman and
Williams (1963a) and Verster (1965).

The findings of uniformity in morphology and *in vivo*
development of *E. granulosus* in Kenya collaborates the
conclusions of McManus and Macpherson (1984) that larval
morphology and *in vitro* developmental studies of the parasite
do not readily detect great variation in the hydatid material
of different host origin in Kenya.

The results of the study of hydatid DNA characterisation,
showed two patterns of *E. granulosus* in Kenya, which
represent distinctly genetically different strains of the
parasite. Similar findings, but based on isoenzyme analysis
studies, were reported earlier by Macpherson and McManus
(1982). In the isoenzyme studies (Macpherson and McManus,
1982) parasite material of camel origin was shown to be
similar to the rare form of the parasite of goat origin. It
is therefore possible that hydatid DNA of camel origin
examined in the present study was of the same affinity.
Available evidence, therefore, points to a sympatric
existence of two genetically and enzymatically distiguishable
strains of the parasite in Kenya. One strain, which appears
be more common, infects cattle, sheep, human and goat.
The rare strain has been identified from goat and camel. The
strains are physiologically and morphologically similar, but

heir significance in chemotherapeutic treatment of the
isease is not as yet clear. This aspect, and the extent of
istribution of the the two strains among the intermediate
sts, require further study. The unique band identified in
A material of the rare strain (Figs. 3.7 and 3.8) may prove
value, if cloned, for characterisation of parasite
ciates from the different intermediate hosts in Kenya.

erimental infection of baboons with *E. granulosus* eggs of
an, goat, cattle, sheep and camel origin from Kenya
cpherson *et al.*, 1986a) have indicated that all the
estic intermediate hosts of the parasite could be
tential sources of hydatid infection to man. It is
refore advisable for the Turkana control programme to
sider the two strains as cross-infecting among the
ermediate hosts until the epidemiological distribution
ure is determined.

atric existence of strains within the *Echinococcus* has
n supported or refuted, depending on ones inclination to
Thompson and Lymbery (1988) call models of intraspecific
ation (Smyth's and Rausch's models). Rausch's model
sch, 1967a; 1986; Thompson and Lymbery, 1988) upholds
s-fertilization as the normal breeding system in all
es of *Echinococcus*. Genetic flow under such state is
maintained and phenotypic differences between
ations would only occur in case of substantial barriers
e flow, such as ecological or geographical segregation.
s model (Smyth and Smyth, 1964; Thompson and Lymbery,
argues that *Echinococcus* spp. are predominantly self-

fertilizing and that this, coupled to the clonal reproduction in the intermediate host, does not only favour the generation of strains as products of mutation, but also their propagation even in the absence of any kind of segregation. The results of the present study seem to favour the latter model. The same can be observed from the results of other workers who have studied strain variations within Echinococcus by examination of the genome or its products (McManus and Smyth, 1979; Macpherson and McManus, 1982; McManus and Simpson, 1985; Bryant and Flockhart, 1986). Thompson and Lymbery (1988) have, however, pointed out that detailed studies of the parasite genome of each isolate, encompassing representative samples of the population under study, are required before conclusive genetic structure of the population can be made.

The morphology of E. granulosus, 35dpi worms of various domestic host origin in Kenya differed in many respects (Table 3.4) from sheep/dog worms of similar infection age from Australia. The domestic sheep/dog strain of E. granulosus is probably the most widely distributed of E. granulosus strains, covering most of Europe, USA, Australia and New Zealand (Thompson and Lymbery, 1988). The Kenyan parasite showed a number of similarities to E. granulosus worms from moose/dog (Canada) and cattle/dog (South Africa and Switzerland) cycle but the highest affinity was to worms from human/dog cycle of South Africa. The most striking similarities are the rate of development in the definitive host, distribution of testes below vitelline glands and the

shape of the ovaries. The reproductive duct of worms from the various intermediate host origin in Kenya, was observed to have a number of loops before and after the seminal vesicle and this was the only significant morphological feature, different from the South African worms of human/dog origin.

The human/dog parasite from South Africa was first described and designated *E. g. granulosus* by Verster (1965). The extent of its distribution in South Africa is unknown (Verster, 1965). It is possible that this is the same parasite found in Kenya, which may further suggest that it has a wide distribution from the south through east, and probably to the northern part of Africa.

The fast development of the Kenyan parasite, as is evident from the presence of thick shelled eggs in 33-35dpi worms, obtained from Turkana dogs, is similar to *E. granulosus* of moose/dog (Canada), cattle/dog (South Africa and Switzerland) and human/dog (South Africa) cycle. The Kenyan worms (37dpi) of camel origin studied by Kumaratilake (1982) and concluded to be retarded, were most probably normal worms with detached gravid segments. As noted above, the rate of development of *E. granulosus* may vary with definitive host species or strain. This puts to question the significance attributed to comparisons of *E. granulosus* worms obtained from different definitive host species or strain.

Comparative genomic analysis is a parasite characterisation method that eliminates the taxonomic problems associated with

henotypic variation and is applicable to any life cycle
tage of a parasite species.  This may well in future provide
he basis for systematic characterization of strains within
he genus *Echinococcus*.

Chapter 4

THE NATURAL INFECTION RATE OF DOGS WITH *E. GRANULOSUS* IN TURKANA DISTRICT

1 INTRODUCTION

agnosis of *E. granulosus* in naturally infected dogs has en used as a surveillance technique for monitoring progress hydatid control programmes in New Zealand, Cyprus, smania and Iceland, (Gemmell, 1979). This parameter gives indication of the infection pressure to dogs (Schwabe, 68) and can also be used to estimate the progress of an ucational programme aimed at discouraging people from eding hydatid cysts to dogs.

1983, a pilot hydatid control programme began in north stern Turkana district in Kenya (Macpherson *et al.*, 1984b). e control area of 9,000 sq. km is occupied by the nomadic rkana people who herd cattle, goats, sheep, donkeys and els. Before the implementation of the control programme, e population of dogs in this area numbered between 6,000 d 8,000 (French and Macpherson, 1983), but due to the 78/82 drought (Appendix 1.1) and subsequent famine, less an 10% of these dogs remained at the start of the control tivities (Macpherson *et al.*, 1986b).

e main purpose of the present control programme in Turkana to destabilise the adult worm population in the definitive

st by reducing the number of dogs.  This is done by
shooting of the unwanted dogs and registering and dosing of
the rest with Droncit$^R$ every six weeks.  In addition,
encouraging the Turkana to have their bitches spayed and not
to feed raw offal to dogs is an important component.

The present study examines the rate of natural infection with
*E. granulosus* and other taeniids in dogs treated within the
pilot control area, to evaluate the necessity of maintaining
the six weekly dog dosing regime, and the impact of the
educational programme.  An attempt is also made to assess the
use of arecoline hydrobromide for surveillance under Turkana
conditions, and the effect of drought on the *Echinococcus*
infection levels in dogs in Turkana.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Prevalence of infection in Turkana dogs

The yearly (1984-1988) infection levels with *E. granulosus* in
dogs were obtained by autopsy of the unwanted dogs killed
within the pilot control area.  The worm burdens were
recorded as either light (<200) or heavy (>200).  To
determine whether there is a relationship between drought and
the incidence of *E. granulosus* and/or the intensity of the
infections, 1979-1982 data (Macpherson *et al.*, 1985) were
included in the analysis.

4.2.2  Natural infection rates with taeniids in Turkana dogs following Droncit[R] treatment

Baseline taeniid infection rates of dogs in the control area were obtained from autopsy of unwanted dogs and the first arecoline hydrobromide (3mg/Kg) treatment of the wanted dogs. Natural infection rates, following Droncit[R] treatments, were then obtained from subsequent arecoline hydrobromide purges and autopsies of dogs killed on request, or those abandoned by the owners.  Comparison between the infection rates from autopsied and purged dogs were made at 6, 12, 18 and 24 week intervals following Droncit[R] treatment.

4.2.3  Diagnosis of *Taenia* spp. in dogs

Intestinal examination for tapeworms in dogs on autopsy was carried out as previously described.  Tapeworm diagnosis in the arecoline purge was, however, done through a series of washings and decantations in water on a black bottomed tray. All tapeworms or segments recovered on autopsy or arecoline purge were preserved in 70% ethanol and later identified morphologically.

4.2.4  Identification of *Taenia* spp.

Identification of specific species of taenia worms was carried out using a *Taenia* species key as provided in Appendix 4.1.

Mounting of the scolex was done as previously described (Chapter 3). Mature segments were stained in aceto-carmine and dehydrated in a series of increasing concentrations of ethanol (30, 50, 70, 90 & 100%). The segments were then cleared in clove oil and mounted in Canada Balsam. Before mounting, the segments were sectioned horizontally with a razor on the dorsal and ventral surfaces, removing tissues to the level of the longitudinal muscle layers, thus exposing the reproductive organs.

## 4.2.5 Statistical analysis

To determine whether there was a relationship between drought and *E. granulosus* prevalence and/or infection intensity, a standard chi-square test was used. The prevalence rates across the years, as well as the proportions of all dogs with light and heavy infections, were compared.

Regression analysis was used to compare the natural infection rates in Turkana dogs as determined either by autopsy or arecoline purge. The proportions of infected dogs were transformed using the relationship:

$$Y = \ln [(z + 1)/(n - z)]$$

where

$n$ is number of dogs observed

$z$ is number of dogs infected

and ln is natural logarithm.

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.3.1  Incidence rate of *E. granulosus* in Turkana dogs

he variation in the yearly *E. granulosus* infection level in
he dogs is depicted in Figure 4.1.  Chi-square analysis
owed no difference among the prevalence rates for the
ifferent years, but there were highly significant
ifferences among the proportion of infected dogs with light
d heavy worm burdens.  The proportions of dogs with heavy
rm loads was highest towards the end of the drought
981/2).

.3.2  Identifications of *Taenia* spp.

total of 200 *Taenia* spp. samples were examined
rphologically and all identified as *Taenia hydatigena*.  All
e samples fitted with the typical morphological description
 *T. hydatigena*  (Appendix 4.1 ), except in six of them
ere the distribution of testes tended to be confluent below
e vitelline glands.
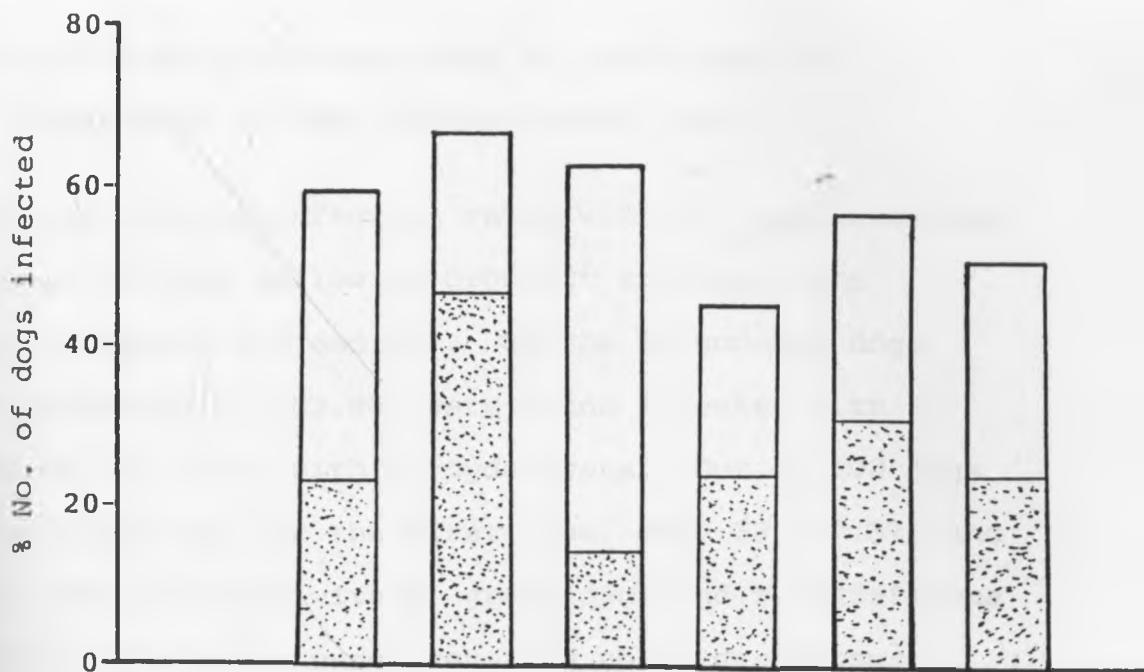
| Time (years) | 1979 | 1980-1982 | 1984 | 1985 | 1986 | 1987 |
|---|---|---|---|---|---|---|
| Number of dogs examined | 124 | 139 | 60 | 37 | 19 | 33 |
| Estimated dog population | 6 - 8000 | | 550 | 300 | 200 | |
| Estimated No of dogs with heavy infections | 1600 | 3300 | 85 | 75 | 65 | 50 |
| Estimated No of dogs with light infections | 2500 | 1400 | 265 | 65 | 50 | 55 |
| Estimated No of dogs not infected | 2800 | 2300 | 200 | 160 | 85 | 95 |

▨  %  dogs with >200 worms

☐  %  dogs with 1 - 200 worms

FIGURE  4.1

Variation in the incidence of *E. granulosus* in undosed dogs in the control area

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4.3.3   Natural infection rates with *E. granulosus* and
        *T. hydatigena* in dogs in the control area

Results of the natural infection rates with *E. granulosus* and
*T. hydatigena* in dogs following Droncit[R] treatment are
presented in Figures 4.2 and 4.3.  Of the 58 undosed dogs
that were autopsied 37 (63.8%) were found infected with *E.
granulosus* and 40 (69%) with *T. hydatigena*.  Out of 190 dogs
purged with arecoline for the first time, only 33 (17.3%) and
73 (38.4%) were infected with *E. granulosus* and *T. hydatigena*
respectively.  Arecoline purge revealed no *E. granulosus*
infection in 100 dogs examined six weeks following Droncit[R]
treatment.  However, two dogs were found infected with
immature stages of *E. granulosus* on autopsy of 12 dogs in the
same category.  The incidence of *E. granulosus* and *T.
hydatigena* in Droncit[R] treated dogs was found to increase
with time (Figs. 4.2 and 4.3) and reverted to the pre-
chemotherapy level by six months.  Comparison of the two
infection rates of the two parasites showed no significant
differences (p>0.05) in the slope of the relationship with
weeks post-chemotherapy for either type of parasite or method
of examination (Figs. 4.4 and 4.5).  However, there were
significant differences (p<0.01) in the levels of reinfection
for both types of parasites and method of examination.
Arecoline purge was shown to detect more dogs infected with
*T. hydatigena* than with *E. granulosus*, but fewer with either
parasite when compared to autopsy (Figs. 4.2 and 4.3).
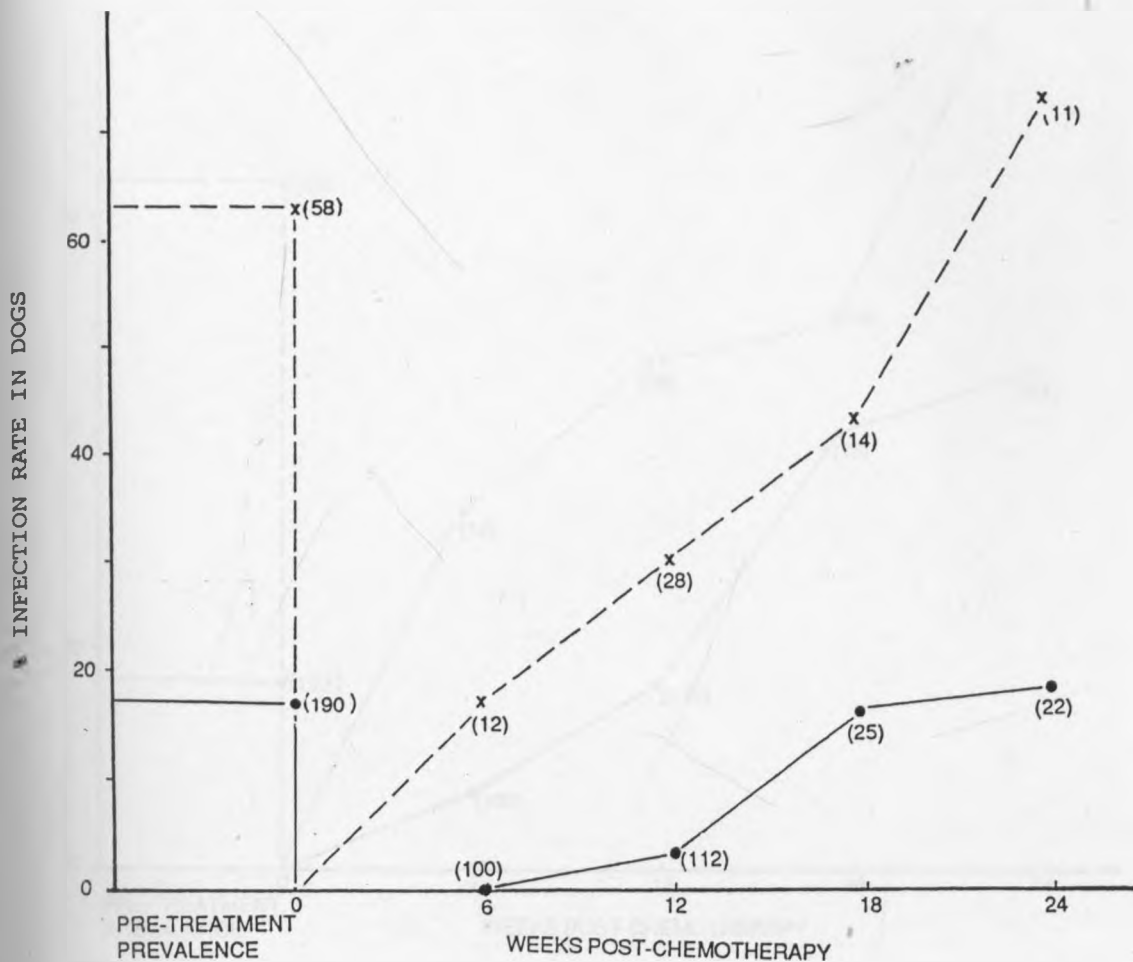
( )      number of animals sampled
(x--x)   diagnosis by autopsy
(●—●)   diagnosis by arecoline purge

FIGURE   4.2

Natural increase in the infection rate of dogs with *E. granulosus* following Droncit[R] treatment
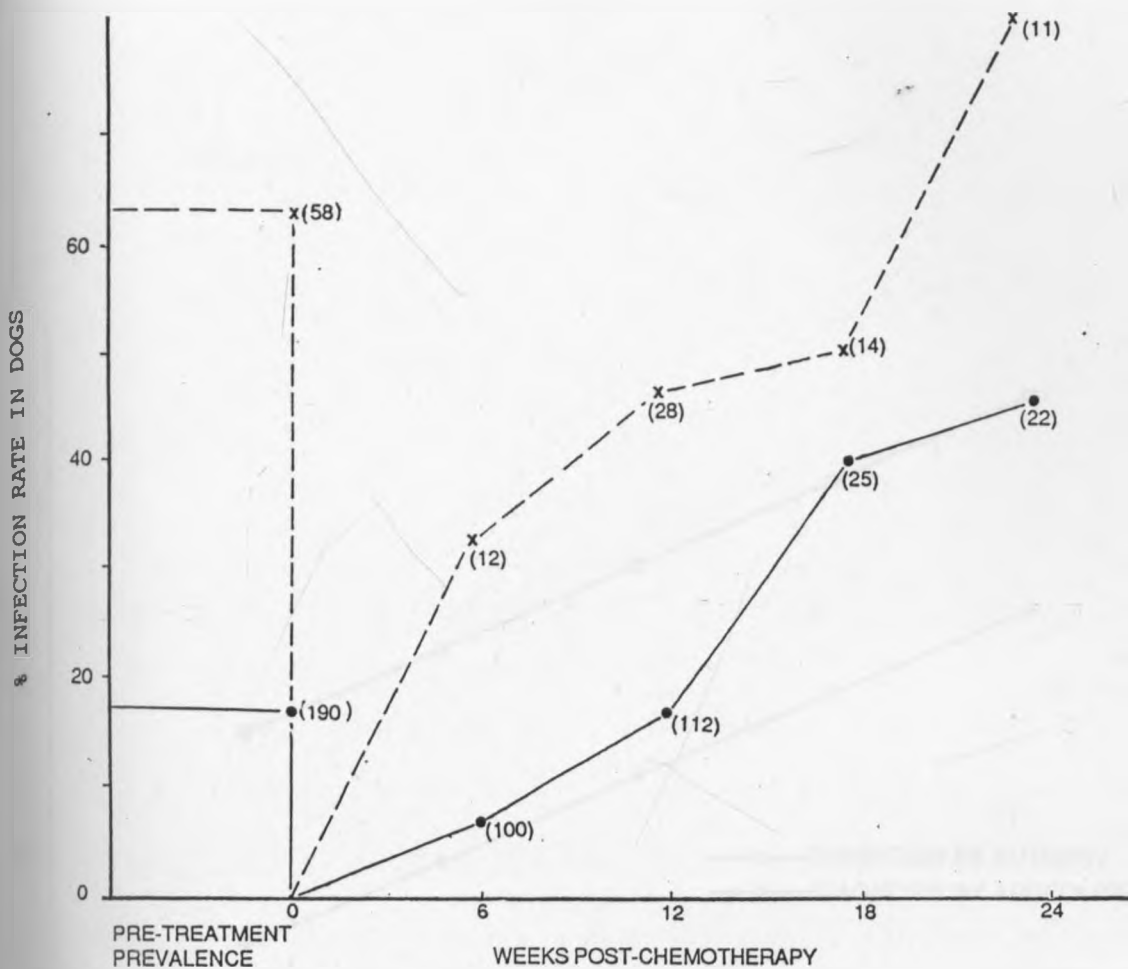
```
( )        number of animals sampled
(x--x)     diagnosis by autopsy
(●—●)      diagnosis by arecoline purge
```

FIGURE  4.3

Natural increase in the infection rate of dogs with *T. hydatigena* following Droncit[R] treatment
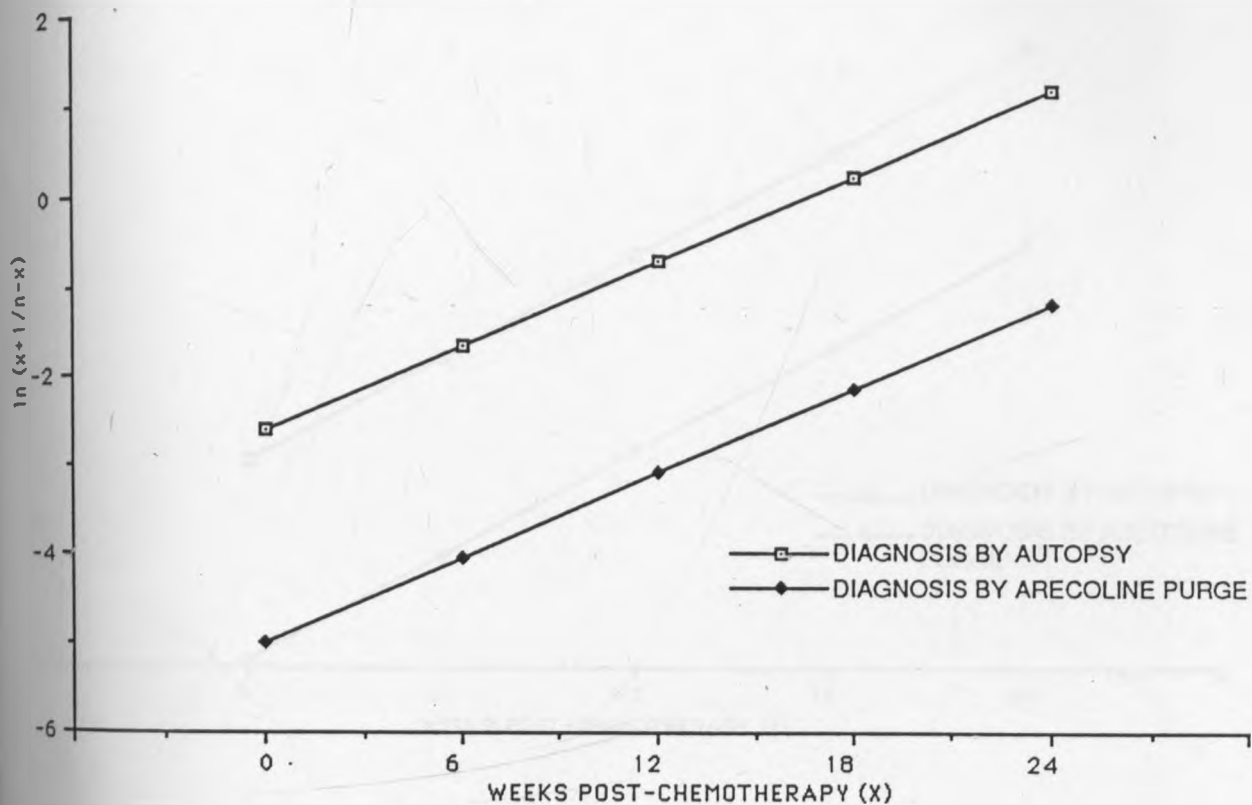
FIGURE 4.4

Natural increase in the infection rate of dogs
with *E. granulosus* following Droncit[R]
treatment: Regression lines

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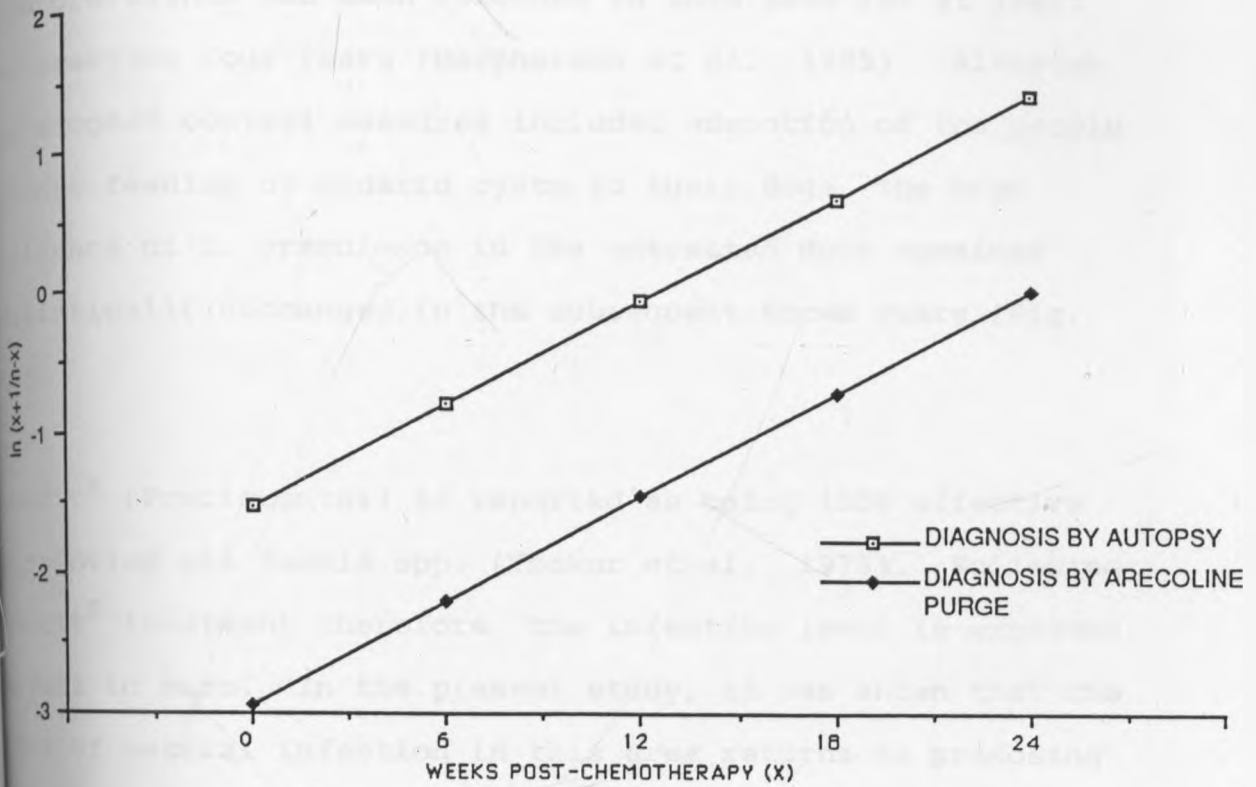
FIGURE 4.5

Natural increase in the infection rate of dogs
with *T. hydatigena* following Droncit[R]
treatment:  Regression lines

y-axis: ln (x+1/n-x)

x-axis: WEEKS POST-CHEMOTHERAPY (X)

Legend: DIAGNOSIS BY AUTOPSY · DIAGNOSIS BY ARECOLINE PURGE

## 4.4 DISCUSSION

Immediately prior to the introduction of Droncit[R] treatment, the natural infection rate of dogs with *E. granulosus* by autopsy in the control area was found to be 63.8%. Such a high prevalence had been recorded in this area for at least the previous four years (Macpherson *et al.*, 1985). Although the adopted control measures included education of the people against feeding of hydatid cysts to their dogs, the high incidence of *E. granulosus* in the untreated dogs remained statistically unchanged in the subsequent three years (Fig. 4.1).

Droncit[R] (Praziquantel) is reported as being 100% effective in removing all *Taenia* spp. (Thakur *et al.*, 1978). Following Droncit[R] treatment therefore, the infection level is expected to fall to zero. In the present study, it was shown that the level of natural infection in this area returns to predosing levels within six months (Fig. 4.2). This indicates that a high infection pressure to dogs exists, which is surprising considering the low prevalence of hydatidosis recorded among the intermediate hosts in this area (Macpherson, 1981).

Macpherson (1985) and Gemmell *et al.*, (1985) have shown that the distribution of hydatid cysts in the intermediate host population is over-dispersed. Since a single hydatid cyst can produce over a million infective protoscoleces, one cyst can potentially infect a large number of dogs. The low incidence in the livestock can therefore produce a much higher incidence in the domestic dog population. In areas

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where there are no abattoirs, such as in the present pilot control area, all cysts are likely to be thrown to dogs which congregate at the site of slaughter. The vastly reduced dog population in the pilot control area means that fewer dogs are competing for the same number of cysts (provided the incidence in man and livestock remains relatively constant). Therefore, infection pressure to dogs in such a case could be expected to be higher than in areas with higher dog populations, resulting in a sustained high infection rate and incidence. This is in agreement with the postulate put forward by Keymer and Anderson (1979), and Keymer (1982), stating that where transmission of parasites from host to host is achieved by ingestion of an infective stage, the rate of infection is dependent on the pressure of infection from the parasite (ie. parasite density) level of exposure of the host to the parasite, the size of the host population and the feeding behaviour of the host. Although the prevalence of *T. hydatigena* among the intermediate hosts is higher than that *E. granulosus*, this difference was not reflected in the rate of infection in dogs. This could be due to the high multiplication potential of larvae within hydatid cysts.

Drought is another factor that might contribute to the high infection rate in dogs by increasing the level of availability of hydatid cysts through livestock deaths. Dogs in the control area were shown to harbour significantly higher *E. granulosus* worm loads (Fig. 4.1) towards the end of the 1978/82 drought (Appendix 1.1) when the effects were most severe. Similar increase in infection intensity following

prolonged drought conditions was observed in Maasailand in dogs infected with *E. granulosus* (Ngunzi, 1985). It is therefore possible that in Turkana, prolonged droughts that are known to occur every ten years or so (data from the meteolorogical department of Kenya), may play a role in periodically raising the prevalence of *E.granulosus* infection in dogs.

The rapid acquisition of *E. granulosus* infection by dogs in Turkana suggests that in order to reduce transmission between dogs and intermediate hosts as much as possible, it would be necessary to reduce the dosing interval. This will be discussed further in the concluding chapter in the light of the studies on prepatent period and effects of the parasite density on egg production.

In New Zealand, inclusion of *T. hydatigena* in the dog infection testing programme was found necessary as an educational measure (Gemmell, 1968a). Due to its large size, the tapeworm is easily noticed in the purge, thus helping the people to appreciate the seriousness of feeding their dogs with raw offal. The identification of *T. hydatigena* as the only other taeniid in Turkana dogs is an added advantage to the hydatid control education programme, as the tapeworm can be an indicator of individuals who have not complied with the teaching of not feeding raw offal to their dogs.

The rapid reinfection rate observed in dogs both with *E. granulosus* and *T. hydatigena* seems to indicate poor immunity against these parasites. This field observation supports

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laboratory studies which have shown that there is little protection against reinfection by dogs against *E. granulosus* (Gemmell and Soulsby, 1968; Herd, 1977; Gemmell *et al.*, 1985). However, specific antibodies to *E. granulosus* have been identified as early as 32 days post-infection (Jenkins and Rickard, 1985). If the levels of these antibodies decrease within the six weekly dosing interval, their subsequent detection indicating reinfection would serve as a useful substitute for the present surveillance technique of arecoline purging. Arecoline surveillance is not a reliable technique (Fig. 4.2) as often dogs fail to purge, and even when they do worms are not always expelled (Batham, 1946; Gemmell, 1968b), and young or light infections can easily be missed. It is also a cumbersome method and presents greater risk of infection to personnel. In the present pilot control programme, however, it appears that arecoline surveillance is necessary as an indicator of the disease in dogs, and hence the infection pressure to the intermediate hosts, there being no abattoirs in the control area.

Chapter 5

THE RELEASE AND SURVIVAL OF *E. GRANULOSUS* EGGS UNDER THE
TURKANA ENVIRONMENTAL CONDITIONS AND THEIR IMPACT ON THE
INCIDENCE OF HYDATIDOSIS IN KENYA

5.1 INTRODUCTION

The incidence of hydatidosis in a population is influenced by
numerous factors, including the infection pressure from
infective *E. granulosus* eggs. The infective pressure depends
on the number and transmission potential of viable eggs to
susceptible intermediate hosts. The number of *E. granulosus*
infected dogs, their worm burden and the biotic potential of
the adult parasite, determine the absolute egg production.
Once released, eggs are exposed to the environment where
temperature, humidity and microorganisms affect their
survival (Lawson and Gemmell, 1983). Dispersal of the eggs
is facilitated by such factors as the movement and the
defaecatory habits of the definitive host, wind, flies and
water (Gemmell and Lawson, 1986). Difference in
sociological, cultural, occupational, dog ownership and
husbandry practices, have been shown to influence the
infection pressure to man and his livestock (Araujo *et al.*,
1975; Schwabe *et al.*, 1981; Watson-Jones and Macpherson,
1988). Coupled to all these factors, the survival of eggs in
the environment must also influence the infection pressure,

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and ultimately the infection levels in the intermediate hosts.

The present study was undertaken to examine the biotic potential, release of eggs from proglottids, and the survival of *E. granulosus* eggs in the Turkana environment. The survival of *E. granulosus* eggs in Turkana was compared with that in the Maasai environment. The results were compared with the survival of *T. hydatigena* and *T. saginata* eggs in the two different climatic regions of Kenya where the prevalence of hydatid disease is very different. The prevalence of hydatidosis is high in man and low in livestock in Turkana district, but paradoxically hydatidosis is high in livestock and low in man in Maasailand (Eugster, 1978; Macpherson, 1981).

## 2 MATERIALS AND METHODS

### 2.1 Numbers of eggs per proglottid

The undetached gravid proglottids of *E. granulosus* and *T. hydatigena* were obtained from naturally infected dogs on autopsy while detached *T. hydatigena* proglottids were collected from the lower rectum. The intensity of *E. granulosus* infections in the autopsied dogs was noted and divided into light (1-200), medium (201-1000) and heavy infections (>1,000). *E. granulosus* proglottids were also collected from freshly deposited faeces, and again 15 minutes later, from faeces from naturally infected dogs. Proglottids

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ol *T. saginata* were obtained from local people prior to niclosamide treatment. Eggs were obtained from a known number of proglottids either by tearing them apart with needles or leaving the *E. granulosus* proglottids in water for two days to disintegrate. Total egg count was made using two separate drops (0.04ml) from a known volume of egg suspension. The average number of eggs per proglottid was then calculated.

## 2.2 Collection of taeniid eggs

Gravid proglottids of *E. granulosus* and *T. hydatigena* were collected from dogs found on autopsy to be monospecifically infected. Proglottids were washed in saline and finally transferred to a saline solution containing antibiotics (final concentration of 50 units penicillin, 50ug streptomycin and 5μg fungizone) for transportation to the lab. Eggs were released by tearing the proglottids apart with pointed needles. The eggs of the same species were pooled.

## 2.3 Hatching and activation

Hatching and activation of the eggs were carried out as described by Stevenson (1983) except that 1.5g sodium taurocholate and 1.5g pancreatin powder were used instead of ml bovine bile. After hatching and activation of the eggs, a drop was examined under the microscope and the number of hatched and unhatched oncospheres counted and recorded. Viability was assessed using hook motility and the eosin

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clusion dye test (Smyth and Barrett, 1980). At least 200
cospheres were examined.

## 2.4 Egg survival experiments

ese were carried out in Turkana and in Nairobi under
fferent environmental conditions (open ground, house and
ter) using about 20,000 eggs for each species and
eriment. To facilitate egg recovery whilst avoiding egg
ntamination, for example by flies, and at the same time
ximally exposing them to the environmental conditions, the
gs were sealed in small nylon bags (Fig. 5.1) with pores of
m diameter. The environmental temperatures and humidity
the various regions, were recorded in the course of the
eriment.

## .5 Proglottids as agents of taeniid egg dispersal

granulosus proglottids passed in dog faeces were observed
m the time of defaecation to the time the proglottids
ed moving. The distance moved by a sample of proglottids
the average number of eggs per proglottid on defaecation
after cessation of proglottid movement were noted.
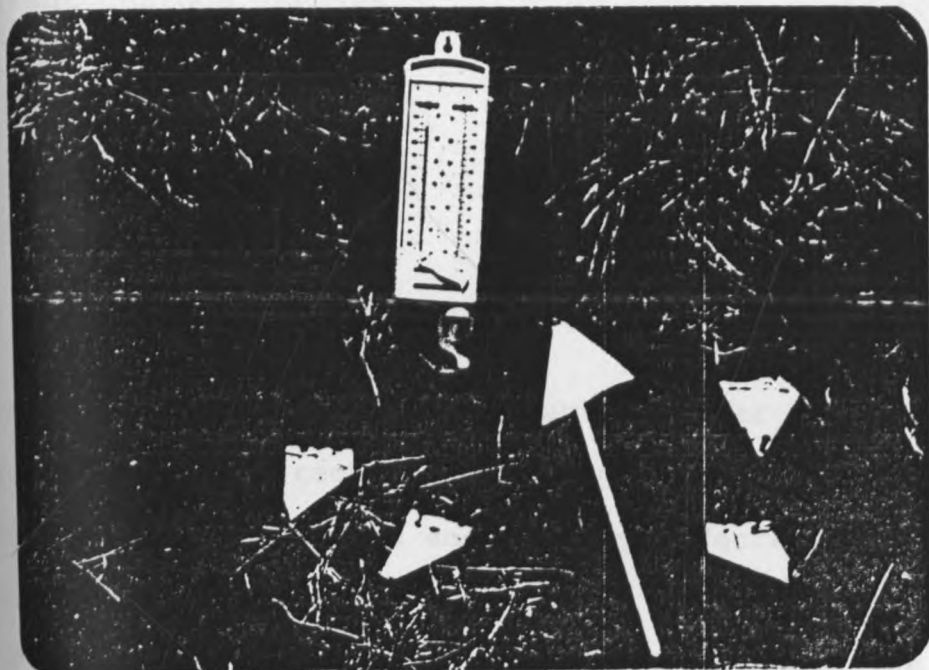
**FIGURE 5.1**

Exposure of taeniid eggs in nylon bags to the Turkana
open ground environment. The sensitive tip of the
thermometer is also covered by a nylon bag

## 5.2.6  Statistical analysis

Comparisons of the number of eggs per proglottid in the different *E. granulosus* infection densities in dogs were carried out using the analysis of variance, while regression analysis was used to compare the egg survival rates among the different cestode species.

## 5.3  RESULTS

### 5.3.1  Egg release from proglottids

As presented in Table 5.1, the average number of eggs per proglottid before detachment for *E. granulosus* was 842, 793, and 835 in lightly, medium and heavily infected dogs respectively. Analysis of variance on the number of eggs per proglottid showed no significant difference among the different infection densities. The average number of eggs per proglottid was shown to be 823 (range, 400-1,300) and 33,929 (range, 20,000-54,000) for *E. granulosus* and *T. hydatigena* respectively. Most of the eggs were found to be released in the intestine before the proglottids passed out of the host. Tables 5.1 and 5.2 summarise the results of the egg counts in *E. granulosus* and *T. hydatigena* segments passed in dog faeces and *T. saginata* segments passed in human faeces. By the time the proglottids are passed out, they were found to have lost 73.3%, 72% and 91% of the eggs for *E. granulosus*, *T. hydatigena* and *T. saginata* proglottids respectively.

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TABLE 5.1

# Number of eggs in *Echinococcus granulosus* proglottids

| Number of dogs | Attached segments | | | Fresh segments in dog faeces | | | Segments after ceasation of movement | | |
|---|---|---|---|---|---|---|---|---|---|
| | Segments examined | Eggs counted | Mean | Segments examined | Eggs counted | Mean | Segments examined | Eggs counted | Mean |
| 1 (H) | 30 | 27240 | 908 | 30 | 7860 | 262 | 22 | 1804 | 82 |
| 1 (H) | 30 | 26400 | 880 | 20 | 6040 | 302 | 10 | 740 | 74 |
| 1 (H) | 30 | 23280 | 776 | 30 | 5520 | 184 | 8 | 448 | 56 |
| 1 (M) | 30 | 28320 | 944 | 50 | 11000 | 220 | 15 | 1395 | 93 |
| 1 (H) | | | | | | | 20 | 1500 | 75 |
| 5 (H) | 217 | 171950 | 792 | | | | | | |
| range (H) | | | 835 | | | | | | |
| 1 (M) | 31 | 23000 | 742 | | | | | | |
| 1 (M) | 24 | 21600 | 900 | | | | | | |
| range (M) | | | 793 | | | | | | |
| 1 (L) | 24 | 20000 | 769 | | | | | | |
| 1 (L) | 6 | 6650 | 1108 | | | | | | |
| 1 (L) | 7 | 6200 | 886 | | | | | | |
| range (L) | | | 842 | | | | | | |
| N/segment (mean) | | | 823 | | 234 | | | 79 | |
| | | | (400-1300) | | (130-300) | | | (30-150) | |
| % remaining* | | | | | 26.7 | | 9 | | |

(H), (M), (L)  Heavy, Medium, and Light infections.

* mean of the remaining eggs/segment as a percent of the mean number of eggs/segment before segment detachment.

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## Number of eggs in *Taenia hydatigena* and *Taenia saginata* proglottids

| T. HYDATIGENA | | | | | | T. SAGINATA | | |
|---|---|---|---|---|---|---|---|---|
| Attached segments | | | Detached segments in lower rectum | | | Segments passed in faeces | | |
| Segments examined per sample | Eggs counted | Mean | Segments examined per sample | Eggs counted | Mean | Segments examined per sample | Eggs counted | Mean |
| 4 | 216000 | 54000 | 8 | 84000 | 10500 | 3 | 144400 | 48133 |
| 6 | 177480 | 29580 | 4 | 16000 | 4000 | 5 | 51800 | 10360 |
| 6 | 180720 | 30120 | 10 | 121300 | 12130 | 2 | 513 | 256 |
| 4 | 80000 | 20000 | 3 | 22350 | 7450 | 3 | 5680 | 1893 |
| 2 | 97320 | 48660 | 4 | 29320 | 7330 | 2 | 2225 | 1112 |
| 3 | 76200 | 25400 | 12 | 121300 | 10108 | 2 | 2435 | 1217 |
| 5 | 196750 | 39350 | 4 | 22680 | 5670 | 1 | 4310 | 4310 |
| 5 | 111000 | 22200 | 7 | 79030 | 11290 | 5 | 42050 | 8410 |
| 5 | 221700 | 44340 | 9 | 75330 | 8814 | 2 | 34240 | 34240 |
| | | | 2 | 26000 | 13000 | 2 | 27640 | 13820 |
| | | | 1 | 9060 | 9060 | 5 | 3795 | 759 |
| | | | 4 | 69200 | 17300 | 7 | 2581 | 369 |
| | | | 7 | 42840 | 6120 | 1 | 20000 | 20000 |

gg/segment
(mean)          33929                  9512                   7859
           (20000-54000)          (4000-13000)           (100-55000)

maining eggs %                       28                     9[+]

The mean of the remaining eggs/segment as a percent of the mean
number of eggs/segment before detachment
number of eggs/segment before detachment is 80,000 (Penfold et al., 1937)

side the host, *E. granulosus* proglottids were seen to
tinue peristaltic movements for less than fifteen minutes
ore they dried up in the suns' heat on the faecal pat,
thout moving any distance.  At this stage, only an average
79 eggs (9%) were left in the proglottid (Table 5.1).


3.2  **Egg survival**

survival times for *E. granulosus*, *T. hydatigena* and *T.*
*ginata* eggs are tabulated in Table 5.3.  No significant
fference in the survival times of eggs among the species
sted was observed.  All the eggs exposed to the Turkana
ound environment lost viability in less than three hours,
le those kept in the house and in water survived for two
d more than twelve days respectively.  In contrast the eggs
pt in the shade in the Maasai environmental conditions were
able even after three weeks.  Loss of hook motility in the
cospheres was observed to precede loss of viability as
termined by the eosin dye test.

# TABLE 5.3

## Survival rates of *E. granulosus*, *T. hydatigena* and *T.saginata* eggs in the Turkana and Nairobi environment

| Environment | | Time | Average Temp.($^{o}$c) and Rel. humidity | | % eggs hatched | | | % viable of eggs hatched | | | % motile of eggs hatched | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | TH | TS | EG | TH | TS | EG | TH | TS | EG |
| **TURKANA** | | | | | | | | | | | | | |
| GROUND | I | 0 hrs | - | - | 45 | | 25 | 88 | | 88 | 18 | | 20 |
| | | 0.5 hrs | 50 | - | 40 | | 20 | 90 | | 88 | 8 | | 25 |
| | | 1.5 hrs | 51 ± 1 | - | 25 | | 3 | 75 | | 100 | 4 | | 0 |
| | | 3 hrs | 53 ± 2 | - | 35 | | 10 | 0 | | 0 | 0 | | 0 |
| | | 6 hrs | 57 ± 5 | - | 50 | | 30 | 0 | | 0 | 0 | | 0 |
| | II | 0 hrs | - | - | 98 | 95 | 100 | 97 | 100 | 100 | 14 | 10 | 12 |
| | | 1 hr | 66 ± 1 | 17 | 97 | 86 | 99 | 96 | 90 | 97 | 13 | 7 | 10 |
| | | 2 hrs | 69 ± 3 | 17 | 98 | 89 | 95 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 2.5 hrs | 68 ± 3 | 18 | 80 | 48 | 88 | 0 | 0 | 0 | 0 | 0 | 0 |
| HOUSE | I | 0 days | - | - | 45 | | 25 | 88 | | 88 | 18 | | 20 |
| | | 1 day | 29 ± 5 | 42 ± 6 | 50 | | 10 | 77 | | 50 | 6 | | 10 |
| | | 3 days | 29 ± 5 | 42 ± 6 | 45 | | 5 | 0 | | 0 | 0 | | 0 |
| | | 5 days | 29 ± 5 | 42 ± 4 | 40 | | 20 | 0 | | 0 | 0 | | 0 |
| | II | 0 days | - | - | 98 | | 100 | 97 | | 100 | 14 | | 12 |
| | | 2 days | 31 ± 5 | 32 ± 8 | 97 | | 23 | 93 | | 34 | 25 | | 0 |
| | | 3 days | 31 ± 5 | 34 ± 10 | 76 | | 64 | 70 | | 0 | 0 | | 0 |
| | | 4 days | 31 ± 5 | 36 ± 12 | 80 | | 50 | 0 | | 0 | 0 | | 0 |
| WATER | I | 0 days | - | | 45 | | 25 | 88 | | 88 | 18 | | 20 |
| | | 2 days | 28 ± 3 | | 60 | | 40 | 88 | | 88 | 8 | | 18 |
| | | 4 days | 29 ± 2 | | 35 | | 15 | 93 | | 90 | 9 | | 47 |
| | | 7 days | 28 ± 3 | | 40 | | 10 | 92 | | 88 | 25 | | 25 |
| | II | 0 days | - | | 98 | | 100 | 97 | | 100 | 14 | | 12 |
| | | 6 days | 29 ± 3 | | 99 | | 97 | 100 | | 99 | 10 | | 9 |
| | | 9 days | 29 ± 3 | | 98 | | 91 | 99 | | 96 | 19 | | 32 |
| | | 12 days | 28 ± 3 | | 87 | | 40 | 97 | | 96 | 14 | | 13 |
| **NAIROBI** | | | | | | | | | | | | | |
| HOUSE | | 0 days | - | | 95 | 99 | 96 | 100 | 100 | 100 | 35 | 28 | 42 |
| | | 19 days | 20 ± 5 | 75 ± 15 | 77 | 70 | 55 | 77 | 93 | 33 | 1 | 2 | 0 |
| | | 29 days | 20 ± 5 | 75 ± 15 | 3 | 5 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 34 days | 20 ± 5 | 75 ± 15 | 10 | 5 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |

# DISCUSSION

average numbers of eggs contained in intact *E. granulosus*
*T. hydatigena* proglottids in Turkana are approximately
and 34,000 respectively.  These are within the reported
ges of average number of eggs per proglottid for these
rasites (Lawson and Gemmell, 1983).  The large variation in
numbers reported for *E. granulosus* (Arundel, 1972;
ompson and Eckert, 1982) may have been due to lack of
tailed studies on the number of eggs per proglottid.  In
present study it was shown that the number of eggs per
oglottid for *E. granulosus* is not density dependent.  The
te of development for *E. granulosus* in dogs is also not
nsity dependent (Gemmell *et al.*, 1986).  It therefore
llows the total egg production in the infected dogs in the
rthwestern Turkana is high as the prevalence of *E.*
anulosus is 63% with more than half of them carrying more
an 200 worms per dog (Fig. 5.2).

onset of egg release from the proglottids is probably
en the anterior end ruptures as the segment detaches
onnert *et al.*, 1968; Mehlhorn *et al.*, 1981).  Studies
rried out with *T. hydatigena*, *T. pisiformis* and *T.*
lticeps in dogs have shown that the majority of eggs are
leased in the gut before the segments are passed out of the
t (Rijpstra *et al.*, 1961; Featherston, 1969; Coman and
ckard. 1975; Gregory, 1976; Willis *et al.* 1981).  This
pends on the rhythmic movements of contraction and
laxations by the proglottid, and the pressure of the
gesta against the gut wall as ft passes down the gut.
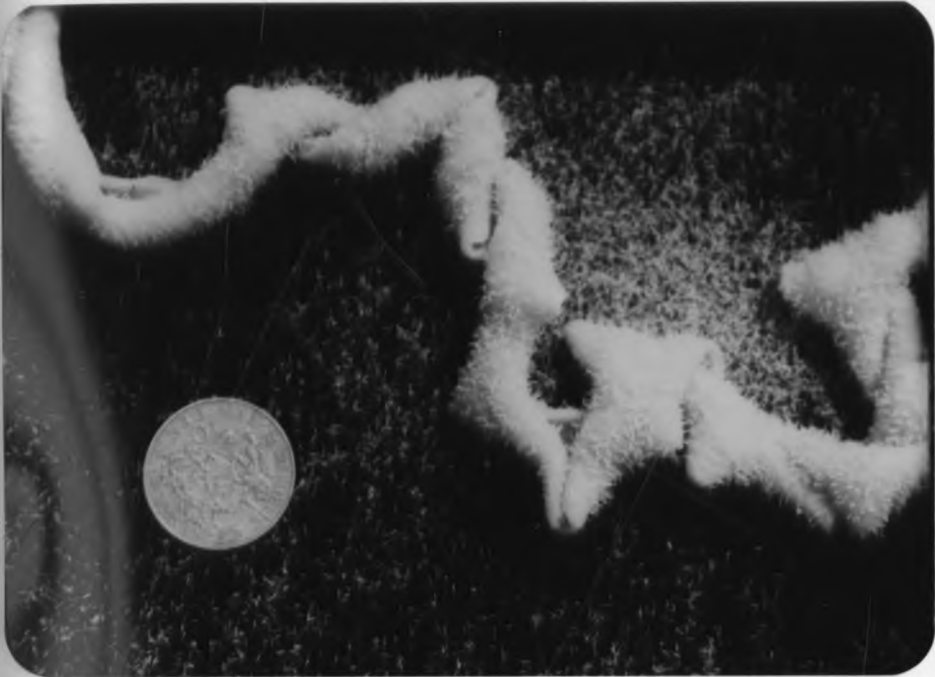
FIGURE 5.2

Longitudinally opened small intestine from
experimental Turkana dog showing a heavy infection
with *E. granulosus*

The present study has shown that about 70% of eggs of *E. granulosus*, *T. hydatigena* and *T. saginata* are released in the gut. Handling of segments by patients at collection, may have contributed to the increased release of eggs in the case of *T. saginata*. Peristaltic movements by the proglottids continue outside the host, releasing most of the remaining eggs, and only about 9% of *E. granulosus* eggs per proglottid were found by the time all movements ceased. This means that although taeniid segments may migrate under favourable conditions for more than 25cm from the faecal pat (Nosik, 1939; 1952; Sweatman and Plummer, 1957; Matoff and Kolev, 1964), this is probably not a very significant method of taeniid egg dispersal as suggested by Mattoff and Kolev (1964) and Lawson and Gemmell (1983). In the hot Turkana climate all *E. granulosus* segments were found to dry up on the surface of the faecal mass before any migration occurred.

Outside the host, many factors may affect egg survival; these include microbial contamination, temperature, desiccation, and age (Silverman, 1956; Laws, 1968; Coman, 1975; Gemmell, 1977; reviews by Lawson and Gemmell, 1983; Gemmell and Lawson, 1986). When the results on the survival of taeniid eggs in days are plotted against temperature (Fig. 5.3), it is apparent that temperature is one major factor determining taeniid egg survival. This, and similar conclusions made by Coman (1975), Gemmell, (1977) and Schaefer (1986), does not support the suggestion by Laws (1968) that the importance of temperature in causing mortality of taeniid eggs is only in its influence on the rate of water loss. Irrespective of taeniid species and method of assay (*in vitro* or *in vivo*),

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the survival of the eggs is shown to be maximum at temperatures between $0^{\circ}$C and $10^{\circ}$C (Fig. 5.3). Within this temperature range *Echinococcus* eggs have been shown to be infective after being held in water for more than seven months (Thomas and Babero, 1956; Sweatman and Williams, 1963b; Schaefer, 1986). In temperatures beyond $45^{\circ}$C, however, taeniid eggs perish within hours of exposure. Humidity does not seem to have any effect on their survival (Nosik, 1952; Meymerian and Schwabe, 1962; Williams, 1963; Williams and Colli, 1970; Colli and Williams, 1972; Gemmell, 1977; Schaefer, 1986). In Turkana, midday sand temperatures usually exceed $60^{\circ}$C and it was therefore not surprising that *E. granulosus*, *T. hydatigena* and *T. saginata* eggs kept on the sand remained viable for only less than three hours.

The eggs kept in the Turkana house environment at $25^{\circ}$C to $35^{\circ}$C and approximately 35% relative humidity, remained viable for two days whereas those in the cooler Maasai climate and higher humidity survived markedly longer. Coman (1975) was able to infect rabbits with *T. pisiformis* eggs kept at $37^{\circ}$C to $39^{\circ}$C for seven days, but they were maintained at a higher relative humidity level than found in Turkana.

The survival of *E. granulosus*, *T. hydatigena* and *T. saginata* eggs in water for more than twelve days at $30^{\circ}$C was similar to that shown by Ross (1929), who was able to infected guinea-pigs with *E. granulosus* eggs kept in water at $30^{\circ}$C for 11 days.

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TIME (DAYS)

324
270
216
162
108
54

-50    -30    -10    10    30    50    70    90
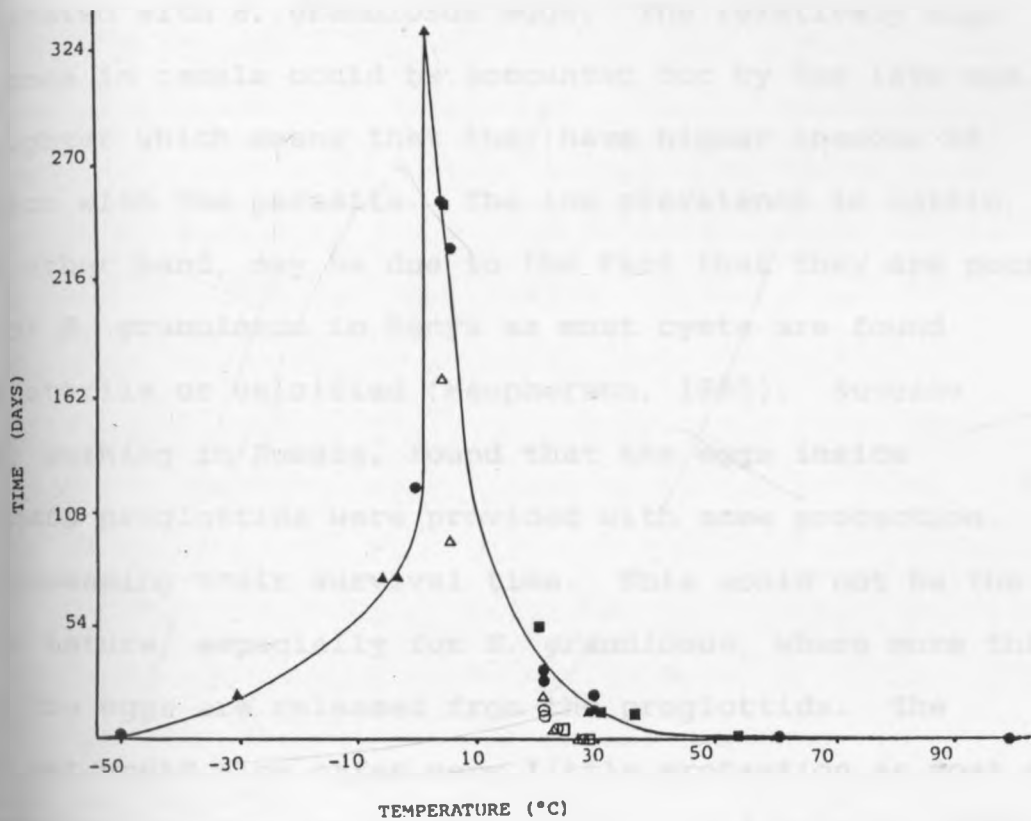
TEMPERATURE (°C)

Survival of E. granulosus (●), T. hydatigena (■) and T. saginata (▲) eggs at different temperatures and relative humidity above 85%. The survival of E. granulosus (○), T. hydatigena (□) and T. saginata (△) eggs at relative humidity below 85%-----these were not taken into account whilst drawing the line of best fit.

(Data from Appendix 5.2 and including results of the present study.)

FIGURE 5.3

Survival rates of taeniid eggs at different temperatures

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The results obtained in the present study help to explain the difference in the prevalence of hydatidosis in man and his livestock in Turkana. In a land that has very little vegetation cover, the low disease prevalence in sheep and goats (Macpherson, 1981) may be due to the "sterilizing" effect of prolonged high temperatures on dog faeces contaminated with *E. granulosus* eggs. The relatively high prevalence in camels could be accounted for by the late age of slaughter which means that they have higher chances of infection with the parasite. The low prevalence in cattle, on the other hand, may be due to the fact that they are poor hosts of *E. granulosus* in Kenya as most cysts are found either sterile or calcified (Macpherson, 1985). Suvorov (1965), working in Russia, found that the eggs inside *T.saginata* proglottids were provided with some protection, thus increasing their survival time. This would not be the case in nature, especially for *E. granulosus*, where more than 90% of the eggs are released from the proglottids. The faecal pat would also offer very little protection as most of the eggs are found on the surface (Lawson and Gemmell, 1983). Comparatively, livestock in Maasailand have a higher incidence of the disease than in Turkana, as the Maasai climate, which is closely comparable to that of Nairobi, is much cooler and more humid, making it more favourable to survival of the taeniid eggs.

Man in Turkana, unlike his animals, is much more exposed to infective *E. granulosus* eggs as a result of his habits and practices. According to Watson-Jones and Macpherson (1988), the Turkana people in the north of the district who have a

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higher disease prevalence than those in the south, also have more contact with their dogs. They were also observed to tolerate the dogs in their "akai" (huts), allowing them to sleep there and lick the food utensils used by the families. Infected dogs are known to carry taeniid eggs on the hairs around the anal region, muzzles and paws, (Nosik, 1952; Matoff and Kolev, 1964). Gravid proglottids also occasionally crawl out of their anus. Due to the shortage of water most of the dogs in Turkana are used as "nurse-dogs", licking babies when they vomit or defaecate, and so spend most of their time around the "akais". The chances of infected dogs contaminating peoples' hands, utensils and living areas with infective *E. granulosus* eggs, are therefore very real. In Turkana, women appear to spend more time with the dogs than men do and they have a higher disease incidence than men (French and Nelson, 1982). However, it is surprising that Watson-Jones and Macpherson (1988) found no correlation between the total time dogs spent in the presence of different people and the disease incidence, but they showed a statistically significant correlation between the incidence of human hydatidosis and the amount of time the dogs spent in the "akais". This supports the point made on the importance of favourable environment for survival of eggs in the disease transmission.

Water is another source of hydatid infection in Turkana. In the heat of the day, the author has observed dogs cooling themselves by dipping in water-holes, the only source of water in Turkana for most people in the dry season.

Stevenson and Macpherson (1982) counted upto 800 taeniid eggs per litre of water drawn from one of the water-holes in the northwestern Turkana. Although the people normally avoid stirring the water while drawing, there are times when the level drops and makes it inevitable, thus resuspending the normally sedimented taeniid eggs. As *E. granulosus* eggs can remain viable in water for days, people may swallow infective eggs with the water.

Different types of flies have been shown to transport infective taeniid eggs either internally or externally (Lawson and Gemmell, 1983). As flies occur in large numbers around the people in Turkana and Maasailand, especially in the rainy season, it is easy to envisage how they could contaminate human food with *E. granulosus* eggs.

The reasons for the large difference between the incidence of hydatidosis in man and his livestock in Turkana are therefore fairly clear, but it is not easy to explain why the incidence among the Maasai people is low despite similar behavioural practices to the Turkana (Macpherson, personal communication). Although the climatic conditions in Turkana do not favour the survival of taeniid eggs, the incidence of human hydatidosis continues to be the highest in the world (French and Nelson, 1982; French *et al.*, 1982; Macpherson *et al.*, 1987) due to human social behaviour. These have thus become the main points stressed in the education of the people as part of the current pilot control programme in Turkana district.

Chapter 6

E. *GRANULOSUS*: TREATMENT IN DOGS BY CONTROLLED DRUG RELEASE
FORMULATION

.1 INTRODUCTION

Up until twelve years ago, there was no highly effective drug
for the treatment of echinococcosis in the definitive host
and control measures against the adult stage consisted mainly
of arecoline purge and destruction of the unwanted dogs
(Gemmell, 1979). With the introduction of praziquantel, a
drug which has 100% efficacy against both immature and mature
stages of *E.granulosus* in a single dose rate of 5mg/kg
(Thakur *et al.*, 1978), it is now possible to achieve a rapid
break in the transmission of hydatidosis. However,
praziquantel given to dogs is rapidly eliminated from the
body in less than two days (Andrews, 1976). In addition,
immunity in dogs against this parasite is poor (Gemmell and
Soulsby, 1968; Herd, 1977; Gemmell *et al.*, 1985) and
reinfection can therefore occur soon after praziquantel
treatment. Thus, to prevent environmental contamination with
Echinococcus eggs, and hence transmission of the disease,
most hydatid control programmes have adopted a dog dosing
interval of six weeks, which is generally accepted as the
prepatent period of the parasite (WHO, 1982).

To be of any value as a control measure, mass dog treatment
needs to be sustained until longer lasting measures, like

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behavioural change in people through education, have fully been effected and the incidence of infection much reduced. The development of a treatment regime or vaccine that would provide prolonged protection against reinfection with Echinococcus would therefore be of real advantage to hydatid control programmes. This would be more so in areas like Turkana where the dog population is sparse and distributed in a large area with poor communication.

Sustained drug release has been experimented on in the treatment of malaria (Powers, 1965; Judge and Howells, 1979), Litomosoides carinii (Collins, 1974), schistosomiasis (Olanoff et al., 1980; Marshall, 1982a), Hymenolepis nana (Marshall, 1982b) and echinococcosis (Belousov, 1985). Belousov (1985) was able to protect dogs from Echinococcus infection for the first 35 days of praziquantel treatment injected subcutaneously with silicone as a prolonger, but in the subsequent 110 days the drug only inhibited the development of the worms.

The present study examines the possible use of controlled release glass encapsulated praziquantel in the treatment of E. granulosus infections, with an aim of obtaining a prolonged dog treatment regime. Due to the high risks of infection involved in maintaining dogs with patent infections of Echinococcus, T. hydatigena is used as a model.

## MATERIALS AND METHODS

### .2.1  Dogs

en puppies aged three to five months and weighing not more
than 8kg, were purchased and reared in Lokichokio as
escribed in Chapter 3.

### .2.2  Praziquantel capsules

lass capsules manufactured by Standard Telecommunication
aboratories, Harlow, Essex, Britain were obtained from Dr.
rshall of Liverpool School of Tropical Medicine, England,
nd used in the present experiment.  The degradable capsules
ere primarily made of oxides of calcium, sodium and
hosphorous. Each capsule measured 14x3mm and contained 20mg
f praziquantel.  The capsules lumen was eccentric to enable
he collapse of one length of wall to ensure rapid release of
he drug, while maintaining the strength of the capsule frame
Fig. 6.1).

### .2.3  Infection of dogs with *T. hydatigena*

he puppies were each fed with five *Cysticercus tenuicollis*
sts obtained from Lokichokio slaughter slab.  Prior to
aziquantel capsule implantations, the infections were
firmed either by laparotomy or presence of the tapeworm
gments in the host faeces, depending on the number of days
st cyst-feeding (Table 6.1).  The surgical operations were
rried out under general anaesthesia and a three day post-
rgical course of procaine penicillin treatment was given as
antibiotic cover.

A

14 mm

3mm

thin walled section
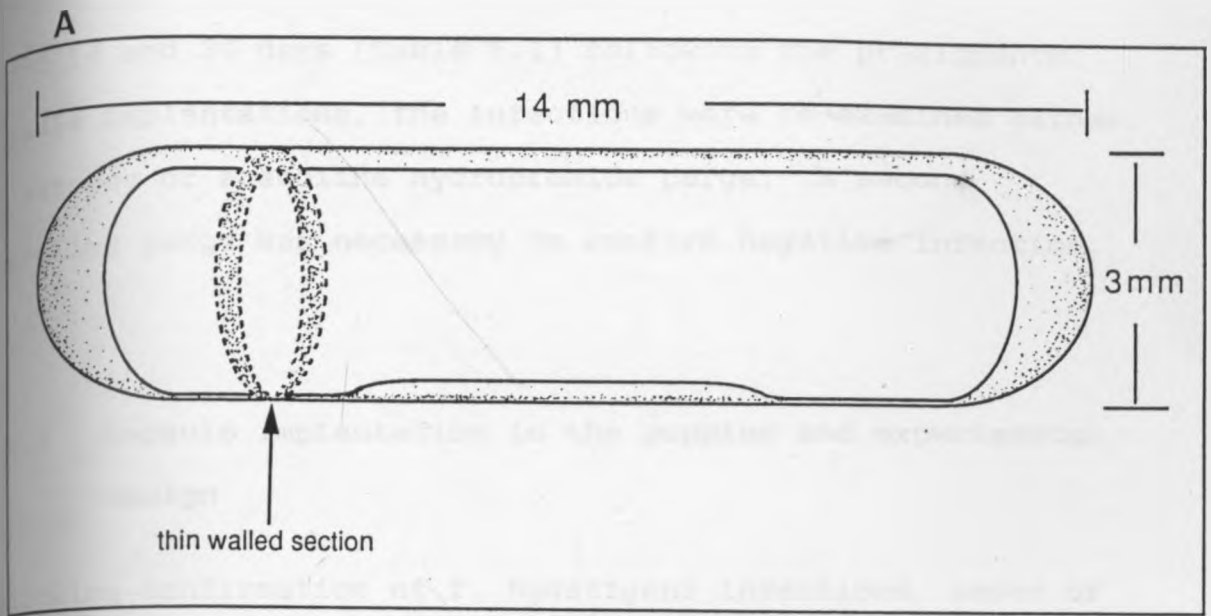
FIGURE  6.1 A.   Diagramatic representation of the degradable
capsule
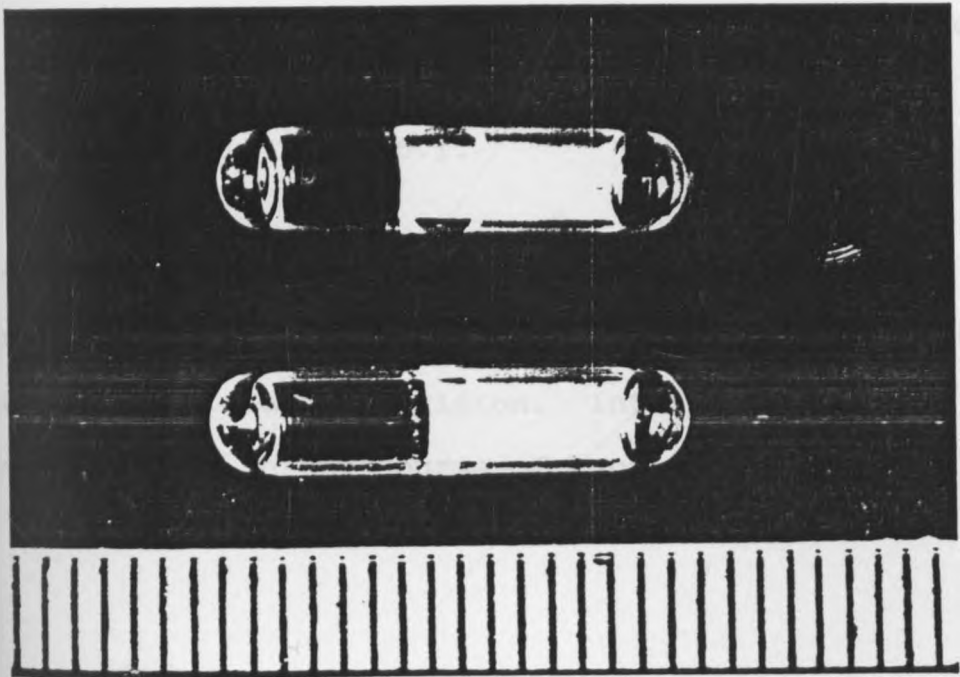


FIGURE 6.1 B.   A photograph of the capsules with millimetre
scale provided

After 14 and 30 days (Table 6.1) following the praziquantel capsule implantations, the infections were re-examined either by autopsy or arecoline hydrobromide purge. A second arecoline purge was necessary to confirm negative infection.

### 6.2.4 Capsule implantation in the puppies and experimental design

Following confirmation of *T. hydatigena* infections, seven of the ten puppies received two subcutaneous capsule implantations each, one received two peritoneal implantation and the last two remained as untreated controls. The capsules were dipped in a solution of penicillin/streptomycin to minimize any bacterial contamination. The location of the implants in each puppy and the rest of the experimental design are summarized in Table 6.1.

Capsules were implanted into the subcutaneous tissue under local anaesthesia. This was a simple operation requiring only two sutures to close the incision. Intraperitoneal capsules were inserted in the course of laparotomy.

TABLE 6.1

## Controlled release glass encapsulated praziquantel treatment in dogs infected with *Taenia hydatigena*

| Dog No. | No. of cysts fed | Examination of the infections in dogs | | | Praziquantel capsule implants | | | Re-examination of the infections in dogs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Method | DPI | +/- | No. | site | | Method | DPI | DPIM | +/- |
| 1 | 5 | Laparotomy | 28 | + | 2 | Sternum | | Autopsy | 42 | 14 | - |
| 2 | 5 | Laparotomy | 28 | + | 2 | B. scapula S/Q | | Autopsy | 42 | 14 | - |
| 3 | 5 | Laparotomy | 28 | + | 2 | Peritoneal | | Autopsy | 42 | 14 | + |
| 4 | 5 | Faecal examination | 60 | + | 2 | B. scapula S/Q | | Arecoline purge | 90 | 30 | - |
| 5 | 5 | Faecal examination | 60 | + | 2 | B. scapula S/Q | | Autopsy | 90 | 30 | - |
| 6 | 5 | Faecal examination | 60 | + | 2 | B. scapula S/Q | | Arecoline purge | 90 | 30 | - |
| 7 | 5 | Faecal examination | 60 | + | 2 | B. scapula S/Q | | Autopsy | 90 | 30 | - |
| 8 | 5 | Faecal examination | 60 | + | 2 | B. scapula | | Arecoline purge | 90 | 30 | - |
| 9 | 5 | Faecal examination | 60 | + | * | | | Arecoline purge | 90 | 30 | + |
| 10 | 5 | Faecal examination | 60 | + | * | | | Arecoline purge | 90 | 30 | + |

| | |
|---|---|
| DPI | Days post infection |
| DPIM | Days post capsule implants |
| S/Q | Subcutaneous |
| B scapula | Between the scapulas |
| + | Infected |
| - | Not infected |
| * | Control dog |

The puppies recovered well from the capsule implant operations and the wounds were hardly detectable after a week. They showed no signs of discomfort or irritation associated with the implants. An exception to this was puppy number 6, which had a bacterial infection on the incision wound; this was, however arrested by a four-day course of procaine penicillin treatment.

All the puppies that received subcutaneous capsule implantation were cleared of the *T. hydatigena* infections in about two weeks (Table 6.1). In contrast, puppy number 3 in which the capsules were implanted peritoneally and the control puppies, were still infected after 14 and 30 days respectively.

Capsules implanted subcutaneously were detectable on palpation immediately after implantation but not after day 17 post-implantation. All the implanted capsules were .pa recovered intact from puppy number 3. Capsules in puppies 1 and 2 were open at the thin walled section (Fig. 6.2) and approximately half of the praziquantel remained in each capsule. No capsules were detected in puppies autopsied 30 days post-implantation. In addition, no tissue reactions were observed at the implant sites in any of the autopsied puppies.

## 4.4 DISCUSSION

Seven of the eight dogs infected with *T. hydatigena* were cleared of the infections following subcutaneous implantation of the praziquantel capsules. The capsules were well tolerated by the puppies, causing no obvious side effects. Capsules implanted into dog number 3 were still intact 14 days later, and the infection in this puppy was retained, as were the infections in the control dogs. Degradation of the capsule wall therefore seems to be better in a subcutaneous rather than peritoneal site, a fact which is probably due to differences in blood supply and tissue contact.

A drug delivery system that exposes the parasite to sublethal drug concentrations, such as described by Belousov (1985), may provides conditions favourable to the development of resistant strains. Although resistance to anthelmintics has never become the problem that one associates with the use of antimalarials, insecticides or antibiotics, isolated cases have been reported over the years (Brander *et al.*, 1982). Development of *Haemonchus contortus* resistant strains against thiabendazole reported from Australia was partly associated to the usage of the drug at sublethal doses (Le Jambre *et al.*, 1978). The use of degradable capsules, however, ensures a fast release of chemotherapeutic dose of the drug into the blood system. This method has an added advantage in that less of the drug is used as opposed to the slow release method.

open sections

remaining praziquantel
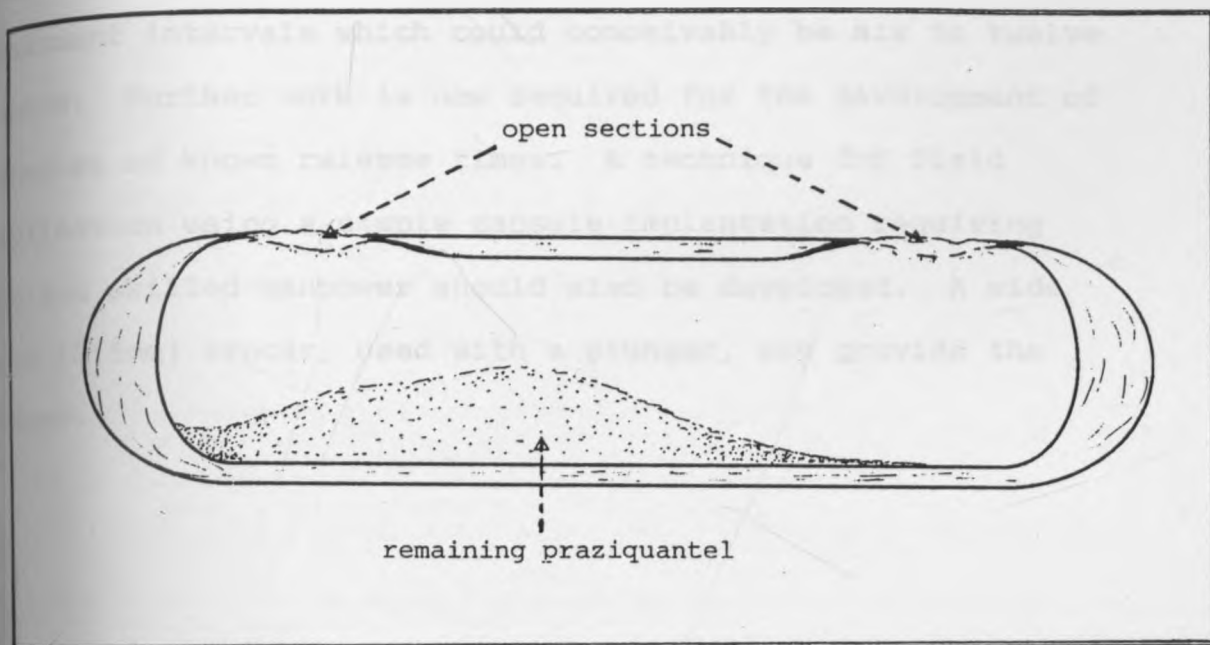
FIGURE 6.2

Diagramatic representation of a degradable
capsule open at the thin sections of the wall
and less than half of praziquantel remaining
14 days post implantion.

The time taken for the release of the drug can be regulated by increasing or decreasing the thickness of the thinner section of the capsule wall or by changing the composition of the capsules. By implanting capsules of known different drug release times, it would be feasible to achieve prolonged dog treatment intervals which could conceivably be six to twelve months. Further work is now required for the development of capsules of known release times. A technique for field application using a simple capsule implantation requiring minimum skilled manpower should also be developed. A wide bore (3.5mm) trocar, used with a plunger, may provide the answer.

# Chapter 7

## GENERAL DISCUSSION

It was recognized right from the onset of the Turkana pilot hydatid control programme that, like in all previous programmes in other parts of the world, education of the local population to the dangers of the disease, and motivating them to do something about it, was the key to its success (Macpherson *et al.*, 1984b).

The high infection pressure to dogs with *E. granulosus* in the control area, though partly explained by the drastic reduction in the dog population (chapter 4), testifies to the fact that long lasting changes in human behaviour usually take time. There is therefore a need for intensification of the educational campaign in order to reduce the amount of hydatid material made accessible to dogs. This need is more acute towards the end of drought periods, when infection pressure to dogs with the parasite seems to be highest (chapter 4). As the aims of the education programme take root, the most important step in the control of the disease in Turkana will remain interruption of transmission of the parasite from the domestic dog. This includes routine mass dog dosing with Droncit[R]. The Turkana dog dosing regime was adopted after the WHO guidelines (WHO, 1982) drawn from the fact that the prepatent period of *E. granulosus* in many parts of the world is shown to be six weeks (Gemmell, 1962; Smyth

*et al.,* 1967). A shorter prepatent period of the parasite in dogs, such as was demonstrated in the present study (chapter 3), has in the recent past been reported by Kumaratilake (1982) and Thompson *et al.* (1984). It is therefore clear that generalization concerning the timing of drug treatments used for controlling *E. granulosus* infections in dogs can no longer be made.

The significance of the prepatent period in the choice of a dog dosing regime, in any hydatid control programme must, however, be viewed in the light of the rate of reinfection in dogs with the parasite, following treatment. Effective dog dosing intervals of durations longer than the prepatent period of *E. granulosus*, can only be envisaged in areas of very low rate of reinfection. The relationship between the dog dosing interval in Turkana and the rate of reinfection with *E. granulosus* can be calculated from a reinfection regression equation describing the autopsy regression line (Fig. 4.4), and given by:

$$y = a + bx + 2.378$$

For a large number of  n, one can assume that  y  in the transformation equation (chapter 4) is very nearly

$$\ln (z/n - z).$$

It therefore follows that

$$\ln (z/n - z) = \ln (p/q)$$

where  p  is the proportion of dogs infected

with *E. granulosus* in the control area

and  q  is the proportion of dogs not infected.

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the proportion of dogs that would be reinfected with *E. granulosus* when a six weekly dosing regime is used, is p in the following equation:

$$\ln (p/q) = -4.977 + 6(0.1589) + 2.378$$

p = 16% (ie 32 dogs out of 200 in the control area).

Both the prepatent period and the rate of reinfection in dogs with *E. granulosus* in Turkana, therefore, indicate that the dosing interval should be reduced from the present interval of six weeks to five weeks. Although at this interval, fourteen percent of the dogs in the control area would continue to be reinfected, the infections would not be patent, and hence there would be no risk of disease transmission to man.

The biotic potential of *E. granulosus* is high, and the production of eggs in the parasite is not density dependent (chapter 5). Gemmell *et al.* (1986) has also shown that the rate of development of the parasite in the definitive host is also not density dependent. It therefore requires only a small number of infected dogs to maintain the disease transmission cycle in Turkana, a region where infections in dogs of 50,000 or more gravid worms are not uncommon (Nelson and Rausch, 1963; Macpherson, 1985). In order to reduce the disease transmission as far as possible, it is then necessary that the regular treatment schedule be maintained in all dogs in the control area. Upto the present time, this has not been practicable in Turkana, due to difficulties experienced

in tracing and reaching the dogs, mainly because of the nomadic way of life of the people and poor road communication in the region (Macpherson *et al.*, 1986b).

In view of the problems encountered in regular treatment of dogs in Turkana, the importance of a more prolonged protection against reinfection in dogs cannot be overstressed. Results obtained in treatment of tapeworm infections in dogs, with controlled release encapsulated praziquantel (chapter 6), show that the drug formulation has a promising future in this area. Further studies are required in the development of capsules of different release times, which would allow for prolonged treatment intervals in dogs.

Purging of dogs with arecoline hydrobromide in the control area is shown to detect only 10% (as in the following formular) of *E. granulosus* infected dogs.

$$y = a + bx \quad \text{(arecoline regression equation, Figure 4.4)}$$

$$\ln (p/q) = -4.997 + 5(0.1589)$$

$$p = 1.5\% \quad \text{(ie 3 dogs out of 200 in the control area)}.$$

Although this information shows that arecoline purge is not reliable in diagnosis of *E. granulosus* infections, it can be used to estimate the parasite infection rate from arecoline surveillance data. Purging of dogs has an added advantage in that it is a useful adjunct for the education programme.

The close association between man and his dog in Turkana has been cited as a major contributing factor to the high prevalence of human hydatidosis in that area (French *et al.*, 1982; Watson-Jones and Macpherson, 1988). This view is supported by the findings of the study on the survival of *E. granulosus* eggs in Turkana, which have shown that the eggs remain viable for less than three hours in the Turkana open ground (chapter 5). This stresses the significance of the education programme, pointing out the need for a decreased dog/man contact, while keeping the dogs out of the houses.

The importance of strain differentiation studies within *E. granulosus* lies in the fact that different strain characteristics may influence local patterns of transmission and the health significance of the parasite (WHO, 1982). Two strains of *E. granulosus* have been shown to occur in Kenya (chapter 3) but their epidemiological significance to the control of the disease is not as yet apparent. Further studies are required to determine the extent of the distribution of each strain among the domestic intermediate hosts, and whether the stains show any differences in their response to chemotherapeutic agents. From the available evidence (Table 3.4), however, it will be advisable for the control programme to regard the strains as cross-infective among all the domestic intermediate hosts in Turkana, and potential sources of infection to man.

## Chapter 8

### CONCLUSIONS

Based on the findings of this study the following observations and conclusions were made.

1.1 At least two strains of *E. granulosus* exist in Kenya and are probably infective to all the domestic intermediate hosts in the country.

1.2 The strains are morphologically and developmentally similar but can be differentiated on the basis of isoenzyme profiles or analysis of parasite DNA material.

2. *E. granulosus* in Kenya develops faster in Turkana dogs than in those from Nairobi.

The rate of natural infection with *E. granulosus* in dogs in the control area is high and infection level reverts to pre-control level in six months.

To reduce transmission of hydatid disease from dogs in the control area, the six-weekly treatment of dogs with praziquantel aught to be reduced to five weeks interval.

*T. hydatigena* is the only other common dog taeniid in Turkana. Diagnosis of this tapeworm in dog faeces or purge can be used in the control programme as an indicator of dogs having been fed on raw offal.

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Although purging of dogs with arecoline hydrobromide is not a reliable echinococcosis diagnostic method, it is a useful surveillance technique for the disease in domestic dog in Turkana and an adjunct for the education programme.

The increased infection intensity in dogs with *E. granulosus* observed towards the end of droughts in Turkana requires that hydatid control measures in this area be intensified at such periods.

Domestic dogs in Turkana have poor immunity against *E. granulosus*.

The use of controlled release glass encapsulated praziquantel in hydatid control programmes in order to prolong protection against reinfection with *E. granulosus* in dogs is now feasible.

The average number of eggs per proglottid in *E. granulosus* in Turkana is 825 $\pm$30. The number of eggs per proglottid is independent of the infection intensity.

Release of *E. granulosus* eggs starts soon after the proglottid detaches from the worm. By the time the proglottid leaves the host, about 70% of the eggs have already been released.

The rhythmic movements in proglottids of *E. granulosus* outside the host do not contribute to the dispersal of the parasite eggs in Turkana.

13. Survival of taeniid eggs is best at temperatures between 0°C and 10°C.

14. Open ground day temperatures in Turkana are inimical to survival of taeniid eggs.

15. Relevant changes, in human behaviour that help in maintaining the transmission cycle of hydatidosis in Turkana, is ultimately the answer to the human hydatid problem in the region.

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APPENDICES

YEARLY RAINFALL VARIATION IN THE TURKANA H
(data from the meteorology departm
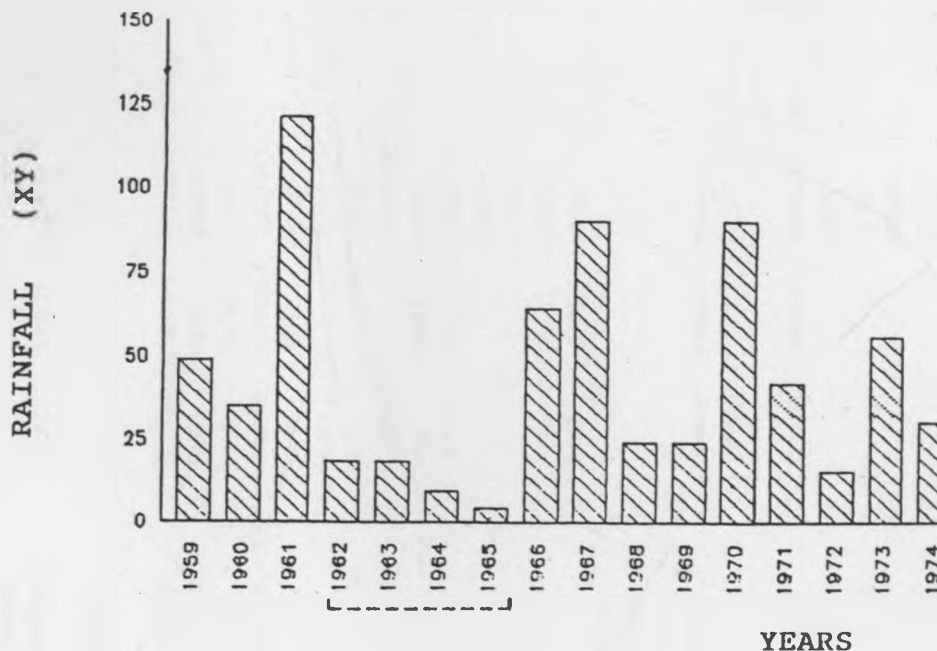


**YEARS**

|  | Data not availabe |
| --- | --- |
| * | |
| L_____J | Drought period |
| | Four consecutive years with a mean XY < |
| X | Number of months in a year with 2 rainfa |
| Y | Number of months in a year with a mean r |

[The transformation of annual rainfall by XY, showed mor
sustained significant rainfall days as this was more he
single day heavy down pour in a year.]

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# Appendix 2.1

## Prevalence and occurrence of echinococcosis/hydatidosis in domestic animals in Kenya

| Origin of animals | Dog | Intermediate hosts | | | | | References |
|---|---|---|---|---|---|---|---|
| | | Camel | Cattle | Sheep | Goat | Others | |
| - | | R | | | | | Leese, 1915. |
| - | | | | R | | | Walker, 1925. |
| - | | | R | R | | | Daubney, 1926. |
| - | | | R | R | | pigs | Hudson, 1934. |
| - | | | 30.6 | 35.1 | 16.6 | | Ginsberg, 1956. |
| whole country* | | | 25.5 (1000) | | | | Froyd, 1960a. |
| whole country* | | | 30.2 (1000) | | | | Froyd 1956b. |
| Turkana | 70.4(27) | R | common | common | common | | Nelson and Rausch, 1963. |
| Nairobi | 50(16) | | | | | | |
| Turkana | | | | | common | | |
| Whole country* | | | common | common | common | | Department of Veterinary Services Annual Reports, Kenya,1962-65; 1969; 1972; 1976-78; 1980; 1981. |
| Rift Valley | | | 29.2 (1766) | 32.3 (1834) | 33.1 (172) | | Mango, 1971. |
| Central | | | 23.7 (215) | | | | |
| Eastern | | | 17.7 (282) | 9.1 (406) | 11.1 (777) | | |
| Coast | | | 16.1(31) | | | | |
| North Eastern | | | 16.8 (622) | | 4.3 (279) | | |
| Whole country* | | | 12.8+ (1162237) | 20.3+ (489702) | 14.7+ (245231) | pigs | Ng'ang'a, 1974. |
| Kajiado | | | 46.7 (1446) | 29.5 (44) | 9.0 (100) | | Eugster, 1978. |
| Turkana | | 80(10) | 10(10) | 1.6(61) | 844(0.8) | | Macpherson, 1981. |
| | 39.4 (695) | | | | | | Macpherson et al., 1985. |
| Maasailand | | | 8.9 (1499) | 8.1 (1798) | 7.1 (2020) | | Macpherson, 1985. |
| Maasailand | 37(92) | | | | | | Ngunzi, 1985. |

R   Reported.

+   Based on Liver and lung hydatids only.

*   Mainly based on animals examined at Athi River, Kenya Meat Commision (KMC) abattoir.

(10)  Number of animals examined.

Appendix 4.1

Key to common *Taenia* species in dogs (data from verster, 1969)

1. Vaginal sphincter absent                              2

   Vaginal sphincter present                             3

2. Testes distributed in a single layer

       and confluent anteriorly only         *Taenia hydatigena*

   Testes distributed in 2-4 layers and

       confluent anteriorly and posteriorly      *T. pisiformis*

3. Vaginal sphincter reduced to a pad with

       diameter <30 um.  Ovary lobes equal        *T. multiceps*

   Vaginal sphincter complete with

       diameter >40 um.  Aporal ovary

       lobe >poral lobe                                 4

4. Large rostellar hooks >210 um and

       primary uterine branches not >10      *T. parenchymatosa*

   Large rostellar hooks <210 um and

       primary uterine branches not <10               5

5. Testes distributed in a single layer

       and primary uterine branches 11-25           *T. ovis*

   Testes distributed in 1-3 layers

       and primary uterine branches 10-18         *T. serialis*

# Appendix 4.2

### Reported maximum survival times for taeniid eggs stored under different laboratory and field conditions

| Species | Condition | Time in days | Viability assay | Reference |
|---|---|---|---|---|
| *Echinococcus* | | | | |
| *sibiriensis* | $-51^{\circ}$C | 1 | *in vivo* (voles) | Schiller (1955) |
| *E. ranulosus* | $-50^{\circ}$C | 1 | *in vivo* (mice) | Colli & Williams (1972) |
| *Taenia* | | | | |
| *saginata* | $-30^{\circ}$C | 16-19 | *in vitro* | Suvorov (1965) |
| *E. sibiriencis* | $-26^{\circ}$C | 54 | *in vivo* (voles) | Schiller (1955) |
| *E. multilocularis* | $-18^{\circ}$C (in tap water) | 240 | *in vivo* (voles) | Schaefer (1986) |
| *T. ovis* | $-9^{\circ}$C | 170 | *in vitro* | Gemmell (1977) |
| *T. saginata* | $-5^{\circ}$C | 76 | *in vivo* | Lucker (1960) |
| *T. saginata* | $-4^{\circ}$C | 77 | *in vitro* | Suvorov (1965) |
| *E. granulosus* | $-1^{\circ}$C to $1^{\circ}$C | 116 | *in vivo* (pigs) | Deve (1910) |
| *E. multilocularis* | $2^{\circ}$C (in water) | 910 | *in vivo* (voles) | Thomas & Babero (1956) |
| *E. multilocularis* | $4^{\circ}$C (in PBS) | 478 | *in vivo* (voles) | Schaefer (1986) |
| *T. saginata* | $4^{\circ}$C (in saline) | 335 | *in vitro* | Silverman (1956) |
| *T. pisiformis* | *$4^{\circ}$C (32-33% R.H.) | 56 | *in vivo* (rabbits) | Coman (1975) |
| *E. multilocularis* | $4^{\circ}$C (in PBS) | 219 | *in vitro* | Schaefer (1986) |
| *T. pisiformis* | *$4^{\circ}$C (29-94% R.H.) | 300 | *in vivo* (rabbits) | Coman (1975) |
| *T. pisiformis* | $4^{\circ}$C (in saline) | 187 | *in vitro* | Silverman (1956) |
| *T. saginata* | *$4^{\circ}$C | 168 | *in vivo* and *in vitro* (cattle) | Froyd (1962) |
| *T. saginata* | *$4^{\circ}$C (in saline) | 96 | *in vivo* (cattle) | Penfold *et al* (1937b) |
| *E. granulosus* | $6^{\circ}$C (in water) | 225 | *in vivo* (sheep) | Sweatman & Williams (1963) |
| *E. granulosus* | $7^{\circ}$C (with surface moisture) | 210-294 | *in vitro* | Gemmell (1977) |
| *T. hydatigena* | $7^{\circ}$C " | 210-294 | *in vitro* | Gemmell (1977) |
| *T. pisiformis* | 5-10$^{\circ}$C (moist chamb.) | 100-150 | *in vivo* (rabbits) | Enigk *et al* (1969) |
| *T. ovis* | $16^{\circ}$C (with surface moisture) | 91-150 | *in vitro* | Gemmell (1977) |
| *T. pisiformis* | $20^{\circ}$C (25% r.h.) | 1 | *in vitro* | Laws (1968) |
| *T. saginata* | (room temp. in saline) | 60 | *in vitro* | Silverman (1956) |
| *E. granulosus* | (sunlight & desiccation) | 11 | *in vivo* (pigs) | Deve (1910) |
| *T. pisiformis* | (room temp. in saline) | 60 | *in vitro* | Silverman (1956) |
| *T. saginata* | (room temp. with no moisture) | 14 | *in vitro* | Silverman (1956) |
| *T. hydatigena* | $21^{\circ}$C (no surface moisture) | 7 | *in vitro* | Gemmell (1977) |
| *E. granulosus* | $21^{\circ}$C " | 7 | *in vitro* | Gemmell (1977) |
| *T. hydatigena* | $21^{\circ}$C (with surface moisture) | 56 | *in vitro* | Gemmell (1977) |
| *E. granulosus* | 10-21$^{\circ}$C (in water) | 32 | *in vivo* (mice) | Batham (1957) |
| *E. granulosus* | $21^{\circ}$C (with surface moisture) | 28 | *in vitro* | Gemmell (1977) |
| *E. granulosus* | $30^{\circ}$C (in water) | 21 | *in vivo* (guinea-pigs) | Ross (1929) |
| *T. pisiformis* | $38^{\circ}$C (90% R.H.) | 7 | *in vivo* (rabbits) | Coman (1975 |

| Species | Condition | Time in days | Viability assay | Reference |
|---|---|---|---|---|
| *T. hydatigena* | 38°C (in water) | 7 | *in vitro* | Gemmell (1977) |
| *T. ovis* | 45-65°C (in water) | (few hours) | *in vitro* | Gemmell (1977) |
| *T. hydatigena* | 45-65°C (in water) | (few hours) | *in vitro* | Gemmell (1977) |
| *E. multilocularis* | 45°C (85-95%R.H.) | (2 hours) | *in vivo* (voles) | Schaefer (1986) |
| *E. granulosus* | 55°C (saline) | (5 minutes) | *in vivo* (mice) | Colli & Williams (1972) |
| *E. granulosus* | 60°C (moist heat) | (10 minutes) | *in vitro* | Meymerian & Schwabe (1962) |
| *T. hydatigena* | 65°C (saline) | (1 minute) | *in vitro* | Williams & Colli (1970) |
| *E. granulosus* | Boiling water | (20 sec) | *in vitro* | Nosik (1952) |
| *E. granulosus* | In sachets on ground | 630 | *in vivo* | Vibe (1968) |
| *T. solium* | Winter in France | 365 | *in vivo* (pigs) | Davaine (1863) |
| *E. granulosus* | Cold & dry New Zealand weather | 365 | *in vivo* (sheep) | Sweatman & Williams (1963) |
| *T. saginata* | Kenyan pasture | 413 | *in vivo* (cattle) | Duthy & van Someren (1948) |
| *E. multilocularis* | German winter (on sunny ground) | 240 | *in vivo* (voles) | Schaefer (1986) |
| *E. multilocularis* | German winter (on shaded area) | 172 | *in vivo* (voles) | Schaefer (1986) |
| *T. saginata* | Denmark winter | 161 | *in vivo* (cattle) | Jespen & Roth (1952) |
| *T. pisiformis* | Australian winter | 125 | *in vivo* (rabbits) | Coman (1975) |
| *T. pisiformis* | Australian summer | 0 | *in vivo* (cattle) | Coman (1975) |
| *T. saginata* | Kenyan pasture | 102 | *in vivo* (cattle) | Penfold *et al* (1937b) |
| *T. hydatigena* | Russian winter (-10 to 10°C) | 60 | *in vivo* (sheep) | Abbasouv (1965) |
| *T. saginata* | in silage (10°C) | 60-80 | *in vivo* (cattle) | Egnigk *et al* (1969) |
| *T. saginata* | in silage (20°C) | 30-40 | *in vivo* (cattle) | Egnigk *et al* (1969) |
| *T. saginata* | stored hay | 21 | *in vivo* (cattle) | Lucker and Douvres (1960) |
| *E. multilocularis* | German summer (sunny or shadded area) | 112 | *in vivo* (voles) | Schaefer (1986) |
| *T. hydatigena* | Russian summer | 30 | *in vivo* (sheep) | Abbasouv (1965) |
| *E. mutilocularis* | German summer (sunny or shadded area) | 15 | *in vitro* | Schaefer (1986) |
| *T. pisiformis* | Australian summer | 7 | *in vivo* (rabbits) | Coman (1975) |

* Average temperature (2 - 5°C