

EPIDEMIOLOGY AND STRAIN DIFFERENTIATION
OF ECHINOCOCCUS GRANULOSUS IN KENYA

BY

CALUM NORMAN LINDSAY MACPHERSON B.Sc. (Hons)

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Department of Zoology and Applied Entomology,
Imperial College of Science and Technology,
Prince Consort Road,
London SW7 2BB.

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'I know of no more futile effort than fishing for specific characters in a cestode or trematode gene-pool. When life-cycles have been worked out, when larval stages and asexual generations are known, when the ranges of possible intermediate and definitive hosts are determined, and the effects of the parasite of nutritional and other physiological conditions of the hosts have been assessed, we can begin to define morphological species with real assurance. In the meantime, specific determination remains exceedingly difficult. Host varieties may be recognized, but in my opinion, subspecies of trematodes and cestodes have no meaning. The problems of intraspecific variation in parasitic flatworms are formidable, but not insuperable.'

Stunkard (1957)

ABSTRACT

The occurrence and prevalence of hydatidosis in man, domestic and wild animals in Africa is discussed with that found in Kenya, where the disease is a major public health and economic problem. A detailed comparative examination of the current prevalence and intensity of infection of the disease in man, domestic and wild animals in Turkana District in north-western Kenya and Masailand in southern Kenya is made. The possible role played by each of the intermediate and definitive hosts in the epidemiology of hydatidosis in these two regions is assessed.

Domestic dogs (Canis familiaris), spotted hyaenas (Crocuta crocuta) and silver-backed jackals (Canis mesomelas) were experimentally infected with hydatid material from several intermediate hosts to assess their susceptibility to infection with Echinococcus granulosus.

Attempts were made to examine whether biological strains of E. granulosus exist in different hosts in Turkana and Masailand. These investigations included; morphological examination of the parasite from various definitive and intermediate hosts; isoelectric focusing of larval and adult parasite material from different hosts and in vitro cultivation of larval material obtained from the numerous intermediate hosts. The significance of the results obtained, is discussed in relation to the prevalence data and the overall epidemiological findings are reviewed.

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GENERAL INTRODUCTION

Echinococcosis (hydatidosis) is a cyclo-zoonotic disease of major economic and public health significance in all the inhabited continents of the world. The causative agent is the metacestode or cystic stage of the parasites which belong to the genus Echinococcus Rudolphi 1801 (Cestoda:Taeniidae).

In man it is a particularly important disease, not only because of the morbidity it causes but also due to the lack of an effective chemotherapeutic agent. At present surgery remains the main form of treatment, which often results in a poor prognosis.

In Kenya, echinococcosis is considered a major economic problem due to the high prevalence in the domestic livestock (Ginsberg, 1956; Mango, 1971; Ng'ang'a, 1974; Eugster, 1978). Hydatid disease in humans, however, is only rarely encountered, except in the north-western desert region of Turkana, which has one of the highest incidences of the disease in the world (Schwabe, 1969; O'Leary, 1976; AMREF, 1978; 1979; 1980).

This study is concerned with assessing this situation and deals with various aspects of the epidemiology and biology of the parasite in Turkana, Narok, Kajiado and Marsabit Districts in Kenya. The overall distribution and prevalence of hydatidosis found in Kenya is compared with the occurrence of the disease in other countries, particularly in Africa.

The causative organism of hydatidosis in Kenya is Echinococcus granulosus (Nelson & Rausch, 1963). However, numerous intra-specific 'variants' of E. granulosus have been reported and a complex speciation pattern exists within this species (Verster, 1965; Rausch, 1967b; Smyth & Smyth, 1968; Thompson 1979).

Smyth & Smyth (1968) state that 'speciation in Echinococcus is a complex matter with perhaps well-defined species at each end of a hypothetical scale having morphological, physiological and immunological characteristics recognisable as belonging to either E. granulosus or E. multilocularis. Between these two extremes are a variety of 'races', 'strains' or 'variants' incorporating characteristics belonging to both species.' The position of strain differentiation in Echinococcus could be regarded as being similar to that found in micro-organisms where it has been stated (Wilson, 1955) 'The conception of species as held by Linnaeus was based on the belief in a fixed natural order in the world of living things. Experience however, has shown that the demarcation of species is often far from clear, and that in many instances gradations can be traced between them... the task of defining species seems to be almost insuperable. To abandon the concept of species would be unfortunate... it seems that the only practicable method of classifying and naming micro-organisms is to establish a series of nodal points along the continuous chain of variants, and to regard the organisms at these nodes, and for some distance on either side of them, as constituting species. The criteria used for selecting these nodes, and for determining the distance on either side of these nodes within which variants of the species might be included, will clearly need considerable discussion.'

Smyth & Smyth (1964) point out that parasites of the genus Echinococcus have a mode of reproduction which favours the expression of mutants, with the result that new intraspecific 'variants' can readily arise. It is hardly surprising, therefore, that a large number of intraspecific 'variants' of E. granulosus have been reported,

and that a complex speciation pattern exists within this species. The taxonomic status of such 'variants' is uncertain although at present it is the practice to refer to such intraspecific 'variants' as 'strains' (Smyth, 1977; World Health Organisation, 1979, 1981). The point at which differences between populations become sufficiently large to justify them being termed a 'strain' is clearly an arbitrary one and cannot be precisely defined (Smyth, 1981). In this context, Smyth (1981) has suggested that a major criteria of a strain difference could be if one 'strain' does not develop in the host of the other and vice versa, although he acknowledges that this may not always hold true. Additionally, there is increasing evidence that not only do 'strains' exist within E. granulosus, but that geographical 'sub-strains' may also exist, even within the same host (McManus, 1981).

During this study an attempt was made to examine certain biological characteristics of the parasite, in an effort to determine whether or not any strain 'variation' existed within the parasite from different hosts in Kenya. In particular, morphological, isoenzymatic and in vitro and in vivo growth characteristics of the parasite from various hosts were examined. Where the identity of the material was not recognised as a 'specific strain', the term 'form' has been used in this text in common with normal biological usage, although the imprecise nature of using such a term is recognised.

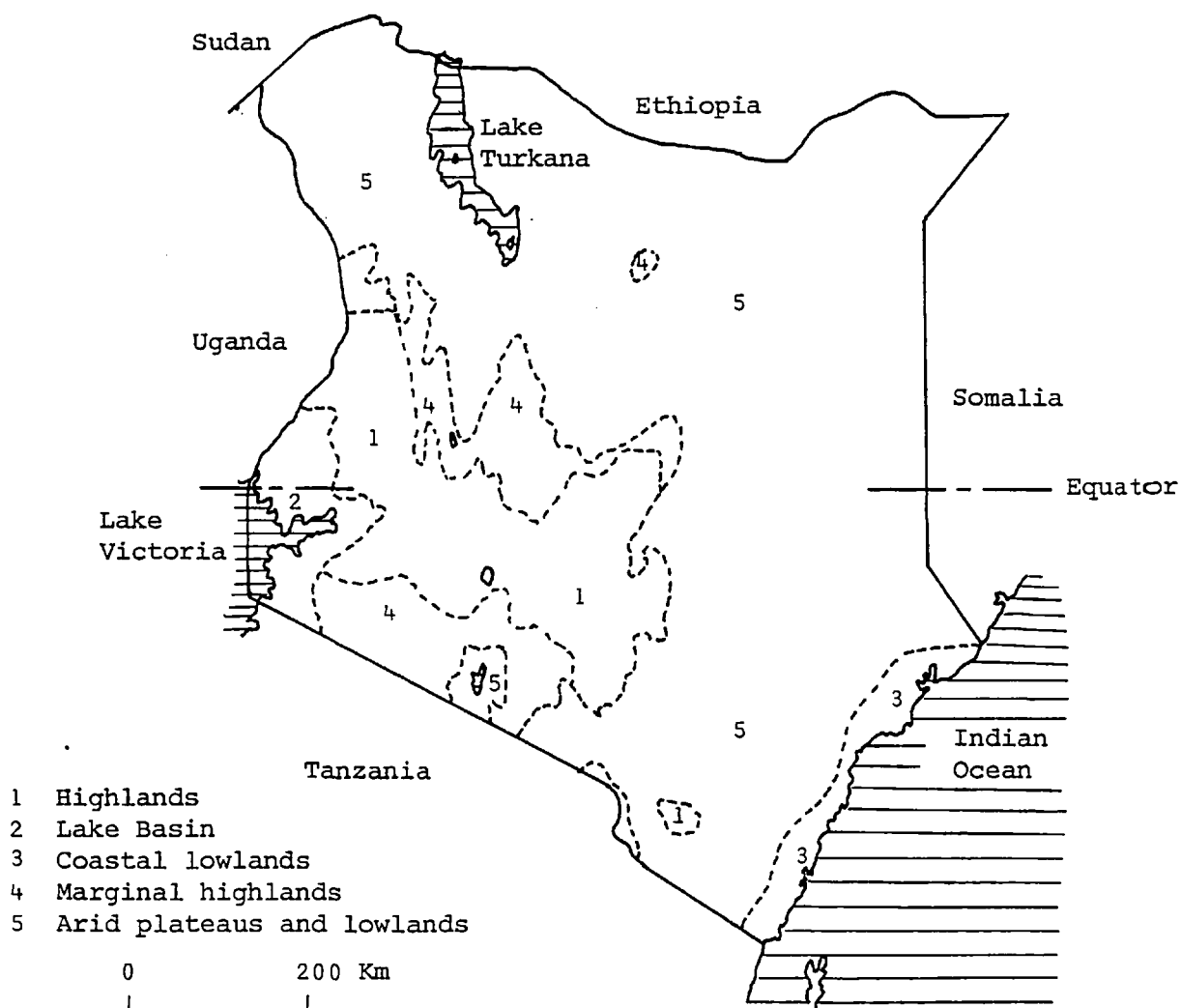
CHAPTER 1

STUDY AREA

1. General features of Kenya

Kenya lies on the equator between approximately $4^{\circ} 30'$ north and $4^{\circ} 31'$ south. The climate is greatly modified by altitude and much of the country enjoys a Mediterranean-type climate. The country can be divided broadly into five natural regions (Figure 1).

Figure 1. Natural Regions of Kenya (After Ojany & Ogendo, 1973)



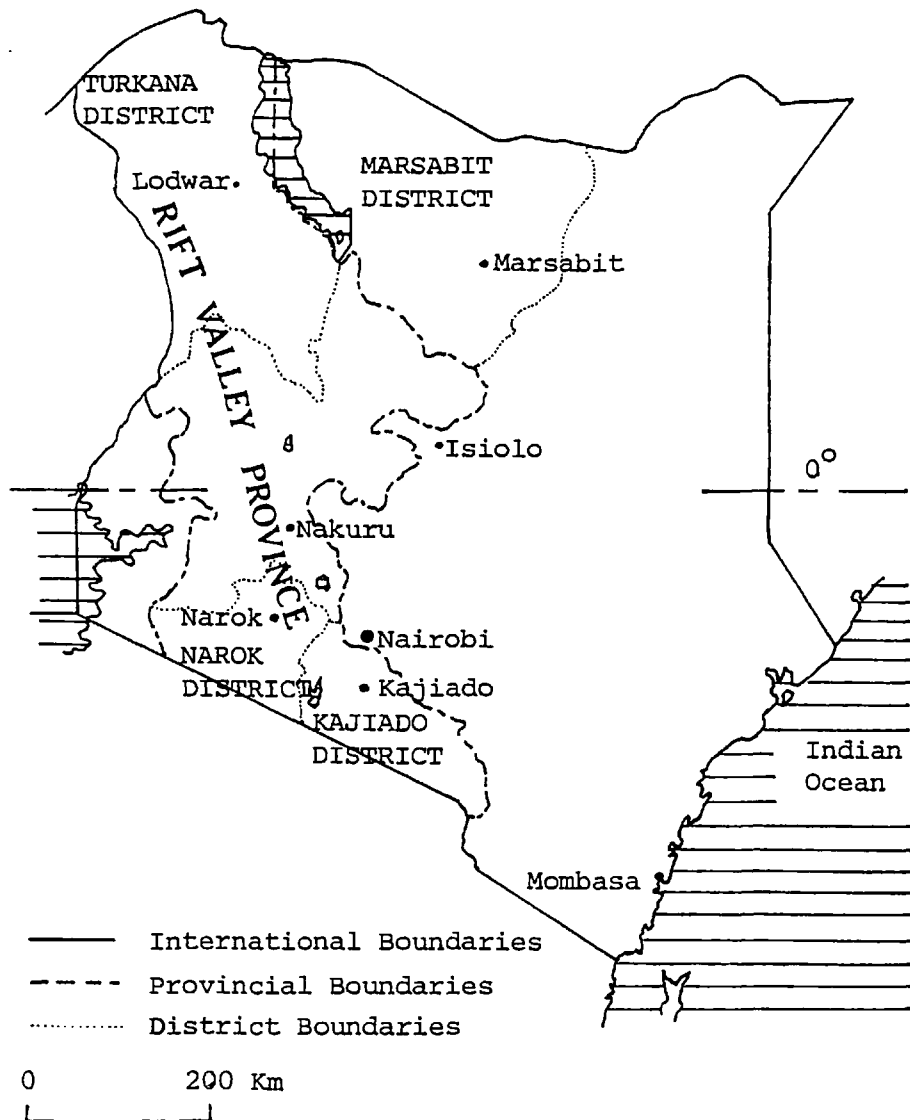
The high-rainfall areas which receive more than 650 mm of rain annually include the coastal lowlands, the highlands and the lake

basin. These areas form the most intensively cultivated and farmed parts of Kenya and although they comprise less than a quarter of the total land area, they contain more than 80 per cent of Kenya's 15.3 million population (1979 census).

The remainder of the country receives an annual rainfall of 650 mm or less and includes, the marginal highlands and the vast arid plateaus and lowlands. These areas are occupied by pastoralists or semi-pastoralists who practise some cultivation.

This study was undertaken between February 1979 and September 1980 in Turkana, Narok, Kajiado and Marsabit Districts (Figure 2) all of which lie in the low rainfall areas of Kenya.

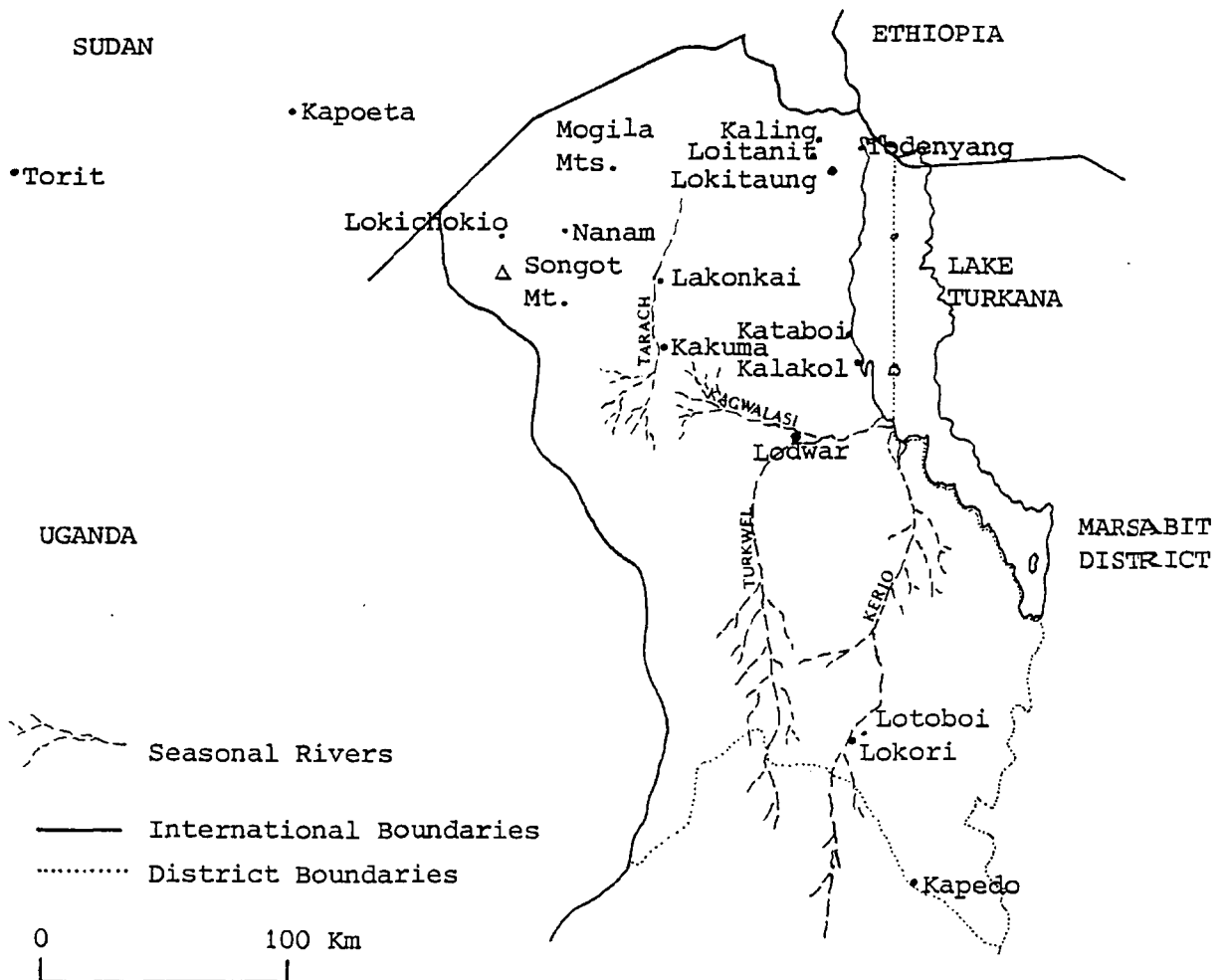
Figure 2. Map of Kenya showing study areas



1.1 Turkana

Turkana District (Figures 2 & 3), occupies the whole of north-west Kenya which lies in the Rift Valley and the escarpment (and Uganda boundary) to the west and Lake Turkana (formerly Lake Rudolf) to the east. To the north are black cotton plains and seasonal marshlands and to the south the Suk Hills. The District covers some 60,824 square kilometers and as such, is the second largest District in Kenya. The Turkana also tacitly occupy the Ilemi Triangle which, although part of the Sudan, has been administered from Kenya since the 1930's. Turkana forms part of the Rift Valley Province, with the District headquarters in Lodwar and the Provincial headquarters in Nakuru (Figure 2).

Figure 3. Map of Turkana District



1.1.1 Topography and climate

Two-thirds of Turkana District is covered by desert scrub, the vegetation consisting mainly of thorn trees and thorn bushes, cacti and bayonet aloes. Numerous dry tree-lined river beds divide the country which supports goats, camels and donkeys. Along the Lake shore and in the central region of the District the soil is mainly sandy; clumps of doum palms grow near the water courses. In this region the people live near the rivers as insufficient vegetation for goats and camels exists elsewhere. In the west and north-west a third type of vegetation is found, dominated by desert and semi-desert grasslands. In a few areas grass cover is thick and tall and shrubs abound, many trees line the river banks. Here the Turkana bring their cattle down from the mountains, whenever the rains have been sufficient. However, they frequently have to move as the grass does not last for any great length of time.

Rainfall in this District is low and unpredictable and although the seasons are divided into 'wet' and 'dry', rain may only fall on five or six occasions during the 'wet' season, from March to May. Rainfall is least in the central and eastern part of the District averaging less than 255 mm per annum. In the north and south rainfall averages between 255-510 mm per annum. In the extreme north-west, around Lokichokio and in the south-west, rainfall averages between 510-760 mm per annum (National Atlas of Kenya, 1970). The relative humidity is low throughout the District, in Lodwar the relative humidity falls from between 60-70% at 6.00 am to 30-40% by mid afternoon, this remains relatively constant throughout the year. The annual mean minimum temperatures vary between 14-22°C and maximum temperatures from 22-34°C. Between January and March the average daily temperature can reach as high as 39°C.

1.1.2 Water supplies

The two main rivers in Turkana, the Turkwel and the Kerio, arise in the escarpment to the south-west and flow into Lake Turkana from about April to September, irrespective of whether it rains in Turkana or not. Many other rivers drain from the escarpment, but flow less regularly; however, these replenish the underground supplies which are again available for water-holes. Such water-holes (akar) are the most usual type of watering place for the majority of the Turkana. They vary in depth from two to over twenty feet; in the latter case it takes several women to pass water to the surface. The water in these particular water-holes is often brackish, like that in the Lake. Other water sources include rock pools (ebur) which are available for a short period after the rains and springs (ecuar), which are found only near the mountainous regions. A relatively new additional source of water for some of the Turkana are boreholes, usually constructed near the larger townships; these were begun in the early 1960 s.

1.1.3 Origin and history

The Turkana originated from the Plains Nilotes. They moved into eastern Africa from the north and split into two groups, one moving further south to form the Masai, the other remaining in the north-west forming the Karamajong cluster. The latter gradually split up into the Karamajong and Jie and moved down the Rift Valley escarpment via the Tarach river valley to the Turkwel and Kagwalasi river valleys, where they probably established their first firm pied à terre.

Ethnographically, the Turkana are classified as Central Nilo-Hamites, their linguistic relations to the north, in southern Sudan

are classified as the Northern Nilo-Hamites and those to the south, the Masai, for example, are classified as the Southern Nilo-Hamites.

The Turkana were already well established when the first European explorers, Graf von Samuel Teleki's expedition, reached Turkanaland in 1888. It is estimated that the Turkana have lived in this region for at least 150-200 years (Gulliver & Gulliver, 1953).

British civil administration in Turkana was established in 1926, but this did little to develop the area, or to improve its contact with the outside world. It was not until a particularly severe drought in 1961 that missionaries and other relief organisations first arrived in the District. Initially the activities of these groups were confined to distributing food, but when the last of the 'maskini' (famine) camps was disbanded at Lodwar, the missionaries remained and became active in medical, social and educational fields.

The Turkana, who number some 169,000 (1969 census), are traditionally a nomadic tribe, a way of life dictated by the environment in which they live. They are pastoralists because they cannot rely on agriculture to support themselves due to the unreliability of and the very low rainfall in the area. The soil is often too poor to yield good crops and thus their animals are their livelihood. Hence, their movements are dictated by the availability of water and food. Since 1970, large developments have occurred and many of the Turkana have abandoned their peripatetic way of life and settled near the larger towns, irrigation schemes and at the fishing industry at Kalakol on the Lake shore. However, for the majority of the Turkana, life is a continual search for water and grazing. Many (an estimated 15,000 tribesmen) have emigrated and now live outside the District, mainly in Samburu and Isiolo.

1.1.4 Diet

Milk, often mixed with blood, forms the staple diet. All types of stock, except donkeys, are bled from the jugular vein, or under the eye, in the case of sheep and goats. If the wet season is especially good, dried 'milk', edodo, is made by drying curdled milk on skins in the sun. This is then stored until required when food supplies are short. The skins employed in the drying process are also used as mats to sleep on at night.

Wild berries and nuts form a large part of the diet and are collected in both the wet and dry seasons. Meat is often eaten and grain is bought occasionally to supplement the diet. Although undernutrition is quite common, malnutrition per se is relatively uncommon.

1.1.5 Livestock

Livestock are kept by the Turkana for meat, milk, blood and trade. They are also kept for more erudite reasons such as the cementing of friendships or the provision of dowry (bride-price). Donkeys are objects of contempt but are indispensable for transporting a family's possessions when moving to a new pasture. Although different areas in Turkana vary in their capacity to support the various types of livestock, almost all Turkana men own some large stock (cattle and camels) and some small stock (sheep and goats). These may be separated into different stock types which are grazed in different localities (Gulliver & Gulliver, 1953).

An aerial survey by the Kenya Rangeland Ecological Monitoring Unit (KREMU, 1979) in 1978 recorded an estimated 434,982 cattle, 60,258 camels, 1,714,752 sheep and goats and 62,692 donkeys in northern Turkana, and 83,913 cattle, 52,125 camels, 962,963 sheep

and goats and 15,644 donkeys in southern Turkana.

Although these numbers must continually change with the vicissitudes of the Turkana climate, they correspond closely with the numbers estimated for Turkana by Watson (1969). In the very severe droughts of 1960-61 and the present drought 1980-81, over 70% of the livestock perished, with only camels surviving in any number. Therefore, it would be expected that the camel, although a relatively recently acquired stock for the Turkana (Gulliver & Gulliver, 1953), would assume greater importance in this area. However, the two aerial livestock counts completed to date by Watson (1969) and KREMU (1979) mentioned earlier, do not reveal any numerical increase and in fact show a fall in the number of camels vis à vis the other livestock. Both of these surveys were performed several years after very severe droughts and may therefore represent the carrying capacity of the District. Indeed, Watson (1969) notes that the biomass density for all domestic herbivores for south Turkana is higher than predicted when a comparison is made with the Samburu or Kajiado Districts.

1.1.6 Domestic dogs

In Turkana almost every family possesses dogs. In addition to numerous pet dogs, many feral dogs can be seen roaming around the manyattas. The dogs in Turkana are small, measuring approximately 45 cm at the shoulder, are invariably brown and white or black and white and have a good natured temperament.

The main use of the dog appears to be to act as 'nurses' for the Turkana infants, when a child vomits or defecates, a dog is called to lick clean the child. This practice may have arisen due to the shortage of water in the District and it provides a very

intimate contact for the Turkana with their dogs from a very early age. It is a very common sight to see a woman with an infant having a dog in close, constant attendance. Dogs are allowed to sleep in the same hut as their master and it is my experience that even feral dogs are tolerated in the huts at night.

The dogs may also be used as guards for the household, warning the occupants of the arrival of strangers or wild animals. It has been shown that they may be effective against wild predators of Gabra stock in north-eastern Kenya (Kruuk, 1980).

One surprising fact is that the Turkana, in common with all the other nomadic pastoralists of Kenya, do not employ their dogs for herding, as is the practice in many sheep rearing countries of the world, including Britain. Some dogs do accompany their masters when herding, but play no active role. The majority of dogs spend most of their time in and around the manyattas.

1.1.7 Wild animals

Unfortunately no surveys of wild carnivores have been undertaken in Turkana District and consequently very little is known of the relationship between the carnivores and the wild herbivores and domestic stock there. However, Nelson & Rausch (1963) observed that there are very few wild carnivores in Turkana. Watson (1969) reported silver-backed jackals (Canis mesomelas), side-striped jackals (C. adustus), Cape hunting dogs (Lycaon pictus), otter (Lutra maculicollis), aardwolf (Proteles cristatus), African wild cat (Felis lybica) and leopard (Panthera pardus) to exist in low numbers in southern Turkana.

Most of the wild herbivores have been eliminated from Turkana; those that remain have a scattered distribution and are few both in

number and species diversity.

In his survey, Watson (1969) reported elephant (Loxodonta africana), hippopotamus (Hippopotamus amphibius), antbear (Orycteropus afer), oribi (Ourebia ourebi), oryx (Oryx biesa), greater kudu (Tragelaphus strepsiceros), ostrich (Struthio camelus), warthog (Phacochoerus aethiopicus) and crocodile (Crocodylus niloticus) in low numbers in southern Turkana. Two antelopes were found to be fairly numerous, these being Grant's gazelle (Gazella granti) and the diminutive dik-dik (Rhynchotragus sp.). The aerial survey conducted by KREMU (1979), reported a population of 15,268 Grant's gazelle in southern Turkana. In addition, populations of lesser kudu (Tragelaphus imberbis) (438), Oryx (188), waterbuck (Kobus spp.) (188), warthog (1,189) and eland (Taurotragus oryx) (688) were observed in the same area.

The population of Grant's gazelle appears to be very healthy in northern Turkana, KREMU (1979) reported from their aerial survey of this area, 29,709 Grant's gazelle, 839 lesser kudu, 839 warthogs and 84 oryx. In both aerial surveys, dik-dik are not reported, probably due to the fact they are too small to be observed from the air.

Watson (1969) speculated that the low population of wildlife in southern Turkana may be due to the relatively higher pastoral efficiency among the Turkana than the Masai or Samburu, and thus the domestic livestock competes with the wildlife much more intensely in southern Turkana than in Masailand or Samburu, where wild animals are much more numerous.

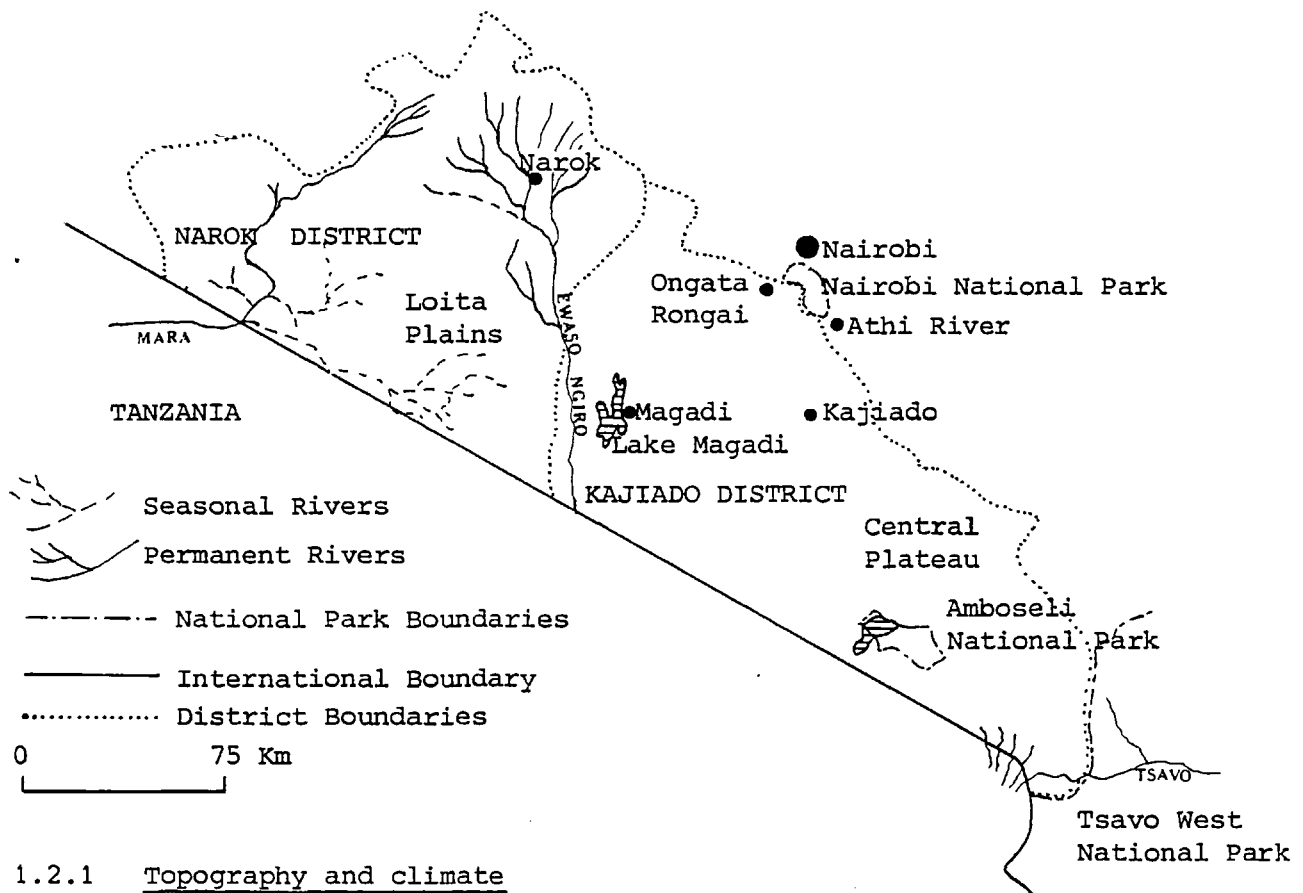
Many Turkana place-names make reference to wild animals, some of which are no longer found in the area, lending support to the idea that the present low wild animal densities are a relatively recent phenomenon. The Turkana are highly skilled hunters and their continuous hunting pressure may be another reason for the reduction

of the wild animal population in this District.

1.2 Masailand

Narok District (18,513 sq km) and Kajiado District (20,963 sq km) make up Kenya's Masailand (Figures 2 & 4) and the District headquarters are based in Narok and Kajiado townships.

Figure 4. Map of Masailand



1.2.1 Topography and climate

The whole area consists mainly of large plains which vary in height from the central lowland arid plain in Kajiado of only 650-1200 m above sea level, to the central plains in Kajiado and Narok of around 13,000-2,100 m above sea level. There are also numerous hill ranges which vary in height from 1,800-2,683 m above sea level.

The lowland arid plain in Kajiado has an average annual rainfall of 255-510 mm, the mean annual temperatures range from a minimum of 17°C-22°C to a maximum of 29°C-34°C and the area is covered with a woody vegetation although perennial grasses can dominate.

The savanna plains on the central plateau in Kajiado and the Loita plains in Narok appear green for part of the year. The average rainfall ranges from 510-760 mm per annum. The average annual temperatures range from a minimum of 10°C-16°C to a maximum of 22°C-28°C. These plains have as natural vegetation dry forms of woodland and 'savanna' or derived semi-evergreen or deciduous bushland. In western and northern Narok District there are large forested areas which receive a higher annual rainfall of around 760-1015 mm.

There are generally two distinct wet seasons occurring throughout the whole area, the long rains from March to May and the short rains from October to December. The main dry season is from January to March. (All data from the National Atlas of Kenya, 1970).

1.2.2 Water supplies

There are three areas with permanent rivers: the west of Narok District (Mara river), the area around the two dividing District borders (Ewaso Ngiro river) and the area around the foothills of Mount Kilimanjaro, which has many small rivers.

During and shortly after the rains in the rest of the District, water is obtained from temporary rivers, surface dams and ponds. In the dry seasons water is drawn from water-holes dug in the dry river beds or from boreholes, tapping the ground water at different depths. (All data from the National Atlas of Kenya, 1970).

1.2.3 The Masai

The Masai are semi-nomadic pastoralists who form the largest tribal group in Masailand, the overall population of which is 149,000 in Kajiado and 213,000 in Narok. The Masai are divided into sections, with each section headed by a 'chief'. Further divisions into clans and families leads to the basic unit of a group of families, usually living together as neighbours in a common enclosure or boma. The boma is surrounded by a strong thornbush fence which helps protect the inhabitants and livestock from cattle raiders and wild carnivores.

1.2.4 Diet

The staple diet of the Masai is very similar to that of the Turkana, consisting mainly of cows' milk mixed occasionally with blood. Goat meat or mutton is eaten once or twice a week, whilst cattle, such as bulls and bullocks are only slaughtered on ceremonial occasions. Cattle, and to a lesser extent sheep and goats, are used to pay dowries before marriage and also represent wealth and prestige.

1.2.5 Livestock

Unlike Turkana, there are no camels in Masailand. From an aerial survey in Kajiado District it is estimated that there are about 550,000 cattle, 150,000 sheep, 250,000 goats and 50,000 donkeys (Savidge, 1975). In Narok District, also from aerial surveys, there are estimated to be 526,179 cattle, 388,291 sheep and goats and 18,859 donkeys (KREMU, 1979).

1.2.6 Domestic dogs

The Masai keep dogs for very similar reasons to the Turkana, using them to warn of approaching strangers or wild predators, especially

at night. Some act as 'nurses' for the children, but the elders are very strict in avoiding close contact with dogs which are not allowed to sleep in the huts at night, as is the case in Turkana. Eugster (1978), estimated that there are between 8,000 and 12,000 dogs in Kajiado District, approximately one dog per family.

1.2.7 Wild animals

In Masailand, there are great concentrations of wild large herbivores and carnivores, of numerous species. The highest concentrations of wild animals are encountered in the Masai Mara Game Reserve, Amboseli National Park and to a lesser extent Nairobi National Park and Tsavo West National Park. The two latter National Parks lie on the Kajiado District boundary. As there are no fences separating the National Parks from the District, all animals are free to migrate in and out at all times. Great annual migrations of wild animals and in particular wildebeest (Connochaetes taurinus) and zebra (Equus burchelli) do take place from the Serengeti National Park in Tanzania to the Masai Mara Game Reserve. The Masai live peacefully amongst large herds of wild herbivores, particularly wildebeest, zebra, hartebeest (Alcelaphus buselaphus), topi (Damaliscus korrigum), impala (Aepyceros melampus), buffalo (Syncerus caffer), Thompson's gazelle (Gazella thompsonii) and Grant's gazelle. In addition, in various regions of Masailand large numbers of eland, giraffe (Giraffa camelopardalis), waterbuck, warthog, gerenuk (Litocranius walleri) and dik-dik are found, so are numerous other species of herbivores, but these are usually rare (Eugster, 1978; KREMU, 1979).

All these herbivores are preyed upon by lions (Panthera leo), leopards, cheetahs (Acinonyx jubatus), spotted hyaenas (Crocuta

crocuta), Cape hunting dogs and silver-backed jackals. Many other carnivores are also found in this region but are not involved in a predator/prey relationship with any of the herbivores mentioned above.

CHAPTER 2

DOMESTIC INTERMEDIATE HOSTS

2. Introduction

The aim of this chapter is to review the reported occurrence of hydatidosis in domestic livestock in Africa, paying particular attention to the position in Kenya. The occurrence of the disease in camels throughout the world is also examined.

An attempt is made to ascertain the importance of the numerous intermediate hosts as reservoirs of infection and the significance each host plays in the continuity of the life cycle of the parasite in Kenya. The intensity of infection and the suitability of each host for E. granulosus is examined.

2.1 Occurrence and prevalence of hydatidosis in camels

The camel (Camelus dromedarius) ranges freely over wide areas of north Africa to northern Kenya and Somalia and west to northern Nigeria. It is also ubiquitous throughout the Middle East and has been successfully introduced to parts of southern Africa, Australia and southern Asia.

Hydatid disease in camels is not uncommon in most Middle Eastern and north African countries, but appears to be very rare in southern Asia and Australia. Data concerning the reported occurrence and prevalence rates in camels are summarised in Appendix 1, Table 1.

There are apparently no references to hydatid disease in camels from Australia or Pakistan and only one infected camel has been reported from India (Gupta, 1979), suggesting that the incidence

of the disease in camels in southern Asia is very low. However, a high incidence has been reported in camels (Camelus bactrianus) from Afghanistan (FAO-WHO-OIE, 1979).

In the Middle East and northern Africa where large numbers of camels are maintained for both food (milk, blood and meat) and for transport, hydatidosis appears to be particularly prevalent in this host. Camels are regarded as important intermediate hosts in Iran (Mobedi et al, 1970; Afshar et al, 1971; Motakef et al, 1976), Syria (Dailey & Sweatman, 1965), Lebanon (Pipkin et al, 1951) and Iraq (Altaif, 1974; Al-Abbassy et al, 1980). They are recognised as optimum hosts for hydatidosis in Egypt (El-Kordy, 1946; Abdou, 1965) and have been reported from numerous other African countries; these include, Chad (Graber et al, 1969), Morocco (Faure, 1949; Senevet, 1951), Nigeria (Dada, 1977; Dada & Belino, 1979; Dada et al, 1979b, 1980), Sudan (Malek, 1959; El-Badawi et al, 1979) and Tunisia (Dévé, 1923; Cousi, 1951; Ben Osman, 1965). Leese (1915) reported that camels were infected with hydatid disease in Kenya, but did not give any further information. The prevalence of the disease is unknown from other African countries.

Camels, in general, are slaughtered at a more advanced age than other slaughterstock (Pipkin et al, 1951; Babero et al, 1963; Mobedi et al, 1970). This has been suggested to contribute to the high prevalence of the disease found in camels, as the prevalence has been shown to increase with age (Mobedi et al, 1970; Afshar et al, 1971; Hamdy et al, 1980). The observed increase with age is thought to be due to the increased opportunity for infection. There have been no reported differences in prevalence between the sexes (Mobedi et al, 1970; Afshar et al, 1971).

2.2 Occurrence and prevalence of hydatidosis in domestic livestock in Africa

Hydatidosis appears to be endemic throughout the entire African continent, the prevalence, however, is unknown in the majority of the African countries. The occurrence and prevalence of hydatid disease reported in domestic livestock in Africa is given in Appendix 1, Table 2.

The disease appears to be particularly prevalent in cattle and sheep in several countries in north Africa, for example, Algeria (Jore d'Arces, 1953), Tunisia (Senevet, 1951) and Morocco (Senevet, 1951; Vaysse, 1955). Hydatidosis has a high prevalence in cattle, sheep and goats in Nigeria (Dada & Belino, 1979; Dada et al, 1980) and Sudan (Eisa et al, 1962; El-Badawi et al, 1979). However, in central and southern Africa, hydatidosis in the domestic livestock is seemingly less prevalent than in the north. Graber et al, (1969) reported a low prevalence in cattle and sheep in southern Chad, northern Cameroon and the northern region of the Central African Republic. The disease is also reported to be low in these food animals in Mozambique (De Castro Amaro, 1960; Buck & Courdurier, 1962), low in cattle in Zimbabwe (formerly Rhodesia) (Chambers, 1978) and low in cattle, sheep and goats in South Africa (Verster, 1962; Verster & Collins, 1966).

There are few reports that the disease occurs in other types of livestock in Africa, however, a low prevalence of the disease has been reported in pigs in Egypt (Sedik et al, 1977; Hamdy et al, 1980), Mozambique (De Castro Amaro, 1960), Nigeria (Dada et al 1979b; Fabiyi, 1979) and South Africa (Verster & Collins, 1966). Donkeys have been found to have a low prevalence of the disease in Morocco (Pandey, 1980), and buffaloes have been found infected in Egypt

(El-Kordy, 1946; Sedik et al, 1977). Finally, in many African countries, wild herbivores are eaten to supplement the diet and many of these have been found to serve as intermediate hosts for the parasite. These will be discussed fully in chapter 5.

2.3 Occurrence and prevalence of hydatidosis in domestic livestock in Kenya

The first recorded incidence of hydatid cysts in Kenya was in camels (Leese, 1915). Ten years later, Walker (1925) reported the disease to be rare in sheep and the following year Daubney (1926) reported hydatid cysts in cattle and sheep. Hudson (1934) noted that hydatids were found occasionally in cattle, sheep and pigs. These reports, however, gave little indication of the prevalence of the disease in the country. It was not until the Kenya Meat Commission (KMC) abattoir at Athi River began operating in 1953 that statistical data became available. After the KMC was opened, numerous other abattoirs were established all over the country, but mainly in the high rainfall areas (Figure 1). Few abattoirs were set up in the low rainfall areas of northern Kenya and today in Turkana District, meat inspection is carried out only in the larger townships. Therefore, a large number of animals slaughtered in this region undergo no meat inspection at all, although all animals slaughtered are required by law to be examined by a meat inspector. This situation will gradually change with an increase in the number of qualified meat inspectors and abattoirs.

Today some information is available on the prevalence of hydatidosis in cattle, sheep, goats and pigs slaughtered at all the major abattoirs in Kenya. Unfortunately, as far as the author is aware, no such records have been published on the prevalence of the

disease in camels and nothing is known concerning the disease in donkeys or horses in the country. The occurrence and prevalence of hydatidosis reported in Kenyan slaughter stock is presented in Appendix 1, Table 3.

Ginsberg (1956) reported the prevalence of hydatidosis in slaughter stock from the first two years meat inspection at KMC. The results showed an extremely high prevalence of the disease amongst the indigenous African cattle (43.9%), sheep (47.5%) and goats (16.6%), which was significantly higher than the recorded figures for European cattle (17.4%) and sheep (26.4%).

The high prevalence of the disease reported by Ginsberg (1956), is supported by the later investigations of Froyd (1960a; 1960b), who found 25.5% of 1000 cattle infected at KMC in 1958 and 30.2% of 1000 cattle infected at KMC in 1959; Mango (1971), who conducted a Provincial survey reported an overall countrywide prevalence of 20.7% in 2916 cattle, 20.7% in 2240 sheep and 16.2% in 1228 goats; Ng'ang'a (1974), presented the abattoir records for KMC between 1959 through 1970, showed that 12.8% of 1,162,237 cattle had hydatid cysts in the liver, while 20.3-37.2% of 489,702 sheep and 14.7-23.6% of 245,231 goats had hepatic or concurrent hepatic and pulmonary cysts. The most recent survey, conducted by Eugster (1978) between 1973-1975, shows that the prevalence has remained high amongst the domestic livestock. His results from Kajiado District showed the prevalence to be 46.7% in 1446 cattle, 29.5% in 44 sheep and 9.0% in 100 goats.

Unfortunately, besides Eugster's (1978) and to a lesser extent Mango's (1971) figures, the origin of the animals brought to the abattoirs has not been recorded. However, since the animals were drawn from all over the country, the prevalence of the disease in all

types of slaughter stock appears to be high and widespread. There are as yet, insufficient data collected to show any indication of areas where hydatidosis has a particularly high prevalence of the disease in domestic animals. Eugster (1978) provided the first data on the prevalence of the disease in one District and his results showed a high prevalence of hydatidosis, particularly in cattle, in Kajiado District.

To date, there are no published records of the prevalence of the disease in livestock from northern Kenya. The 1962 annual report of the Veterinary Department of Kenya mentioned that hydatid cysts were found in camels and cattle killed at a field abattoir in this region. In Turkana, hydatids have been reported to be common in domestic animals (Nelson & Rausch, 1963) and widespread in goats (Department of Veterinary Services, Annual Report, 1967).

There are a few records regarding the incidence of infection in cattle, of different ages; sheep and goats are both usually slaughtered at an early age (up to 15 months). Froyd (1960a) found that the incidence of hydatidosis in cattle increased with age, an observation that has been made by other authors from various countries (Pullar & Marshall, 1958; Gemmell, 1961; Verster & Collins, 1966). The increase in incidence of infection is, however, thought to be related to the opportunity for infection rather than any increased susceptibility dependent on age (Pullar & Marshall, 1958).

2.4 Economic effects of hydatidosis in livestock in Kenya

The economic loss due to hydatidosis in livestock is considerable for a developing country such as Kenya. The losses result from the reduction in the quality of the meat and the condemnation of offal. Although there are no data concerning the effect on meat categorisation

in Kenya, several authors have shown that the disease does cause a diminution in the quality of meat, which would therefore adversely affect the economy due to the high prevalence of the disease, especially in cattle. Ul'yanov (1959) reported that only 6.5% of meat from infected cattle could be placed in the 'prime category' compared to 22.4% from healthy animals. This observation was supported by Pozdnyakova (1965) who also found a reduction in the quality of meat from infected cattle. Hydatidosis has also been reported to cause a deficiency in the meat from infected lambs (Vibe, 1960) and to increase the amount of water in meat from infected pigs vis à vis control animals (Kostyák & Adám, 1965).

In addition to the uncalculated economic effects on meat quality, hydatidosis causes considerable economic losses due to offal condemnations. Figures provided by the Veterinary Services Division (1980) for the condemnation of cattle and smallstock (sheep and goats) livers, from both export and many local slaughterhouses throughout Kenya, show that a total of 12,892 cattle livers and 4,016 smallstock livers were condemned due to hydatidosis alone during 1979. These figures represent 4.9% of all cattle and 1.9% of all smallstock livers. As such, condemnation of livers because of hydatidosis is second only to condemnation due to Fasciola hepatica in cattle, and second to infection with Stilesia hepatica as the main reason for liver condemnations in smallstock. Cattle livers weigh on average 4.5 Kg, smallstock livers 1.0 Kg, and have a retail value of 12/50 and 15/- (Kenya shillings) respectively (10/- Kenya \equiv £0.50p U.K.). Thus, the economic loss caused by the condemnation of reported hydatid infested livers for 1979 alone amounted to approximately 800,000/- (£40,000 U.K.). This sum represents a very serious loss in Kenya's economy as well as a

considerable loss of protein from the national diet.

2.5 Materials and methods

Source of material

Statistics concerning domestic intermediate hosts were obtained from three geographically separated regions in Kenya: Turkana District, Masailand and to a much lesser extent, Marsabit and Isiolo Districts.

Information from Turkana District was obtained during numerous safaris to the District. On each occasion the local 'abattoir' was visited in the early morning to examine the animals slaughtered that day. Few animals are slaughtered in these 'abattoirs' outside Lodwar and therefore only a few statistics on the occurrence of hydatid disease amongst the Turkana livestock were obtained. However, numerous animals were examined from Lodwar abattoir and the two 'abattoirs' just outside Lodwar - Napatet and Nairobi village. Any cysts found whilst on a three day surgical safari, were collected and stored in a refrigerator in the newly constructed hydatid laboratory in Lodwar and then flown back to Nairobi for processing. Cysts discovered during overland safaris usually had to be processed immediately and, if fertile, protoscoleces were preserved in both liquid nitrogen for isoenzyme analysis (see chapter 7) or in 70 per cent ethanol. The public health technicians at Lodwar and Kakuma occasionally preserved cysts found in the slaughterstock in 10% formalin, or sometimes cysts found in Lodwar were kept for up to a week in the laboratory refrigerator in Lodwar, for collection by the author.

A limited number of livestock slaughtered in Marsabit and Isiolo townships were examined during an overland safari to this region

between 25th and 30th August, 1979. Any hydatid cysts found were processed in the same manner as those discovered during the overland safaris to Turkana.

The Kenya Meat Commission (KMC) abattoir at Athi River and the two co-operative society abattoirs at Ongata Rongai were frequently visited between February and December, 1979. During this period any hydatid cysts found were placed in labelled plastic bags, stored in a cool box and transported back to Nairobi as soon as possible. During the second visit to Kenya between April and September, 1980 only Ongata Rongai abattoirs were visited. This was due to numerous factors. Firstly, Ongata Rongai was only a few kilometers from the author's home and was therefore readily accessible, whereas the KMC abattoir at Athi River was over 40 km away. Secondly, the operation at Ongata Rongai was a lot smaller than the one at KMC in fact small enough for the author to become acquainted with the regular livestock vendors personally, which helped in discovering the origin of their animals. A similar operation may have been possible at KMC, but would have proved more difficult. Thirdly, it was known that the majority of the livestock slaughtered at Ongata Rongai originated from Kajiado District and included, in addition to cattle, large numbers of smallstock (sheep and goats), whereas KMC deals mainly with cattle, which are brought to the abattoir from all over the country. Finally, it was possible to get to know the staff at Ongata Rongai, who were extremely helpful and allowed the author to examine the viscera on the carcasses. Therefore some indication of the prevalence of hydatid disease in the livestock was obtained.

The two abattoirs at Ongata Rongai were visited regularly on different days. The lungs, liver, heart and spleen from cattle and additionally the kidneys from smallstock were examined thoroughly.

Collection of data on the incidence of hydatid cysts in the rare localisations such as the bones or brain, and the determination of the sex and age of infected animals were impracticable. Any cyst found was examined macroscopically and small cysts of uncertain identity were not kept. Therefore the figures obtained represent the least possible prevalence of the disease from these animals.

Hydatid cysts recovered were individually labelled and then placed into large plastic bags according to the host species and transported in a cold box back to Nairobi.

Examination of material

Infected organs were usually examined on the day of collection, but if this was not possible then they were stored overnight at 4°C and examined the following day.

First, the material was evaluated with regard to the size and shape of the cysts in each organ. For each animal and each organ the cysts were counted and the data were collated as follows:-

animals with 1 cyst only
 animals with 2-4 cysts
 animals with 5-10 cysts
 animals with more than 10 cysts (per organ)

The various sizes of hydatid cysts encountered were also further divided into four groups, namely: small (diameter of less than 2.0 cm), medium (diameter 2.0-6.0 cm), large (diameter 7.0-10.0 cm) and extra large (diameter greater than 10.0 cm).

The terminology used to describe the various types of hydatid cysts that are known to occur is somewhat confusing, and has been reviewed by Smyth (1964). The types of cyst found in the Kenyan

animals were divided into three groups based on classification provided by Smyth (1964): a) unilocular, characterised by having only one bladder, or many completely isolated bladders, each enclosed in its own envelope. This envelope, the laminated membrane, is solid and continuously restricts the living germinal epithelium from invading adjacent tissue; b) lobulated, unilocular cysts but having one or many pouches or lobules (multilobular) as described recently by Vaněk (1980); c) multilocular, characterised by having many bladders embedded in a common adventitious membrane.

For in vitro culture, infected organs were examined and opened in an open 'laminar flow' (sterile air) cabinet, using sterile dissecting instruments and sterile containers throughout. All subsequent procedures, with only minor alterations, were performed as described by Smyth & Davies (1974b).

After the number, location, shape and size of the hydatid cysts had been recorded, the cyst and organ surfaces were coated twice with 1% iodine in 95% ethanol, allowing the surfaces to dry after each coat. Approximately half of the hydatid fluid was then aspirated from each cyst, using a wide-bore needle (No. 17-19) and hypodermic syringe of a size (2-20 ml) appropriate to the cyst size, in order to reduce intracystic pressure and so help to avoid spillage of fluid.

The cysts were opened using scissors or a scalpel and a piece of cyst measuring approximately 2.0 cm square was completely removed and stored in Bouin's fluid in 20 ml screw-capped vials (universal containers) (Sterilin). The cysts were then examined for the presence of protoscoleces and if fertile (protoscoleces present), all the protoscoleces, which were normally contained in brood capsules, were removed using a 1 ml pipette and transferred with at

least 5 ml of hydatid fluid, to a 20 ml universal container.

When necessary these containers were stored at 4°C.

Non-fertile cysts were either classified as being sterile (fluid filled but no protoscoleces), or calcified (cysts either calcifying, completely calcified or caseated).

Tests for viability

After the sedimented volume of brood capsules from each fertile cyst had been recorded, the viability of the freshly removed protoscoleces was determined using any of the three following methods.

1. Flame cell activity as observed microscopically, counting the number of viable and non-viable protoscoleces in five separate fields of view.

2. Eosin exclusion test for cell death (Hanks & Wallace, 1958) was performed by applying a drop of 0.1% aqueous Eosin directly to protoscoleces suspended in hydatid fluid on a microscope slide. Eosin is not absorbed by viable tissue, but non-viable tissues absorb the dye and turn pink. After five minutes, slides were examined and viability determined by counting the number of viable and non-viable protoscoleces in five separate fields of view.

3. Ability of the protoscoleces to evaginate when placed into 10 ml of warm (37°C) sterile Hank's balanced salt solution (HBSS) (Difco), in a 20 ml universal container and placed on a roller in an incubator for two hours at 37°C. The majority of viable protoscoleces evaginate during this period (Thompson, 1975).

2.6 Results

2.6.1 Turkana District

The number of domestic animals examined from the 'abattoirs' visited throughout Turkana District are presented in Table 1.

Table 1. Domestic livestock examined for the presence of hydatid cysts in Turkana

HOST SPECIES	No. EXAMINED	No. INFECTED	% INFECTED	ABATTOIR
CAMEL	10	8	80.0	LODWAR
CATTLE	8	1	12.5	LODWAR
	2	0	-	KAKUMA
	10	1	10.0	TOTAL
SHEEP	50	1	2.0	LODWAR
	10	0	-	KAKUMA
	1	0	-	NANAM
	61	1	1.6	TOTAL
GOAT	784	5	0.6	LODWAR
	32	2	6.3	KAKUMA
	15	0	-	LOKITAUNG
	12	0	-	LOKORI
	1	0	-	NANAM
	844	7	0.8	TOTAL

2.6.1.1 Camel

Eight out of 10 (80.0%) camels examined in Turkana District were found to harbour hydatid cysts. Fresh material from another infected camel was received from the public health technician in Lodwar.

Details from all the infected organs examined are shown in Table 2.

Table 2. Location of hydatid cysts in nine infected camels from Turkana

	Total	Liver	Lungs	Spleen
Total cysts	93	14	61	18
Fertile	63	-	52	11
Sterile	11	1	5	5
Calcified	19	13	4	2

A total of 93 hydatid cysts were recovered from the nine infected camels (9.7 cysts per animal). Of these 63 (67.7%) were fertile, having an overall viability of 74.3%; 11 (11.8%) were sterile and 19 (20.4%) calcified.

The most frequently infected organs were the lungs (65.6%), followed by the spleen (19.4%) and liver (15.0%); no other organs were found infected. Two of the 9 camels harboured concurrent pulmonary and splenic infections, three had only pulmonary infections and the other four camels had: splenic infection only (1), concurrent hepatic and splenic involvement (1), pulmonary and hepatic involvement (1) and pulmonary, hepatic and splenic involvement (1).

Pulmonary involvement

A total of 61 pulmonary hydatid cysts were recovered from seven infected camels (8.7 cysts per camel). Fifty-two (85.2%) cysts were fertile and had an average viability of 75.5%.

In most cases, one to three pulmonary cysts were found in each infected camel. However, one camel harboured 45 pulmonary, four splenic and two hepatic cysts.

The majority (96.7%) of pulmonary cysts were 1.0-4.0 cm in

diameter, with the largest cyst having a diameter of 7.0 cm. The number of brood capsules obtained from each fertile cyst tended to increase with increasing cyst size. This general trend was not always followed, for example, one 3.0 cm cyst yielded 1.0 ml of sedimented brood capsules, whilst a 7.0 cm cyst from another cyst yielded only 0.5 ml of brood capsules (Appendix 1, Figure 1a). Heath (1970b) calculated that in 1.0 ml of sedimented brood capsules there were approximately 660,000 protoscoleces and, therefore, these two cysts contained approximately 660,000 and 330,000 protoscoleces respectively.

Very few of the pulmonary cysts were sterile (8.2%) or calcified (6.6%). Such cysts were found in three animals with pulmonary involvement only and in one case of concurrent pulmonary and hepatic infection. All pulmonary cysts were unilocular and spherical in shape.

Hepatic and splenic involvement

Three of the 9 infected camels harboured a total of 14 hepatic cysts (4.7 liver cysts per camel), none of which was fertile. All but one of the hepatic cysts were small (approximately 1.0 cm) and calcified. The single large cyst measured 6.0 cm and was sterile.

Five of the 9 infected camels harboured a total of 18 splenic cysts (3.6 spleen cysts per camel), most (61.1%) of which were fertile, with an average viability of 73.2%.

The majority (77.8%) of splenic cysts were between 1.0-4.0 cm in diameter, the other cysts measuring 4.5, 5.0, 7.0 and 9.0 cm respectively. As with the pulmonary cysts the number of brood capsules removed from a cyst increased with an increase in cyst size. Once again the trend was not always observed and in two cases

approximately 660,000 protoscoleces were obtained from each of the 4.5 and 5.0 cm cysts, whereas only 200 protoscoleces were recovered from a 9.0 cm cyst. The smallest fertile cyst found measured 1.0 cm (Appendix 1, Figure 1a).

Five (27.8%) splenic cysts were sterile, whilst only two (11.1%) were calcified. All hepatic and splenic cysts were spherical and unilocular, except for three splenic cysts which were lobulated. None of the camel cysts contained daughter cysts.

2.6.1.2 Cattle

Only two small (2.0 cm) sterile pulmonary hydatid cysts were obtained from a single infected animal out of 10 examined.

2.6.1.3 Sheep

A single small (1.5 cm) calcified hepatic cyst was obtained from the 61 sheep examined.

2.6.1.4 Goat

Seven of 844 (0.8%) goats examined throughout Turkana District were found to harbour hydatid cysts. Fresh hydatid cysts from a further five goats were received from the public health technician in Lodwar. Details of all the infected organs examined are shown in Table 3.

A total of 25 cysts were obtained from the 12 infected goats (2.1 cysts per goat). Seventeen (68.0%) out of these 25 cysts were fertile having a mean viability of 91.6%. The volume of brood capsules obtained did not appear to be dependent on the location of the cyst, but tended to increase with an increase in cyst size (Appendix 1, Figure 1b).

Table 3. Location of hydatid cysts in 12 infected goats from Turkana

	Total	Liver	Lungs	Spleen
Total cysts	25	10	14	1
Fertile	17	6	10	1
Sterile	8	4	4	-
Calcified	-	-	-	-

Of the 12 infected goats, five harboured only hepatic cysts, five only pulmonary cysts, one harboured a concurrent hepatic and pulmonary infection with a single cyst in each location and one goat had a single splenic cyst. Three each of the five hepatic and five pulmonary infested goats had only single hydatid cysts.

The hepatic cysts tended to be larger than the pulmonary cysts. The four largest hepatic cysts having a diameter of 6.0 cm or more, whereas the three largest pulmonary cysts were 5.0 cm in diameter. The single splenic cyst had a diameter of 4.0 cm. All other cysts measured 4.0 cm or less.

2.6.2 Marsabit and Isiolo

The number of domestic animals examined from this area is shown in Table 4.

The single infected bovine had a small (1.0 cm) pulmonary cyst which was sterile. Four cysts were found in three out of four infected sheep examined at Isiolo. Three were small (1.0 cm) calcified hepatic cysts and one a small (2.0 cm), sterile pulmonary cyst. The single pulmonary cyst found in the goat was large (6.0 cm) and very fertile, yielding 0.75 ml brood capsules. The viability of this material was

not examined when fresh, but inspection of the preserved material revealed few yellow/brown, small triangular protoscoleces, which are characteristic of dead larvae (Smyth, 1962a).

Table 4. Domestic livestock examined for hydatids from Marsabit and Isiolo

HOST SPECIES	No. EXAMINED	No. INFECTED	% INFECTED	ABATTOIR
CAMEL	1	0	-	MARSABIT
	1	0	-	ISIOLO
CATTLE	8	1	12.5	MARSABIT
	4	0	-	ISIOLO
SHEEP	13	0	-	MARSABIT
	4	3	75.0	ISIOLO
GOAT	37	1	2.7	MARSABIT
	16	0	-	ISIOLO

2.6.3 Masailand

Hydatid cysts from 21 infected cattle, 19 infected sheep and 32 infected goats slaughtered at Ongata Rongai and from 41 infected cattle slaughtered at KMC (Athi River) were collected during the author's first visit to Kenya. The infected animals seen at Ongata Rongai probably all originated from Masailand. Unfortunately, the origin of most of the infected cattle from KMC was not established although some of the cattle were known to have come from Masailand. The data obtained from all the KMC cattle are included with the rest of the data on cattle from Masailand.

A total of 64 (6.6%) out of 964 cattle, 42 (8.2%) out of 514 sheep and 31 (3.6%) out of 855 goats slaughtered at Ongata Rongai between April and September 1980, were found to harbour hydatid cysts. The majority, if not all, of these animals originated from Masailand (mainly Kajiado District). During this period, of 104 sheep and 322 goats brought by lorry to Ongata Rongai from north-eastern Kenya by Somali traders, none harboured hydatid cysts.

2.6.3.1 Cattle

Five hundred and eighty-seven hydatid cysts were obtained from 126 (4.7 cysts per animal) infected cattle. Ninety (71.0%) out of the 126 infected cattle had pulmonary infections only, 19 (15.1%) had hepatic infections only, 13 (10.3%) had concurrent hepatic and pulmonary infections, and there were two cases (1.6%) of hepatic, pulmonary and splenic involvement. Two cows harboured single splenic and cardiac infections. Details of the infected organs are shown in Table 5 and in Appendix 1, Tables 4 and 5.

Table 5. Location of hydatid cysts in infected cattle from Masailand

	Total	Liver	Lungs	Spleen	Heart
Total cysts	587	170	411	5	1
Fertile	66	2	63	-	1
Sterile	401	70	328	3	-
Calcified	120	98	20	2	-

The majority of the 587 hydatid cysts were sterile (68.3%) and 120 (20.4%) were calcified. Only 66 (11.2%) were fertile having a mean viability of 63.0%.

Pulmonary involvement

Most (79.8%) of the 411 pulmonary cysts were sterile, 63 (15.3%) were fertile and only 20 (4.9) were calcified. The majority of all pulmonary cysts were of medium size (64.0%), some were large (15.3%) and five were extra large. All pulmonary cysts with a diameter of less than 2.0 cm (19.3%) were either sterile or calcified. The smallest fertile cysts found had a diameter of 2.0 cm. There were two instances where fertile cysts contained only dead protoscoleces. As with the camel and goat material from Turkana, the volume of brood capsules obtained from fertile cattle cysts tended to increase with an increase in cyst size. The two largest cysts examined had diameters of 13.0 cm and contained 0.2 ml and 0.5 ml brood capsules. The majority of fertile cysts measured 5.0 cm or more (Appendix 1, Figure 1c). Cyst viability did not decrease with increasing size.

Other infection sites

Of 170 hepatic cysts, only two (1.2%) were fertile and these were obtained from a single infected animal which harboured 24 hepatic cysts. Both fertile cysts were of medium size (3.0 cm) and the material had an average viability of 58%.

The majority of small hepatic cysts were calcified, whereas most medium sized cysts were sterile and none was calcified.

Two large hepatic cysts were obtained, both had a diameter of 8.0 cm and were sterile.

Only six cysts were not found in hepatic or pulmonary localisations. These included five splenic cysts (three sterile, two calcified) and a single fertile cyst in the left ventricle of a heart (Plate 1). The cardiac cyst measured 7.0 cm and contained 0.25 ml

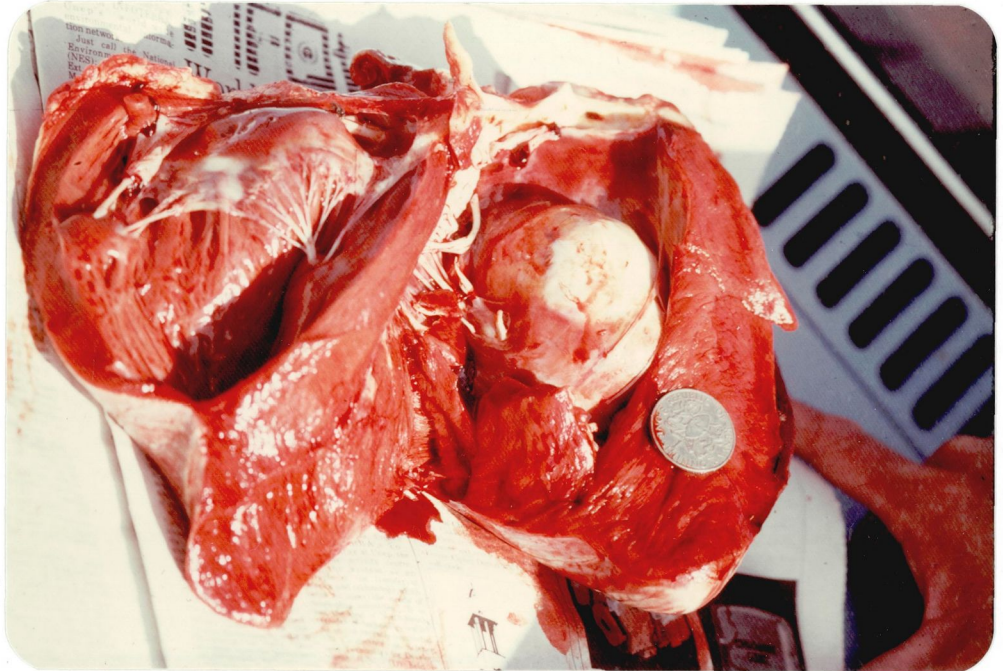


Plate 1. Hydatid cyst in the left ventricle of the heart from a cow.



Plate 2. Concurrent hepatic, pulmonary and renal hydatid infection from a goat.

sedimented brood capsules which had an average viability of 52.3%.

Number of cysts per organ per animal

When only pulmonary involvement was found, the majority of infections were single cysts (45.6%), 34 cattle (37.8%) had 2-4 pulmonary cysts, 11 (12.2%) harboured 5-10 pulmonary cysts and a few cattle (4.4%) harboured more than 10 pulmonary cysts. A similar picture was observed in cattle with hepatic infections only and also those with concurrent hepatic and pulmonary infections (see Appendix 1, Table 5).

Cyst type

All but two of the hydatid cysts examined from cattle were unilocular and spherical in shape. The two exceptions were both pulmonary cysts, which were lobulated.

Four large pulmonary cysts were found to be packed with daughter cysts. The largest daughter cysts (2.0-3.0 cm in diameter) were usually fertile containing only a few protoscoleces, which were always viable.

2.6.3.2 Sheep

Four hundred and ninety-six hydatid cysts were obtained from 57 (8.7 cysts per animal) infected sheep. Thirty (52.6%) of the 57 infected sheep harboured concurrent hepatic and pulmonary infections, 16 (28.1%) had hepatic infections only, eight (14.0%) had pulmonary infections only and three separate sheep harboured concurrent hepatic and splenic, hepatic, pulmonary and splenic and hepatic, pulmonary and renal infections. Details of the infected organs are shown in Table 6, and in Appendix 1, Tables 4 and 6.

Table 6. Location of hydatid cysts in infected sheep from Masailand

	Total	Liver	Lungs	Kidney	Spleen
Total cysts	496	206	285	1	4
Fertile	374	139	231	-	4
Sterile	90	45	45	-	-
Calcified	32	22	9	1	-

The majority of the 496 hydatid cysts examined were fertile (75.4%), having a mean viability of 85.0%. Ninety (18.1%) were sterile and 32 (6.5%) calcified.

Pulmonary involvement

Pulmonary cysts accounted for 57.5% of all sheep cysts examined. The majority (81.0%) were fertile, 45 (15.8%) were sterile and nine (3.2%) were calcified. Most pulmonary cysts were of medium size (74.4%) or small (23.9%), only five had a diameter greater than 6.0 cm, the largest being two fertile 10.0 cm cysts. The volume of brood capsules obtained from a fertile cyst tended to increase with an increase in cyst size. The smallest fertile pulmonary sheep cyst measured 1.0 cm and the majority of fertile cysts measured 5.0 cm or less (Appendix 1, Figure 1d).

Hepatic involvement

Two hundred and six (41.5%) hydatid cysts were found in the liver. Of these, 139 (67.5%) were fertile, 45 (21.8%) sterile and 22 (10.7%) calcified. Like the ovine pulmonary cysts, the majority of hepatic cysts were of medium size (76.7%) and were usually fertile (84.8%) having a mean viability of 83.7%. Only one medium sized cyst was calcified. The small hepatic cysts, accounting for 20.4%

of all hepatic cysts, tended to be calcified (50.0%) or sterile (42.9%), with only three being fertile with a mean viability of 80.5%. The smallest fertile hepatic cyst had a diameter of 1.0 cm. Of the six large hepatic cysts examined, four were fertile (mean viability 72.3%) one was sterile and one calcified (caseated). The largest hepatic cyst measured 8.0 cm and was sterile.

Hepatic cysts tended to yield more brood capsules per cyst than similar sized pulmonary cysts and as with pulmonary cysts, the majority of fertile hepatic cysts measured 5.0 cm or less. Most of the larger fertile cysts were located in the liver (Appendix 1, Figure 1d).

Other infection sites

The kidney was involved only once and the cyst was small and calcified. Two sheep had two medium sized splenic cysts each. All four splenic cysts were fertile with a mean viability of 89.3%

Number of cysts per organ per animal

Six animals harboured single hepatic cysts, whilst five harboured single pulmonary cysts. The number of cysts found in single organ infections were markedly lower than the number of cysts found per organ in concurrent organ infections. There were five infected sheep with more than 10 pulmonary cysts with concurrent pulmonary and hepatic infections and three cases where there were more than 10 hepatic cysts with a similar concurrent infection. None of the single organ infections harboured more than 10 cysts per organ per infected animal.

Four infected sheep harboured 29.6% of the total number of cysts obtained from the 57 infected animals. One infected sheep had 105 (21.2%) cysts, the majority (95.2%) of which were pulmonary.

Cyst type

The majority of hydatid cysts were spherical and unilocular (70.2%). Many pulmonary (16.3%) and few hepatic (5.6%) cysts were lobulated. In addition, some pulmonary (7.9%) cysts were multilocular. These multilocular cysts were usually divided by numerous internal median septae and had irregular lobulated surfaces.

One small (1.0 cm), two medium (2.0 and 3.0 cm) and one large (7.0 cm) hepatic cysts harboured numerous daughter cysts. Only the large hepatic cyst contained fertile daughter cysts, the smallest fertile daughter cyst having a diameter of 1.0 cm.

One large pulmonary cyst (10.0 cm) was packed with large daughter cysts, one of which had a diameter of 6.5 cm and contained 0.25 ml viable brood capsules. Two other sheep had pulmonary cysts containing daughter cysts, many of which were fertile and viable.

2.6.3.3 Goat

One hundred and sixty-one hydatid cysts were obtained from 56 (2.9 cysts per animal) infected goats. The majority (73.2%) of infected goats harboured pulmonary infections only. Eight (14.3%) animals had concurrent pulmonary and hepatic infections, five (8.9%) only hepatic infections, one (1.8%) had a pulmonary, hepatic and splenic infection and one animal harboured a pulmonary, hepatic, splenic and renal infection. Details of infected organs are given in Table 7, and in Appendix 1, Tables 4 and 7.

A total of 77 (47.8%) cysts were fertile, 71 (44.1%) were sterile and 13 (8.1%) were calcified.

Table 7. Location of hydatid cysts in infected goats from Masailand

	Total	Liver	Lung	Kidney	Spleen
Total	161	47	109	2	3
Fertile	77	20	54	1	2
Sterile	71	20	50	-	1
Calcified	13	7	5	1	-

Pulmonary involvement

Pulmonary cysts accounted for 67.7% of all caprine cysts examined, most were fertile (49.5%) or sterile (45.9%) and a few (4.6%) were calcified. The majority of pulmonary cysts were of medium size (78.0%), the others being either small (14.7%) or large (7.3%), none being larger than 10.0 cm.

The smallest fertile cyst measured 1.0 cm and the majority of fertile caprine cysts measured 5.0 cm or less, the volume of brood capsules generally increased with an increase in the size of the cyst (Appendix 1, Figure 1e).

Hepatic involvement

Of the 47 hepatic cysts examined, 20 (42.6%) were fertile, having an average viability of 74.0%. Twenty (42.6%) hepatic cysts were sterile, whilst only seven (14.8%) were calcified. The seven calcified cysts were all small in size. Most (70.2%) hepatic cysts were of medium size and fertile. Two fertile hepatic cysts were large and the only extra large cyst examined measured 11.5 cm and was sterile.

Other infection sites

Three (two fertile, one sterile) splenic cysts and two (one fertile and one calcified) renal cysts, were the only other localisations of hydatid cysts found in goats. The fertile renal cyst yielded 0.1 ml

brood capsules, which contained viable protoscoleces with a mean viability of 98.0%. The two fertile splenic cysts yielded 0.25 ml brood capsules (Appendix 1, Figure 1e). The protoscoleces of one had a mean viability of 60% the other had a mean viability of 96.0%.

Number of cysts per organ per animal

Twenty-two goats harboured single pulmonary cysts only and four harboured single hepatic cysts only. Single pulmonary and single hepatic cysts were most common in concurrent pulmonary and hepatic infections. The heaviest caprine infestation seen was the concurrent pulmonary, hepatic and renal infection (Plate 2), which had over 130 hydatid cysts.

Cyst type

The majority of caprine hydatid cysts were spherical and unilocular (93.8%). A few were lobulated (3.7%) and three pulmonary and one hepatic cysts were multilocular, having many internal median septae.

Two pulmonary and two hepatic cysts contained numerous daughter cysts. A single fertile pulmonary cyst and a single fertile hepatic cyst contained fertile daughter cysts with viable protoscoleces. The two other cysts that contained daughter cysts were sterile, as were the daughter cysts contained within them.

2.7 Discussion

2.7.1 Turkana

Unfortunately only a small number of domestic animals were examined in Turkana District for the presence of hydatid cysts. However, the prevalence obtained here for the various types of livestock are supported by the prevalence figures given by the public health technicians' in Lodwar and Kakuma. For the periods August

through November, 1979 and March through April, 1980 they reported 13 (24.1%) out of 54 camels, one (1.2%) out of 82 cattle, none of 455 sheep and 19 (0.4%) out of 4,525 goats infected with hydatid cysts (Shadrack (Lodwar) and Wilson (Kakuma) personal communication).

This low prevalence appears to have altered since the 1960s for Nelson & Rausch (1963) commented that the disease amongst the domestic livestock was common in Turkana and the Veterinary Services Report (1967) records the disease as being widespread amongst goats in Turkana. Changes in the prevalence of hydatidosis amongst cattle, sheep and goats have been reported by Verster (1962). She noted that between 1951-1952, 1.6% of sheep at Durban abattoir had hydatid cysts. This figure increased to 11.1% in 1954-1955, and decreased to 1.9% in 1957-1958. A similar rise and fall was also noted for goats slaughtered at the same abattoir. In cattle, between one and three per cent were infected during the entire study except for 1950-1951 when 5.5% were found infected. She speculated that these fluctuations might have been due to the different conditions prevailing in the areas of origin of the slaughter animals. She was unable to substantiate this, since it proved impossible to trace the origin of the infected animals.

The number and type of livestock found in Turkana fluctuates annually. During the 1979-1981 drought the only animals to survive in any great number were camels.

From the data collected, the camel has the highest prevalence of hydatidosis amongst the slaughter stock in Turkana. A similar situation is found in most other African and Middle Eastern countries which slaughter camels in addition to cattle, sheep and goats (see Appendix 1, Tables 1 and 2).

Cattle have the next highest prevalence of the disease after camels followed by sheep and finally goats, which have the lowest

recorded prevalence. No fertile hydatid cysts were found in cattle or sheep in Turkana during this study. This may be due in part to the small number of animals examined especially in the case of cattle, but the public health technicians' figures do suggest that cattle and sheep are only rarely infected in Turkana. They are certainly not as good intermediate hosts as camels, which are also slaughtered in small numbers, but which have been found to harbour numerous fertile cysts.

Goats have the lowest prevalence of hydatid disease, but due to the large number of goats slaughtered daily, the number of infected animals seen is similar to that of the camel. However, the number of hydatid cysts from goats were few in comparison to the number examined from camels.

In camels, fertile cysts were most frequently encountered in the lungs, a predilection site also reported by other authors (Al-Abbassy et al, 1980; Dailey & Sweatman, 1965; El-Badawi et al, 1979; El-Garhy & Selim, 1958; El-Kordy, 1946; Graber et al, 1969). Mobedi et al, (1970) reported that 34.0% of their camel hydatid infections had concurrent pulmonary and hepatic localisations followed by 24.0% pulmonary and 6.0% hepatic infections. Mobedi et al, (1970) also noted that whilst 73.0% of pulmonary cysts were fertile only 20.0% of hepatic cysts were fertile. No fertile hepatic cysts were found during this study. Altaif (1974) and Hassounah & Behbehani (1976) both reported higher numbers of hepatic than pulmonary infections. The only other organ found infected in camels was the spleen, which has also been reported as a site of infection by numerous other authors (Dada & Belino, 1979; Dada et al, 1979b; Dailey & Sweatman, 1965; El-Garhy & Selim, 1958; El-Kordy, 1946; Gupta, 1979; Malek, 1959).

Goats had usually either hepatic or pulmonary hydatid

localisations only, a similar situation found by Eugster (1978). Verster & Collins (1966) stated that the majority of goats in South Africa had hepatic cysts, whereas pulmonary localisations have been reported to be most common elsewhere (El-Badawi et al, 1979; Dada et al, 1979b; 1980). From the limited information available the majority of pulmonary cysts found have been fertile, whilst hepatic cysts are usually sterile or calcified (El-Badawi et al, 1979; Eugster, 1978).

The number of hydatid cysts examined per animal was much higher for camels than for goats in Turkana. Eugster (1978) also recorded a low number of cysts in goats in Masailand, de facto, all nine infected goats he examined harboured single hydatid cyst infections only.

Although many authors have reported that the majority of camel and, to a lesser extent goat, cysts are fertile, there are to my knowledge, no previous reports on the proportion of viable protoscoleces within these cysts, an important consideration when assessing the suitability of these and other animals as intermediate hosts.

The majority of camel and goat cysts examined were fertile, containing numerous brood capsules per cyst, which usually increased with an increasing size of the cyst (Appendix 1, Figure 1a & b). The average viability of the protoscoleces within these brood capsules was extremely high in goats and high in camels, making them both potentially good intermediate hosts of the parasite in Turkana.

The evidence from prevalence rates therefore, suggests that the principal domestic cycle operating in Turkana at present is likely to involve camels and goats, with cattle and sheep playing a subsidiary role. However, since the infection in the livestock fluctuates with time, as in fact does the number of domestic animals, the situation

is unstable and may change at any time, with different animals assuming the principal role. Hydatid infections are likely to be most stable in camels, as these animals survive the long Turkana droughts in greater numbers than the other livestock. It is known that hydatid cysts develop slowly in long-lived intermediate hosts, with maximum productivity attained in older individuals (Rausch, 1967a). Camels in Turkana, which are usually older than sheep and goats at slaughter, do have a high prevalence of hydatid disease, with large numbers of viable fertile cysts. This finding suggests that the camel is probably responsible for the continuous perpetuation of the domestic cycle, even though they are slaughtered infrequently.

2.7.2 Marsabit and Isiolo

As so few animals were examined from this region no comparisons can be made with the prevalence of the disease found in other areas of Kenya. However, the small survey undertaken confirmed that hydatid cysts were present in cattle, sheep and goats in this north-eastern region.

2.7.3 Masailand

The prevalence of hydatidosis in cattle, sheep and goats slaughtered at Ongata Rongai between April and September 1980, was found to be considerably lower than the prevalence of hydatidosis reported by Eugster (1978) for domestic livestock slaughtered in Kajiado District from 1973 to 1975. The largest variation was between the number of infected cattle. One likely explanation of the decrease in the number of infected cattle reported, is due to the sampling techniques employed. Eugster (1978), gives a detailed account of the hepatic cysts he examined and records 340 (86.3%) of these cysts to be fully calcified, with a diameter of 1.0 cm or less.

Due to the difficulties in readily identifying such small calcified cysts, from those belonging to other calcified lesions, only a few small (1.0 cm or less) calcified cysts were included in this survey. The present study therefore represents only the minimum prevalence of the disease in cattle in Masailand. This problem was not encountered to such an extent with the data from sheep and goats, since the majority of cysts from these hosts were not calcified. Even accounting for the difference in sampling methods, there does appear to have been a definite decrease in the prevalence of the disease in slaughterstock since 1975. The observed decrease may be due in part to the Government's effort to control the ever increasing stray dog population. Such a programme was introduced to prevent the spread of rabies in this area (Eugster, 1978). Further explanations for the observed decrease in infection rates are not clear, however, numerous factors may have contributed to this reduction. Amongst these can be included: the increased awareness of the Masai to limit the size of their cattle herds and to slaughter their animals at registered slaughterhouses; the severe drought of 1974 which may have affected the prevalence rates in the older cattle now being slaughtered; the possibility of a decrease in the prevalence rates in the domestic dogs, since Nelson & Rausch (1963) reported 50.0% of dogs infected in Masailand in 1961 whereas Eugster (1978) reported 27.3% of dogs infected in Kajiado during 1974 to 1975.

Even with the reduction in prevalence rates in the domestic livestock found in Masailand, the present rates of infection are many times greater than those observed amongst the cattle, sheep and goats in Turkana District. Possible explanations for this regional difference will be considered in the general discussion.

In Masailand, sheep had the highest prevalence of hydatidosis recorded and the largest number of cysts per infected animal. Concurrent pulmonary and hepatic hydatid cyst localisations were the most commonly found, followed by hepatic and then pulmonary localisations. The spleen was rarely involved and only a single renal infection was seen. Many authors record hepatic hydatid localisations in sheep as being the most common (Abdou, 1965; El-Badawi et al, 1979; El-Kordy, 1946; Eugster, 1978; Ginsberg, 1958; Sedik et al, 1978; Verster & Collins, 1966), and a few record more pulmonary localisations than any other site (Dada et al, 1979b; 1980; Verster, 1962). A high proportion of ovine cysts were fertile and only a few were calcified; similar findings are recorded by El-Kordy (1946) and Eugster (1978).

Some of the pulmonary cysts were multilocular, this has also been observed by El-Kordy (1946) who found the condition in livers from infected sheep.

The smallest recorded diameter for a fertile ovine cyst was 1.0 cm, a size which is attained some four to five months after infection (Soulsby, 1965). The majority of fertile sheep cysts had a diameter of less than 5.0 cm and those fertile cysts with a larger diameter were usually located in the liver (Appendix 1, Figure 1d). A high proportion of fertile ovine cysts were viable and the percentage viability did not decrease with an increase in cyst size.

Goats had the lowest prevalence of infection in Masailand and also fewer cysts per infected animal. A similar pattern was also found in goats in Turkana.

In Masailand, the majority of caprine cysts were found in the lungs, followed by concurrent pulmonary and hepatic localisations,

whereas in Turkana the majority of goats had either pulmonary or hepatic localisations. The findings of other authors have been described earlier.

The majority of cysts were fertile although many were sterile and a few were calcified. The smallest fertile cyst was 1.0 cm and the majority of fertile cysts measured 5.0 cm or less (Appendix 1, Figure 1e). As with fertile cysts from the Turkana goats, the viability of the protoscoleces were very high.

A higher prevalence of hydatidosis was found in cattle, than goats, but it was lower than that found in sheep. However, a larger number of cattle were slaughtered and consequently, a larger number of infected cattle were seen. Single hydatid cysts were usually found particularly when only pulmonary infections were observed. Fewer cattle were found to have 2-4 cysts per organ and fewer still with 5-10 cysts or more than 10 cysts per organ. A similar finding was reported by Eugster (1978), but he found more livers harbouring 2-4 cysts than single cysts. Whereas in this study pulmonary locations of hydatid cysts were most common, earlier workers in Kenya found hepatic localisations to be most prevalent (Ginsberg, 1958; Froyd, 1960a, b; Ng'ang'a, 1974; Eugster, 1978). Although hepatic cysts were most numerous, Froyd (1960a) mentioned that single organ infections were normally located in the lungs. Elsewhere in Africa, mostly hepatic localisations have been reported by, Abdou (1965), El-Kordy (1946) and Masaba et al (1977), whilst others have reported the lungs as the main predilection site (Dada et al, 1979b, 1980; El-Badawi et al, 1979; Owor & Bitakaramire, 1975; Sedik et al, 1977; Verster & Collins, 1966). Mishra & N'Depo (1978) record the kidneys as the usual predilection site of hydatid cysts in cattle in Ivory Coast.

Hydatid cysts in cattle have been found to be mostly sterile or calcified and Gemmell (1960) and Cameron & Webster (1961) stated that cattle are not very suitable intermediate hosts, as a high percentage of bovine cysts are sterile. In Egypt, cattle cysts are usually calcified or sterile and cattle are regarded as the least important intermediate hosts in that country (El-Kordy, 1946). However, El-Badawi et al, (1979) and Verster (1962) recorded fertility rates of 65.9% and 96.8% for bovine cysts in central Sudan and the Republic of South Africa respectively, showing that in some countries bovine cysts are mostly fertile and a 'strain' difference may be involved here.

The majority of bovine cysts examined during this study were found to be sterile, or to a lesser extent, calcified. Very few fertile cysts were seen and those that were fertile were normally single unilocular pulmonary cysts. These results support the earlier findings of Eugster (1978).

The smallest fertile bovine cyst had a diameter of 2.0 cm, twice the size of the smallest fertile ovine or caprine cysts seen. Unlike caprine cysts, the majority of fertile bovine cysts had a diameter of 5.0 cm or more. Cattle protoscoleces were not as viable as those from sheep or goat cysts. In fact, a few cattle cysts contained only dead protoscoleces. The volume of brood capsules obtained from each fertile bovine cyst was similar to the volume obtained from similar sized ovine and caprine cysts.

From the above evidence, cattle do not appear to be very suitable intermediate hosts for E. granulosus in Masailand. However, due to the large number slaughtered they undoubtedly could play some part in the perpetuation of the parasite in this region.

2.8 Summary

2.8.1 Turkana

1. Eight of 10 camels, one of 10 cattle, one of 61 sheep and seven of 844 goats examined at various abattoirs throughout Turkana were found to harbour hydatid cysts.

2. Camels harboured 8.5 hydatid cysts per infected animal, the majority being fertile pulmonary cysts with a high viability. Hydatid cysts from goats were the next numerous (2.1 cysts per animal) being located either in the liver or lungs and on one occasion in the spleen. The majority of caprine cysts were fertile and the protoscoleces had a high viability. Only two small sterile pulmonary bovine hydatid cysts were obtained from single infected animal and a single calcified cyst was obtained from an infected sheep.

3. Camels have the highest prevalence of hydatidosis in Turkana and although goats have the lowest prevalence many more goats than any other livestock are slaughtered and a comparatively high number of caprine cysts were examined. These two hosts are probably the principal intermediate hosts perpetuating the domestic cycle in Turkana District at present, with cattle and sheep playing a subsidiary role.

2.8.2 Masailand

1. Hydatid cysts were found in 6.6% of cattle, 8.2% of sheep and 3.6% of goats slaughtered at Ongata Rongai between April and September, 1980.

2. The 126 infected cattle examined harboured an average of 4.7 cysts per infected animal, the majority of which were located in the lungs and were sterile. Infected sheep harboured 8.7 cysts

per animal and the majority of the cysts were fertile containing viable protoscoleces. Caprine cysts were also usually fertile with viable protoscoleces. Infected goats harboured an average of only 2.9 cysts per animal.

3. Hydatid cysts of both sheep and goats must attain a diameter of at least 1.0 cm before they become fertile, whilst those of cattle must be at least 2.0 cm. The number of brood capsules per cyst tended to increase with increasing cyst size. No marked differences in the number of brood capsules recovered per cyst were seen for similar sized cysts of the different host species. The majority of fertile sheep and goat cysts had a diameter of less than 5.0 cm, whereas the majority of fertile cattle cysts had a diameter greater than 5.0 cm.

4. The majority of cattle harboured only a single hydatid cyst per infected organ, especially when only the lung was involved. Fewer cattle had 2-4 cysts per organ and less had 5-10, or more than 10 cysts per organ. Most infected sheep had 2-4 or 5-10 cysts per organ; some harboured single cysts and a few had more than 10 cysts per infected organ. Single cyst infections were most commonly found in goats, especially when the lungs were affected. Fewer had 2-4 cysts and only a very few had 5-10 cysts per organ. Only a single goat had more than 10 cysts in any location.

5. The majority of all cysts examined were unilocular, although some animals from each species had lobulated cysts, including many ovine pulmonary cysts. Multilocular cysts were found in the lungs of some sheep and in the lungs and liver of some goats. Daughter cysts were seen in four pulmonary bovine cysts, four hepatic and three pulmonary ovine cysts and in two pulmonary and two hepatic

caprine cysts, many of which were fertile and contained viable protoscoleces.

6. Sheep and goats and to a lesser extent cattle are all considered important intermediate hosts of E. granulosus in Masailand. Cattle are not thought to be as suitable an intermediate host for the parasite as sheep and goats, due to the high percentage of sterile and calcified cysts found in this host.

2.8.3 Marsabit and Isiolo

During the small survey undertaken in this area, neither of two camels, one out of 12 cattle, three out of 17 sheep and one out of 53 goats examined from this region were found to harbour hydatid cysts.

CHAPTER 3

MAN AS AN INTERMEDIATE HOST

3. Introduction

The aim of this chapter is to review the reported surgical cases of hydatidosis in Africa, in order to obtain some indication of the seriousness of the health hazard presented by hydatid disease. Particular attention is paid to the incidence of hydatid disease amongst the various tribesmen of Kenya. An attempt is made to evaluate the suitability of man in Kenya as an intermediate host for E. granulosus, and to ascertain whether or not man plays a part in the continuity of the life cycle of the parasite in certain parts of the country.

3.1 Human hydatidosis in Africa

Distribution maps of hydatidosis such as the one produced by Cameron & Webster (1959) suggest that hydatid disease is rare or absent over much of Africa. However, a recent review by Matossian et al (1977), shows that far from being rare, hydatid disease in man is more prevalent in parts of Africa than in many other parts of the world.

The reported human infections in Africa besides those seen in Kenya are given in Appendix 2, Table 1.

Human hydatidosis in Egypt was first reported by Madden (1909) who stated that one of every 1000 surgical cases were hydatid. Twenty-five years later Khalil Bey (1934) reported the death of a man in Egypt due to the presence of a hydatid cyst in his heart. There are few other references to human hydatidosis in Egypt, and

this together with the serological findings of Cahill et al (1965) suggest that the disease in humans there is low. In other north African countries, Algeria, Libya, Morocco and Tunisia, for example, hydatid disease appears to be extremely common (Appendix 2, Table 1).

In comparison to these north African countries, there are very few reports of human hydatid cases from Chad, Ivory Coast, Mali, Nigeria and Senegal, which suggests that the disease is rare in these countries.

In addition to the high prevalence of human hydatidosis in certain north African countries surgical figures indicate that a high prevalence of the disease exists amongst some East African tribesmen. In north-western Uganda, it has been reported in the Anchoi, Karamajong and Lango tribes (Owor & Bitakaramire, 1975) and in the Taposan and Latuka tribes in southern Sudan (Eisa et al, 1962). A serological survey by Cahill et al (1965) supports the hospital data, confirming a high incidence of hydatidosis in southern Sudan. In neighbouring Ethiopia, human hydatidosis has been reported to occur 'time and again' in all parts of the country (Schaller & Kuls, 1972). But, clinical and serological studies indicate that a particularly high prevalence of hydatidosis exists amongst the Dassanetch, Nyangatom, Kerre and to a lesser extent the Hamar tribesmen in south-west Ethiopia (Fuller, 1976). This whole region of Africa is recognised as having a particularly high prevalence, especially amongst the Turkana tribe of north-western Kenya (Wray, 1958; Nelson & Rausch, 1963; Nelson et al, 1965; Röttcher, 1973; Mann, 1974; O'Leary, 1976).

Owor & Bitakaramire (1975) also recorded a single hydatid case in a European who had lived in Sudan, and a single case from

Rwanda. Other records of human hydatidosis in East Africa include the first reported cases from Tanzania (Foley, 1944) and Uganda (Snell & Mukasa, 1948).

Elsewhere, the disease has been reported in Madagascar (Brygoo et al, 1971) and in southern Africa from Zambia (Patel, 1969), Zimbabwe (Holmgren et al, 1971) and by numerous authors in the republic of South Africa (Appendix 2, Table 1). Recently Kayser (1980) reported that on average about 20 new cases of hydatidosis are seen in the republic of South Africa each year, indicating a fairly high incidence of the disease in the country.

3.2 Human hydatidosis in Kenya

The first statistics concerning human hydatidosis in Kenya were published by Wray (1958) who presented details of hydatid operations performed in 11 Government Hospitals throughout the country from 1952 to 1955. The results indicated that the Turkana and to a lesser extent the Masai are much more affected than any of the other tribes in Kenya. Between 1955 and 1958 Wray (1958) personally operated on 25 patients at Kitale Hospital. Nelson & Rausch (1963) mentioned 64 hydatid operations performed by Cummins also at Kitale Hospital between 1957 and 1961, all of which were probably Turkana tribesmen.

Due to the lack of medical facilities in Turkana most hydatid patients were referred to hospitals outside the District, such as the one at Kitale. In 1957, a 32 bed Government District Hospital was built at Lodwar and a 12 bed Sub-District Hospital constructed in Lokitaung; these replaced the two dispensaries which, up to then had been giving mostly an out-patient treatment service. In the 1960s, three Mission Hospitals, one at Kakuma in north-western

Turkana, and two in the south at Lokori and Kapedo were built. These new Hospitals provided the facilities for most hydatid operations in Turkana, excluding those cases which required X-ray or specialised surgery. For example, lung, retro-orbital and intracranial cases, which in a recent report (O'Leary, 1976) accounted for approximately 13% of all cases.

The Lodwar and Kakuma Hospitals are served by a surgical safari team of the Flying Doctor Service (African Medical and Research Foundation, AMREF), and the Lokori and Kapedo Mission Hospitals are attended by surgeons of the African Inland Mission (AIM). The AIM also operate a flying doctor service to some of the remoter northern parts of the District which have limited medical facilities and bring back patients to the Lokori and Kapedo Hospitals.

From 1958 to 1961, 55 cases of hydatidosis were reported in the former King George VI Hospital in Nairobi (Nelson & Rausch, 1963). These increased to 60 cases in 1961 and 78 cases in 1962 (McClatchie & Rajpal, 1965). Three orbital hydatid cases were reported by McClatchie & Manku (1967) in two Turkana and one Masai, and three intracranial cases in two Turkana and a Pokot (Clifford, 1968).

Röttcher (1973) provided the first report of a large number of hydatid operations. During the years 1968 to 1972, whilst a surgeon with AMREF, he participated in 163 hydatid operations at eight different hospitals. One hundred and forty-two of these operations were carried out on Turkana patients and 17 were Masai.

Irvin (1974) provided details of 34 operations performed at Lokori Mission Hospital from January 1964 until May 1970. The majority of these cases had been brought down from northern Turkana, by the AIM.

O'Leary (1976) provided further detailed information of 789 cases during the five years 1971 to 1975. Of these, 776 cases were Turkana, 85% of whom came from one of two areas in northern Turkana. One area extending from Lokichokio in north-west Turkana to the Pelekech mountain range, the other in the north-east corner along the shore of Lake Turkana, including Lowarengak, Todenyang and Lokitaung (Figure 3). The other cases included 10 Merille (from Ethiopia) and three West Pokot.

O'Leary et al, (1979) also provided data on 241 patients who underwent hydatid surgery in Lodwar and Kakuma Hospitals between 1st January 1976 and 31st November 1978. AMREF (1980) extended this study to include all patients operated on for hydatid disease in these two hospitals until May 1980, an increase of 216 cases since 31st December 1978; AMREF (1980) also record 48 Turkana patients operated on for the disease at Lokori and Kapedo Hospitals in southern Turkana from 1st January 1976 until 1st May 1980, with most of these cases being flown in from Lokichokio.

Thus, during the years 1958 to 1980 a considerable amount of information has accumulated on the incidence of hydatidosis in Turkana. Schwabe (1969) using in-patient figures from Kitale and Lodwar Hospitals together with the Turkana population given by Gulliver & Gulliver (1953), estimated the incidence of human hydatidosis in the District to be at least 40 per 100,000 per annum. O'Leary (1976) regarded this to be a gross underestimation and recalculated the incidence rate to be 96 per 100,000 per annum, basing this figure on all the diagnosed patients in the District over the five year period 1971 to 1975 and using the 1969 population census figure of 165,000 people as the denominator. AMREF (1979) took into consideration the incidence of the disease

in different geographical areas of Turkana and confirm O'Leary's (1976) observation that the majority of patients with hydatidosis in Turkana District come from two areas in the north-east and north-west. Basing observations on the geographical distribution of patients who underwent surgery for hydatid disease for the first time, AMREF (1979) report incidences of 92 to 220 per 100,000 per annum for the areas north of Kakuma to Lokichokio in the west and the area around Lokitaung in the east. The incidence along the Turkwel and Kerio river systems and in the south of Turkana generally was notably lower, being only 30, 18 and 23 per 100,000 per annum respectively.

All these data, however, represent only that proportion of infected patients seeking treatment in hospital, and must therefore represent the minimum rate of the occurrence of the disease. The use of diagnostic methods (radiology, ultrasound, serology etc) to detect asymptomatic cyst carriers indicate that symptomatic hospital cases represent only a fraction of total infections. For example, in Rio Negro Province Argentina, where the annual incidence of hospital cases was 143 per 100,000, a mass miniature radiography survey of 15,000 persons indicated a pulmonary incidence of the disease of 460 per 100,000 and pulmonary localisations represent only one-third or less of all infections (Schantz et al, 1973). The extensive records compiled for Turkana patients since 1958, provide very valuable data on the incidence of the disease in the District, which undoubtedly has one of the highest incidences of the disease in the world. A high recurrence rate is also noted for the region (O'Leary et al, 1979). Recently, scolicides (particularly Cetrimide (R)) have been introduced into the operating procedure in an attempt to reduce the risk of recurrence.

Unfortunately, very little information is available concerning the

incidence of human hydatidosis amongst the other tribes in Kenya, but it has been reported in the Suk of West Pokot, the Rendille and Shagilla (O'Leary, 1976), and the Masai (Röttcher, 1973; O'Leary, 1976; Eugster, 1978).

Seventy-eight cases of hydatidosis were recorded from Kajiado and Narok Districts in Kenya and from Loliondo and Machame Mission Hospitals in the neighbouring Masai area of northern Tanzania, during the period 1962 to 1976 (Eugster, 1978). All but two of these patients were Masai. On the basis of data recorded at these hospitals during the period 1968 to 1975, Eugster's (1978) study indicated an average annual incidence of new hospital cases of only 2.0-3.7 per 100,000, based upon the 1969 population census.

All the human hydatid operations reported in Kenya and discussed above are summarised in Appendix 2, Table 2.

3.3 Materials and methods

Source of material

Human hydatid material was collected from 64 operations performed on Turkana tribesmen at Lodwar, Kakuma and Kapedo Hospitals from February 1979 to September 1980. Further fresh material was received within hours of the operation from one Samburu and four Masai. The sex, age and origin of all patients was obtained from hospital records.

At operation, the location, number and approximate size of any hydatid cysts found by the surgeon were recorded. Occasionally the approximate size of the cyst was calculated from the volume of hydatid fluid aspirated ($\frac{4}{3} \pi r^3$). Hydatid cysts were sometimes removed whole, or more usually were drained prior to removal of the laminated membrane and closure of the pericyst (Plates 3-6). As

Plate 3-6. Surgical removal of a hepatic hydatid cyst from a 19 year old Turkana female. Kakuma Hospital, March 1979.

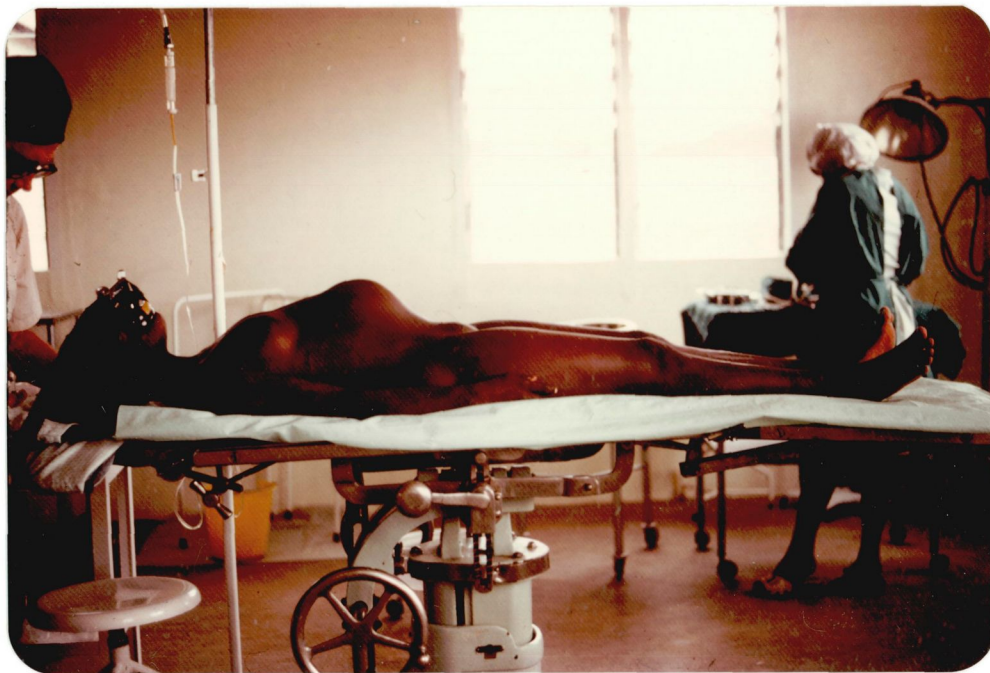


Plate 3. Patient receiving general anaesthesia.

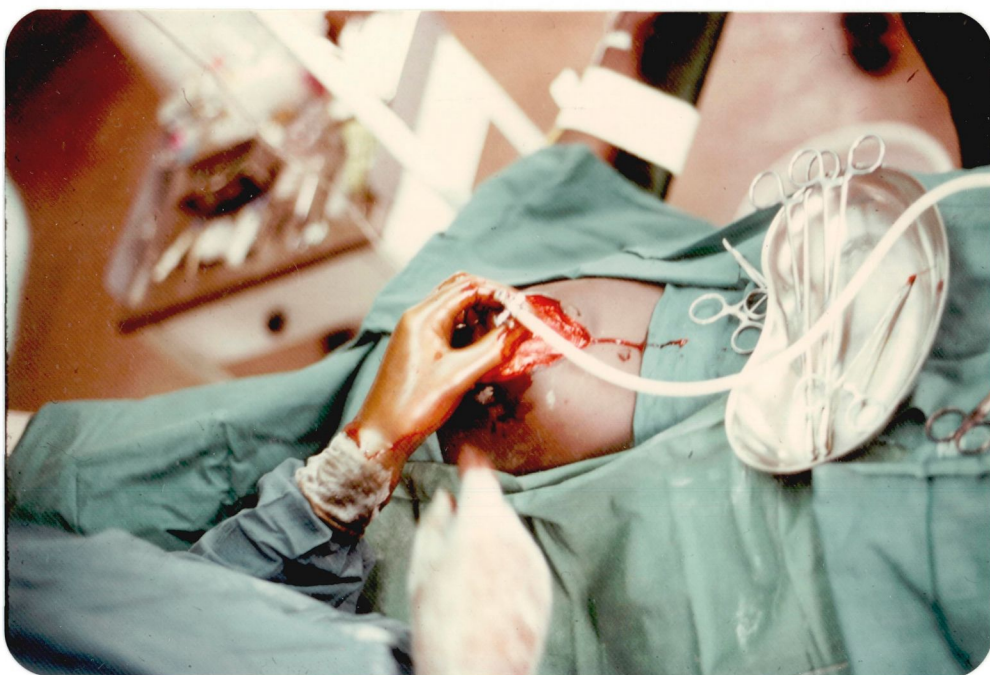


Plate 4. Aspiration of hydatid fluid through a cannula attached to a drainage tube. Hydatid cysts are often under considerable intracystic pressure and the fluid is quickly drained.



Plate 5. Removal of the endocyst. This particular cyst was fertile (containing protoscoleces) and unilocular.



Plate 6. Closure of the incision, the hydatid cyst excised.

much of the hydatid material as possible was kept following each operation, stored in a refrigerator and then flown back to Nairobi, for subsequent examination.

Examination of material

All human hydatid material obtained was examined in an open 'laminar flow' (sterile air) cabinet, using sterile dissecting instruments and sterile containers throughout.

Whole human hydatid cysts were treated in the same manner as whole animal cysts, described in chapter 2. The volume of sedimented brood capsules from fertile cysts was recorded and viability testes were performed using the same methods, also described in chapter 2. Similar viability tests on human material have been employed by others (Smyth & Barrett, 1980).

3.3.1 Evaluation of the effect of Cetrimide (R) on human protoscoleces in vitro

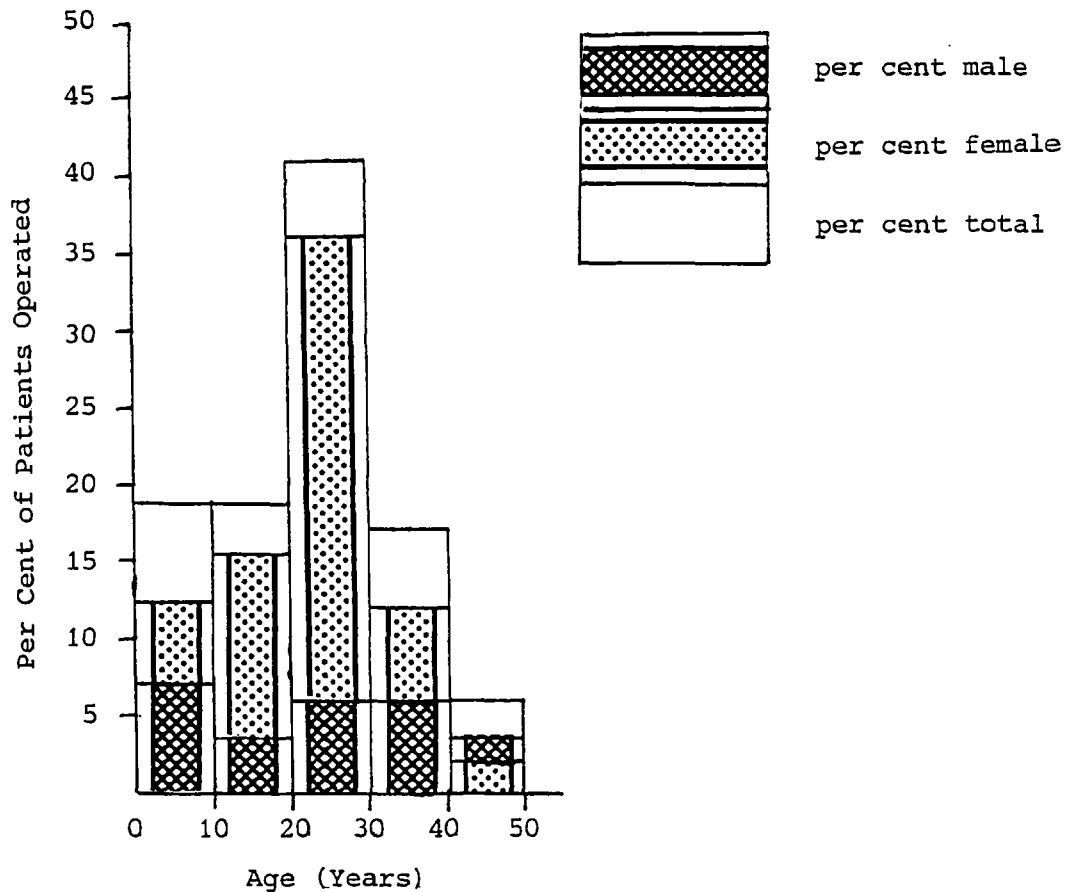
Fresh, viable protoscoleces from two human hydatid cysts were transferred to two separate 20 ml universal containers and washed three times, in warm HBSS. One drop of protoscolex suspension from each cyst was then added separately to warm Cetrimide (R) (cetyl-trimethyl-ammonium bromide) testing solutions (0.1%, 0.2% and 0.3%), and placed on a roller in an incubator at 37°C. Control testing solutions contained warm HBSS instead of Cetrimide (R). After 0.5 and 5 minute intervals the protoscoleces were washed thoroughly in warm HBSS and examined immediately for viability, using flame cell activity and the uptake of 0.1% aqueous Eosin as indicators of viability. The method was repeated several times, using separate aliquots of protoscoleces from the two hydatid cysts.

3.4 Results

3.4.1 Turkana hydatid cases

A total of 64 operations for the removal of hydatid cysts from Turkana patients were attended at Lodwar, Kakuma and Kapedo Hospitals. Of these, 49 (76.6%) were female and 15 (23.4%) were male, a male:female ratio of 1:3.3. The sex and age distribution of 59 of the above hydatid infected patients is given in Figure 5.

Figure 5. Age and sex distribution of 59 patients with surgically confirmed hydatid disease



As can be seen from Figure 5, a large number of the patients (35.6%) were females aged between 20 and 30 years old.

The location of each hydatid cyst is presented in Appendix 2, Table 3. The condition of the hydatid cysts examined in relation to their location is shown in Table 8.

Table 8. The condition of human hydatid cysts examined in relation to location

	TOTAL	HEPATIC	MESENTERIC	OTHER LOCATIONS
TOTAL CYSTS	88	35	35	18
FERTILE	53	28	17	8
PER CENT VIABILITY	73.9	82.4	52.9	87.4
STERILE/INFECTED	35	7	18	10
CALCIFIED	-	-	-	-

The liver was the usual predilection site of the hydatid cysts; 39.0% of patients harboured only hepatic cysts, 21.9% had only mesenteric cysts, and 39.1% harboured cysts in numerous other locations (Appendix 2, Table 3).

Hepatic involvement

Of the 35 hepatic cysts examined, the majority (80.0%) were fertile and had a high mean viability (82.4%). Many of the larger hepatic cysts yielded a large number of brood capsules per cyst (Appendix 1, Figure 1f). In one particular case, two large (15 and 16 cm) hepatic and mesenteric cysts removed from an eight year old boy, yielded 3.0 and 5.0 ml brood capsules with an overall viability of 99%.

The hepatic cysts were usually single, unilocular and extremely large, having diameters between 10.0-20.0 cm. Four patients harboured more than a single hepatic cyst, one of these was a recurrent case

with a multiple hepatic infection.

Thirteen of the 35 hepatic cysts examined contained daughter cysts (Plate 9) which were invariably fertile, containing viable protoscoleces. The smallest fertile daughter cyst measured 1.0 cm and the largest 10.0 cm. One cyst contained fertile daughter cysts in which none of the protoscoleces were viable.

Mesenteric involvement

Mesenteric cysts were encountered in 19 patients, including five recurrent cases. Nine patients harboured single cysts only, five had numerous mesenteric cysts and five harboured mesenteric cysts in addition to cysts in other locations.

The cysts varied in size from small (1.5 cm) to one which was extra large (35.0 cm) (Plate 7). This particular cyst contained approximately 11 litres of hydatid fluid and a lot of pus (Plate 8). The majority of mesenteric cysts were between 5.0-15.0 cm in diameter and many were sterile (Table 8).

Daughter cysts varying from 0.2-5.0 cm were found in 10 out of the 19 cases; some were fertile containing viable protoscoleces.

Other infection sites

Hydatid cysts in other locations were usually single, unilocular and often sterile (Table 8). The cysts varied in size from a 3.0 cm cyst in the left breast to a 15.0 cm uterine cyst.

Three primary cases had hydatid cysts containing daughter cysts, these included a single cyst located in the chest wall (superficial), a single cyst lying outside the peritoneal cavity, and two large (10.0 cm) lumbar cysts.

Although no pulmonary cysts are included in this series, two patients with obvious pulmonary hydatid cysts were seen in the

Plate 7. Turkana woman with a large abdominal hydatid cyst.
Lodwar Hospital, March 1979.

Plate 8. Surgical removal of the above abdominal hydatid cyst by
Mr. A. Michael Wood (Director General, AMREF). The cyst
contained approximately 11 litres of pus infected hydatid
fluid. No viable protoscoleces were seen in the material
examined. The patient survived the operation.

Plate 7



Plate 8



Plate 9. Daughter cysts being surgically removed from a hepatic hydatid cyst. Kakuma Hospital, March 1979.

Plate 10. Turkana woman with multiple recurrent hydatid cysts. Recurrent cases account for approximately 14 per cent of all hydatid operations performed in Turkana.

Plate 9



Plate 10



hospital wards in Lodwar and Kakuma Hospitals (diagnosed by Mr. A.M. Wood); one of these coughed up and discharged several daughter cysts. These cases together with a single intracranial case and one with hydatid bone involvement in the femur were referred to Nairobi or Nakuru Hospitals, for specialised surgery. Unfortunately, no hydatid material was obtained from these operations.

Recurrent cases

Nine out of 64 (14.1%) operations were performed on patients who had previously undergone hydatid surgery. All these patients had hydatid cysts which developed in or near the original operation site and are termed recurrent (Plate 10).

One patient had hydatid surgery in 1970, nine years before returning for a subsequent operation. However, most of the others returned some three to four years after the first operation. The shortest interval between a primary and subsequent operation was only one year and eight months. This case was especially interesting as the patient was given mebendazole treatment for a month after the initial operation. The recurrent cyst was a single unilocular, hepatic cyst which measured 6.0 cm in diameter and contained hundreds of small daughter cysts. Two of the larger (3.0 cm) daughter cysts contained dead and decaying protoscoleces. One other recurrent patient had had mebendazole treatment during the previous admission for surgery.

Six of the nine recurrent cases involved multiple cysts, often in several locations. Recurrent cysts varied in size from 1.5 cm to 15.0 cm, the majority measuring between 2.0-5.0 cm in diameter. The multiple cysts from the same patients tended to be the same size.

Seven of the nine recurrent cases had cysts which contained

daughter cysts, some of which were fertile containing viable protoscolecemes.

3.4.2 Masai hydatid cases

Seven abdominal hydatid cysts removed from four adult female Masai patients were examined. All cysts were large (2 x 6.0 cm, 2 x 10.0 cm, 3 x 12.0 cm), unilocular and all but one were fertile, yielding 1.6 ml packed brood capsules. The average viability of the protoscolecemes from the fertile cysts was extremely high (92.7%).

Two cysts (1 x 10.0 cm, 1 x 12.0 cm) harboured daughter cysts. The smaller cyst was sterile and contained pus, but the daughter cysts were clear, fertile and the protoscolecemes contained within them had a viability of 84.0%. The larger cyst contained only a single, small, sterile daughter cyst, but was itself fertile.

3.4.3 Samburu hydatid case

A single hydatid cyst was examined from an 11 year old Samburu girl. The cyst was removed from the liver and was fertile with an average viability of 99.0%. Unfortunately the size of the cyst was not recorded.

3.4.4 Cetrimide (R) results

The results of all incubations with the three concentrations of Cetrimide (R) are shown in Table 9.

Only a few protoscolecemes remained viable after incubation for 30 seconds with 0.1 and 0.2% concentrations of Cetrimide (R). None appeared viable after five minutes incubation, as examined by the loss of flame cell activity and the rapid uptake by the protoscolecemes of 0.1% aqueous Eosin. In contrast, the control protoscolecemes incubated at the same temperature, showed no decrease in viability

during the same time.

Table 9. Comparative scolicial effect of Cetrimide (R) on human protoscoleces at 37°C

TIME (MINS)	Cetrimide (R) concentration (per cent)						Control HBSS		AVERAGE PERCENT VIABILITY
	0.1		0.2		0.3		A	B	
	A	B	A	B	A	B			
0.5	2.0	3.0	1.0	0.5	0.0	0.0	96.0	80.0	
5.0	0.0	0.0	0.0	0.0	0.0	0.0	96.0	80.0	

3.5 Discussion

The data obtained from hospital records showed that the 'age at operation' of males varied only slightly. However, the incidence of infection amongst females was considerably higher for the 20-30 year group than for any of the other female or male age groupings (Figure 5).

Röttcher (1973), O'Leary (1976) and French (1980) all recorded a higher percentage of females than males infected in Turkana. In addition, O'Leary (1976) and French (1980) both showed that the proportion of infected females to males was much higher in adults than children. This trend has also been shown by Fourati et al, (1977) for hepatic cases in Tunisia. It is thought that this apparent sex difference is unlikely to be due to the females being intrinsically more susceptible to the disease, since in Masailand, Eugster (1978) reported slightly more infected males than females, and a similar pattern has been noted in several other countries (Belding, 1965; Schwabe & Abou Daoud, 1961; Najah & Sawicz Birkowska, 1976; Burridge & Schwabe, 1977). Cameron & Webster (1961) stated that

both sexes are equally prone to infection, and the proportion of males to females infected in any given group is, for the most part, determined by local customs involving the housing and handling of dogs.

Frayha et al, (1971), showed that male mice are more susceptible than are females to intra-peritoneal infection with protoscoleces of E. granulosus. This result has not been found by other workers (De Rosa et al, 1972) although male mice have been reported to be more susceptible to infection with E. multilocularis (Ohbayashi & Sakamoto, 1966).

Data concerning 'age at operation' for the Turkana, were based on estimations of the patients age by hospital staff, and may therefore be a little inaccurate; notwithstanding this, the 'age at operation' data obtained is of limited value when looking for possible cultural differences, as they provide little indication about the age of infection. Clinical symptoms of hydatid disease can remain undetected for many years; for example, Spruance (1974) recorded a latent case which exceeded 50 years.

It was previously assumed, that although persons of all ages could be infected, there was evidence suggesting that although most cases were diagnosed between the ages of 20-30 years, most infections occurred during childhood (Cameron & Webster, 1961). This reasoning was based upon the rarity of primary brain cysts after the age of 15 years. The incubation period for hydatid disease of the brain is, presumably, of relatively short duration, and Dew (1928) stated that intracranial cysts are the least likely to be carried into adult life. Recently, Beard (1978) showed that the incidence of infection of all age groups in New Zealand and Tasmania fell with the introduction of control programmes. This indicated that cysts of short latency were

present in susceptible individuals of all ages, and therefore the disease is likely to be contracted at any time, and not necessarily during childhood.

Schwabe et al, (1959) found that there was a decrease in the number of mice that became infected from intra-peritoneal hydatid infection after reaching maturity. Heath (1970a) however, did not observe any difference in susceptibility between young and old mice.

In a study of 355 Turkana patients, French (1980) found the highest incidence in females and the lowest male to female ratio in the 24 to 44 year age group. He suggested that this could be due to increased contact with dogs by women during child rearing. Unfortunately, no data is available comparing the number of infected women who have children, with those who do not, and it seems likely that this suggestion may be an oversimplification of the situation. For, although the mothers with infants usually have one or more dogs in close attendance, it is the infants who have a closer contact with the dogs, and therefore a greater opportunity to become infected. Thus, in early childhood children of both sexes would appear to have equal chances of becoming infected. This is reflected in the smaller discrepancy in infection rates found between males and females in the younger age bracket. Young boys soon take to herding sheep and goats in the surrounding countryside, whilst the girls usually remain at the manyatta to help the mother, who may have yet another infant to look after. From this time onwards the girls probably have a higher frequency of contact with dogs than do the boys, since dogs are usually always present in the manyatta.

Hence, excluding the possibility of hormonal influences, the reported higher infection rates in females in Turkana, is more likely

to be related to an increased opportunity for infection over many years, rather than due to an increased exposure at any given age.

The small numbers of patients over 40 years infected with hydatid disease, is probably due to the fact that few Turkana survive beyond this age (O'Leary et al, 1979).

The most frequently infected site of all the hydatid cases was the liver (40.6%), which is also the most commonly reported predilection site (45.1-66.4%) in all previously published reports of surgical case series in East Africa (Wray, 1958; Röttcher, 1973; Irvin, 1974; Owor & Bitakaramire, 1975; O'Leary, 1976; Eugster, 1978; O'Leary et al, 1979). Cysts in other abdominal locations were next prevalent, followed by occasional cases in other anatomical sites.

The relative frequency of specific predilection sites of hydatid cysts varies from one country to another, largely because of the differences in the availability and use of diagnostic techniques. In most other published reports of primary surgical cases, hepatic cysts are most common (50-70%) followed by pulmonary (10%) and less frequently other locations (Dew, 1937; Cameron & Webster, 1961). The small number of pulmonary cases reported from Turkana and Masailand to date is probably due to difficulties in diagnosis. There are no X-ray facilities in Turkana, or in many of the hospitals in Masailand, and consequently many pulmonary hydatid cysts remain undiagnosed.

One of the most striking features of hydatid disease in Turkana besides its prevalence, is the extraordinary large size of some of the hydatid cysts. Cysts measuring 10-15 cm in diameter are not uncommon and occasionally extremely large ones are seen

(Plate 7). These cysts are considerably larger than those from humans in Australia where the largest cyst reported contained only three litres of fluid (Soulsby, 1965). The large size that some of the cysts in Turkana achieve could be due to the people's ignorance of the condition and their decision not to seek medical help. For example, one woman was admitted complaining that she had been pregnant for the previous nine years (Wray, 1958). The extremely large sizes that are seen, is indicative of the remarkable tolerance of some of the infected people to the disease and the balance that the parasite has achieved with its host.

The high fertility and viability of the cysts found in man in Kenya, together with the fact that few Turkana, or (to a lesser extent) Masai, are buried suggests that they are suitable as intermediate hosts for the parasite in these regions. In Turkana, man has been previously regarded as a possible alternative intermediate host (Nelson & Rausch, 1963; Nelson et al, 1965; Mann, 1974), whereas in most other regions of the world, he is regarded as a dead end host and an accidental host in the epidemiological sense.

The high fertility of the hydatid cysts coupled with the relatively unsophisticated operating conditions, must increase the risk of postoperative recurrence of the disease. It is the remarkable ability of each protoscolex of Echinococcus to develop in vivo into a hydatid cyst, which causes the major drawback to the successful surgical management of this disease. One hundred years ago Volkmann warned surgeons against the risk of sowing hydatid elements by puncture of fertile cysts (Dew, 1928). Great care, therefore, is taken during surgery, to reduce the spillage of hydatid fluid and protoscoleces. Occasionally a cyst may rupture

due to trauma, and the patient may die from anaphylaxis. If the patient survives, the dissemination of protoscoleces may result in multiple secondary hydatidiasis (Plate 10). Such secondary hydatidiasis resulting either from spillage of protoscoleces during surgery, or due to cyst rupture, is often very difficult to remove and in some cases it is inoperable. Operative recurrence of osseous hydatid cysts are the rule, rather than the exception (Mottaghian & Saidi, 1978), and therefore osseous involvement usually requires amputation. Such cases highlight the considerable morbidity associated with hydatid disease.

The recurrence rate in Turkana has been previously reported to be at least 20% (O'Leary et al, 1979). This is twice the recurrence rate of pulmonary hydatid cysts reported by Barrett & Thomas (1952), and considerably higher than the 11.3% recurrence rate recorded in 106 patients by Mottaghian & Saidi (1978). The latter authors suggest that a recurrence rate greater than 10 per cent should clearly indicate a reassessment of the operative approach.

In Turkana, cysts were removed intact whenever possible but unfortunately this was feasible in a few cases only. The usual operative technique consisted of initially placing abdominal pads around the cyst, to prevent the spillage of cyst contents into the operational area. Once in place, the hydatid fluid was aspirated through a large gauge needle to reduce the often considerable intracystic pressure. A small incision through the pericyst layer was then made and the rest of the fluid removed using suction. The laminated membrane and any daughter cysts, if present, were then removed from the pericyst and the latter closed, sometimes with a drainage tube protruding to the exterior.

In previous attempts to prevent or reduce recurrences many patients were treated with the oral drug mebendazole to kill or inactivate the cyst. In some cases a 5% formalin solution or occasionally a 5% Cetrimide (R) solution was introduced into the partly evacuated pericystic space to kill any protoscoleces present (O'Leary et al, 1979; Gilchrist, 1979). Unfortunately, insufficient time has elapsed to evaluate the results of these measures. One benefit shown by the mebendazole and formalin treatments was that they did appear to reduce the occurrence of serious postoperative complications (O'Leary et al, 1979).

All treatments with scolicedal agents was halted when three patients developed sterile chemical peritonitis with massive adhesions and obstruction following the treatment of cysts with 5.0% Cetrimide (R) and the use of a 0.5% washout (Gilchrist, 1979). However, with the provision of further evidence of the high scolicedal value, and low toxicity of a very much lower concentration of Cetrimide (R) (Eslami et al, 1978), and the confirmation of the in vitro effect of Cetrimide (R) on human protoscoleces removed from Turkana patients (this study), a 0.1% Cetrimide solution was reintroduced as an adjunct to the surgical procedure.

Recently, more evidence has emerged to indicate the usefulness of suction or cryogenic cones, or similar devices designed to prevent spillage of fluid, with the adjuvant use of a scolicedal agent (0.5 per cent silver nitrate, or 0.1% Cetrimide (R), being the most commonly used) as the most promising method of treatment of hydatid disease (Saidi & Nazarian, 1971; Mottaghian & Saidi, 1978; Aarons, 1979; Frayha et al, 1981). Such techniques using suction cones and a 0.1% solution of Cetrimide (R) are at present being evaluated for their applicability in Turkana. To date, 22 patients

have been treated in Lodwar, Kakuma and Kapedo Hospitals, using a 0.1% solution of Cetrimide (R). No complications were observed in any of the 22 patients, but studies on the in vivo toxicity of Cetrimide (R) to human tissues need to be conducted. In Lebanon, no untoward effects have been reported in any of the 378 cases so far treated with this scolocidal agent (Frayha et al, 1981).

3.6 Summary

1. During the course of this study of 64 hydatid operations on Turkana patients, 49 were female and 15 were male. A large number of females aged between 20-30 years underwent hydatid surgery. Possible explanations for this, and the observed sex difference are discussed.
2. The liver was the only organ infected in 39.0% of the patients, and 80.0% of these cysts were fertile, having a high mean viability (82.4%). The hepatic cysts were usually extremely large (10-20 cm) and single. Almost twenty-two per cent of cases were located in the mesentry; the remainder of cases (39.1%) were located in numerous other locations. Many of these cysts were sterile. The cysts varied in size from small (1.5 cm) to extra large (35.0 cm).
3. Daughter cysts varying in size from 0.2-10.0 cm were found in 26 of the 88 cysts examined. Many were fertile containing viable protoscoleces.
4. Nine of the 64 (14.1%) cases were for recurrent hydatid operations. The time interval between the primary and subsequent operation varied from one year eight months to nine years. The

majority of the recurrences had multiple cysts, which varied in size from 1.5-15.0 cm. Seven of the recurrent cases had cysts containing daughter cysts.

5. Seven hydatid cysts were examined from four Masai females; their cysts were large, unilocular and all but one were fertile. A single fertile cyst from a Samburu girl was examined.

6. In vitro experiments with low concentrations (0.1-0.3%) of Cetrimide (R), showed that its scolicial action on viable human protoscoleces was rapid and effective. Most protoscoleces being destroyed after only half a minutes incubation. The use of scolicial agents as adjuncts to the surgical procedure is discussed.

CHAPTER 4

THE DOMESTIC DOG AS A DEFINITIVE HOST FOR E. GRANULOSUS4. Introduction

The occurrence and prevalence of Echinococcus infections in domestic dogs in Africa is presented. In Kenya, the dog is regarded as the most important host for adult E. granulosus (Nelson & Rausch, 1963; Eugster, 1978). Previous surveys have shown an extremely high prevalence of the parasite in dogs in Turkana (Nelson & Rausch, 1963), and an attempt was made to re-examine the current prevalence and intensity of infection in dogs in this District. A smaller survey to examine the existence of the parasite in dogs in Marsabit District was also undertaken.

The infectivity of hydatid material from man, camels, cattle, sheep and goats to dogs in Kenya, is unknown. Consequently, a study of the susceptibility of dogs to experimental infection, with material from all these hosts was undertaken.

4.1 Occurrence and prevalence of E. granulosus in dogs in Africa

Various authors have reported Echinococcus infections in dogs in North Africa from; Algeria, Morocco, Tunisia, upper Egypt and in Sudan particularly in the south of the country (Appendix 3, Table 1). There are only a few reports of infection in dogs from Central and West Africa. In Chad, four out of (3.4%) 117 dogs examined, harboured the adult parasite (Troncy & Graber, 1969). In Nigeria, prevalences of 0.6% and 2.0% have been reported in dogs in Zaria and Kaduna States (Dada et al, 1979a, b) and a prevalence of 6.2% in dogs in Kano state (Dada et al, 1980).

In South Africa, Ortlepp (1934) recorded that five of 25 dogs examined in Pretoria harboured E. granulosus infections. Verster (1979) found 10 of (0.9%) 1063 dogs examined, from various regions in South Africa, harbouring the adult parasite.

There are two previous records of E. granulosus infections in dogs in East Africa, and both of these are from Kenya. Nelson & Rausch (1963) found eight of (50%) 16 dogs examined in and around Nairobi and 19 of (70.4%) 27 dogs examined in Turkana infected with the adult parasite. Eugster (1978) reported 45 of (27.3%) 165 dogs examined, between 1974 and 1975 in Kajiado District, to be infected.

The reported cases of the occurrence and prevalence of E. granulosus infections in dogs from the various African countries are summarised in Appendix 3, Table 1.

The Turkana keep a large number of domestic dogs, numbering at least one per family (Nelson & Rausch, 1963). The role of these dogs has been described earlier (see Chapter 1).

4.2 Materials and methods

4.2.1 Natural infections

During the period April 1979 and July 1980, seven surveys were conducted to examine the prevalence of E. granulosus infections in domestic dogs (Canis familiaris) in many regions throughout Turkana, and a single survey was made to Marsabit District.

In northern Turkana, dogs were examined in Kakuma, Lakonkai, Lokichokio, Lokitaung and Nanam (see Figure 3). These areas were selected for study because they are regions where extraordinarily high incidences of human hydatidosis had been reported (O'Leary, 1976;

AMREF, 1979). For a comparative study in Turkana, dogs were examined from two townships, Lokori and Lotoboi (see Figure 3) in the south of the District, where the human incidence of the disease had been reported to be considerably lower (AMREF, 1979).

Additionally, some dogs were examined from Marsabit township in Marsabit District (see Figure 2), an area where very few human hydatid cases have been reported.

The dogs examined in Marsabit were shot, but in Turkana, only occasionally were dogs shot. All other dogs were brought to us for euthanasia by their Turkana owners for a nominal payment, usually 5-10 Kenya shillings (25-50p), plus a small gift of chewing tobacco.

During the early surveys dogs brought to us were killed immediately with intravenous injections of Euthatal (pentobarbitone sodium, May and Baker Ltd., Dagenham, UK). In each case the effective dose according to body weight was used. In the later surveys, many of the dogs were kept near the camp, and starved for 24 hours (as recommended by Schwabe, 1969) before being killed with a saturated solution of magnesium sulphate injected directly into the heart.

The small intestines were then removed in toto and after rinsing in normal saline, the gut was opened under fresh saline in large black-bottomed trays. If no Echinococcus were observed, the intestinal mucosa was scraped and examined by washing and decanting with fresh saline. After relaxation, the worms were counted unless obviously exceeding 1,000 individuals; they were then fixed in 70% alcohol as recommended by Vogel (1957), or in 10% formal saline. Care was taken to secure container tops as formalin has been shown to be ineffective as an ovicide (Hercus et al, 1962).

Objects which appeared on gross examination to be either whole worms or segments of E. granulosus were kept for microscopic examination later.

In the last survey at Lokichokio and Nanam, some of the dogs' intestines were examined by eye only, and the presence or absence of E. granulosus adults protruding from the mucosa recorded. In all cases where adult worms were visible, their position along the intestine was measured from the pylorus.

Other parasites present in the small intestine were processed in the same manner as the E. granulosus specimens. These were later identified microscopically, using the classifications of Verster (1969).

4.2.2 Experimental infections

A total of 34 puppies (all less than 16 weeks old), were purchased in litters of three or more individuals, from Turkana tribesmen and from people living in Nairobi. The puppies were dosed with canex (R) (pyrantel pamoate, Pfizer Agricare Pty Ltd., UK) to remove roundworm and hookworm infections. Additionally, in the later studies, every puppy received 10 mg per Kg bodyweight of droncit (R) (Praziquantel, Bayer, Leverkusen, Germany) prior to experimental infection, in order to eliminate any possible naturally acquired Echinococcus infections.

The animals were starved for 21 hours before being fed a gelatine capsule containing approximately 160,000 packed protoscoleces, obtained from a hydatid cyst from one of the intermediate hosts. Immediately before administration, all hydatid material was checked for viability using the methods described in Chapter 2. Puppies from the same litter were infected with the same source of hydatid

material. The number of puppies infected with each source of protoscoleces is shown in Table 12.

Puppies were examined post mortem between 34 and 38 days later i.e. before the adult worms became ovigerous. Three puppies fed protoscoleces from a human patient were examined 40 days post infection (p.i.).

Puppies were starved for 18 hours prior to autopsy, and killed with an intravenous injection of Euthatal. The small intestines were removed in toto, opened longitudinally, divided into 30 cm lengths from the pylorus and incubated in separate beakers of prewarmed (37°C) HBSS for one and a half hours. After this time the intestines were scraped, and the numbers of worms estimated by suspending all the worms from each section in 100 ml HBSS and counting the number of worms present in 10x1 ml aliquots. Worms were counted individually in light infections.

4.3 Results

4.3.1 Natural infections

Of 281 dogs examined in Turkana District, 164 (58%) were found harbouring E. granulosus infections. The intensity of infection was variable; 71 dogs (25.3%) harboured light infections (less than 200 worms), 27 (9.6%) harboured medium infections (200 to 1000 worms) and 66 dogs (23.5%) had heavy infections (more than 1000 worms). The sex of the dog did not appear to have any effect on the prevalence or intensity of infection found (Table 10). Unfortunately it proved impossible to determine the ages of any of the dogs examined.

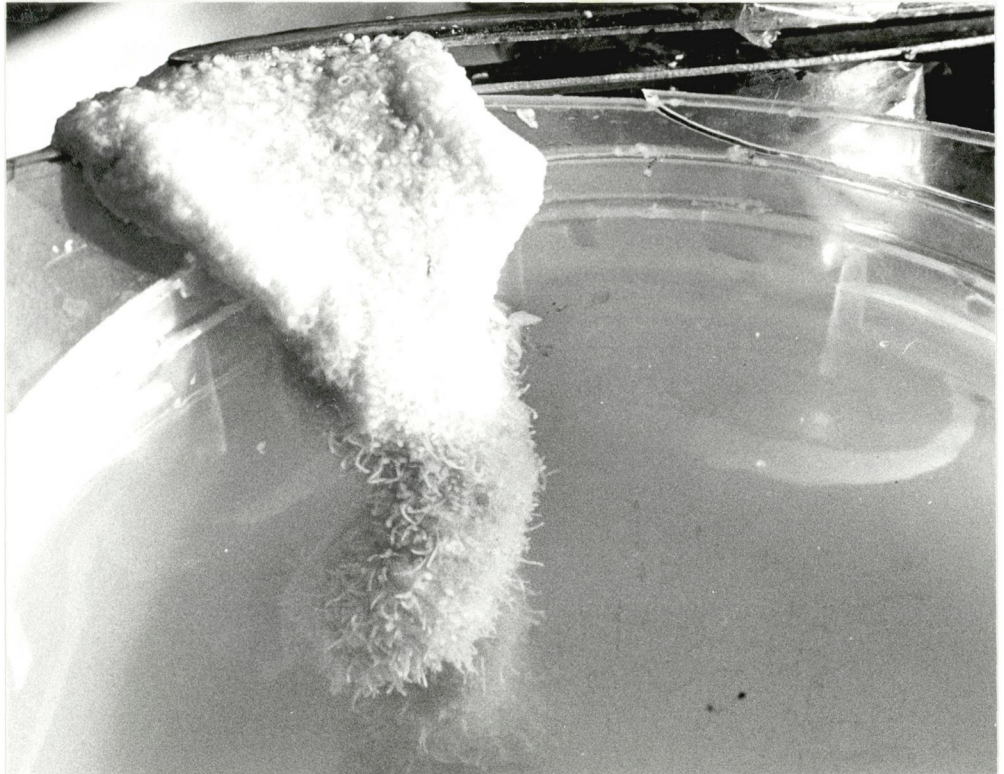


Plate 11. Turkana dog small intestine completely 'carpeted' with adult E. granulosus. Heavy infections such as this were found in 66 out of 281 dogs examined in this District.



Plate 12. Hundreds of adult E. granulosus worms removed from the small intestine of a dog, lying on the bottom of a dissecting dish. The bottle top gives an indication of their small size.

Table 10. Prevalence and intensity of *E. granulosus* infections in domestic dogs examined in Turkana with regard to the sex of the host

SEX	No. EXAM	% OF TOTAL	No. NEG	No. INF	% INF	INTENSITY OF <u>ECHINOCOCCUS</u> INFECTION		
						HEAVY (>1000 WORMS)	MEDIUM (200-1000 WORMS)	LIGHT (<200 WORMS)
MALE	121	43	53	68	56	29	8	31
FEMALE	160	57	64	96	60	37	19	40
TOTAL	281	100	117	164	58	66	27	71

The prevalence and intensity of the *E. granulosus* infections in the dogs examined from the six different areas in Turkana were all extremely high, except for the comparatively low prevalence found in the 36 dogs examined around Lokitaung (Appendix 3, Table 2).

The distribution of adult *E. granulosus* worms along the intestine varied between hosts, but were usually located between 37 and 127 cm from the pylorus. In heavy infections worms completely 'carpeted' almost the entire length of the small intestine (Plate 11 and 12) beginning at 5.0 cm from the pylorus and extending to 210 cm from it (Table 11). From sedimented volumes, it was calculated that approximately $3-5 \times 10^4$ individuals were present in such infections.

Table 11. Distribution of *E. granulosus* along the small intestine of dogs examined in Turkana (measured from the pylorus).

<u>ECHINOCOCCUS</u> INFECTION	INFECTION BEGINS (CM)	INFECTION ENDS (CM)	AVERAGE DISTRIBUTION OF WORMS (CM)
LIGHT	29	94	48 - 68
MEDIUM	5	158	46 - 99
HEAVY	5	210	37 - 127

In this series very few nematodes were found. Nineteen dogs had hookworms, which were identified as Ancylostoma caninum. The infections were light, the highest number in any dog being five. A few dogs harboured ascarids. The number of Taenia spp. recovered was recorded from all the dogs examined. All specimens were later identified as Taenia hydatigena. Only 26 dogs did not harbour T. hydatigena. The number of T. hydatigena present was counted in 192 dogs, and burdens ranged from one to 98 worms, with a mean of 9.4.

None of the seven dogs examined from Marsabit was positive for E. granulosus, but all seven were infected with T. hydatigena, with a mean worm burden of 5.9 per dog.

4.3.2 Experimental infections

The results obtained from the experimental infections are shown in Table 12.

Twenty-six of the 34 puppies fed viable protoscoleces from various hosts were found to harbour numerous E. granulosus worms at post mortem. Five puppies fed human protoscoleces and three fed cattle protoscoleces were negative for E. granulosus at examination.

In the infected puppies, the majority of worms were located in the first 90 cm of the small intestine. On average 7×10^3 - 1×10^4 worms were recovered from each infected puppy, but this varied considerably between animals.

Table 12. Experimental infection of dogs with hydatid material from domestic animals and man from Kenya

Source of larvae	Origin of dog infected	No. dogs exposed to infection and no. infected ()	% worm population along gut (cm) from pylorus				Av. No. worms recovered per infected dog
			0-30	31-60	61-90	91-END	
HUMAN (TURKANA AND MASAI)	NAIROBI	12 (8)	(44.6)	(54.3)	(1.1)	-	10,233
	TURKANA	7 (6)	(16.0)	(57.2)	(26.0)	(0.8)	8,081
	TOTAL	19 (14)	(26.3)	(56.0)	(17.3)	(0.4)	9,311
CAMEL (TURKANA)	NAIROBI	3 (3)	(12.5)	(45.6)	(33.2)	(8.7)	10,565
	TURKANA	0 (0)	-	-	-	-	-
	TOTAL	3 (3)	(12.5)	(45.6)	(33.2)	(8.7)	10,565
CATTLE (MASAI)	NAIROBI	4 (3)	(28.0)	(30.0)	(41.6)	(0.3)	7,087
	TURKANA	2 (0)	-	-	-	-	-
	TOTAL	6 (3)	(28.0)	(30.0)	(41.6)	(0.3)	7,087
SHEEP (MASAI)	NAIROBI	2 (2)	(24.7)	(46.3)	(29.0)	-	11,868
	TURKANA	1 (1)	(46.7)	(45.0)	(7.3)	(1.0)	8,180
	TOTAL	3 (3)	(30.3)	(46.0)	(23.4)	(0.3)	10,024
GOAT (TURKANA)	NAIROBI	0 (0)	-	-	-	-	-
	TURKANA	3 (3)	(49.3)	(27.6)	(22.4)	(0.7)	10,221
	TOTAL	3 (3)	(49.3)	(27.6)	(22.4)	(0.7)	10,221

4.4 Discussion

The experimental infections showed that Turkana and Nairobi puppies were susceptible to infection with hydatid material obtained from humans, camels, cattle, sheep and goats. The locations of the worms along the intestine was similar to that reported by Yamashita et al, (1956), Sweatman & Williams (1963) and Pandey (1972).

The results of the examination of the naturally infected dogs in Turkana support the earlier findings of Nelson & Rausch (1963) and showed that an extremely high number of dogs were

infected with E. granulosus from this region of Kenya. The single exception was the area around Lokitaung, where fewer dogs were infected. The reasons why such a comparatively low prevalence was found in the dogs examined in the Lokitaung area are unknown. The high prevalence found in the dogs examined around Lokori and Lotoboi was unexpected considering the low incidence of human infections reported from these areas. The overall prevalence reported from Turkana is considerably higher than prevalences reported from most of the other African Countries (Appendix 3, Table 1), and also from the Nairobi and Kajiado regions of Kenya. It is also considerably higher than the estimates from other regions of the world, where human hydatidosis has been regarded as an important public health problem. For example, the prevalence recorded in dogs from Iceland was only 28.0% in 1863, when approximately 22.0% of the human population was affected (Krabbe, 1865). In New Zealand, prior to any control programme Barnett (1938) estimated that about one third of the stray dogs of that country were spreading hydatid disease. Between 1944 to 1953, the human incidence in New Zealand (surgical cases in public hospitals only) was 7.5 per 100,000 per annum (Schwabe, 1969). A higher human incidence of 9.3 per 100,000 per annum was recorded from Tasmania for the decade 1950-1959 (Bramble, 1974). The same author reported that the infection rate in dogs in Tasmania in 1964 was approximately 11.28%

In addition to the higher prevalence, the intensity of E. granulosus infection appears to be heavier in dogs from Turkana than those from Nairobi and Kajiado. Nelson & Rausch (1963) reported that, 'only in Turkana were the infections so heavy that the intestines were 'furred' with thousands of worms'. Eugster (1978), found that 16 of the 45 infected dogs examined in Kajiado had worm burdens of

20 or less, and heavy infections consisted of those dogs harbouring more than 20 worms. In the present study, 96 of the 164 positive dogs harboured infections of more than 200 worms, and some of the heavy infections consisted of approximately 5×10^4 individuals. Therefore, it would appear that the Echinococcus infections in the domestic dogs in Turkana were heavier than those reported in dogs from Nairobi and Kajiado. It has been previously speculated (AMREF, 1978) that since the dog in Turkana breeds true, this particular type of dog may be more susceptible to infection with E. granulosus than other dog breeds. Different dog breeds have been known to show variable susceptibilities to artificial infection (Heath, D.D., personal communication). That the Turkana dog is more susceptible to infection may also explain the very high T. hydatigena worm burdens found in them, in comparison to the mean T. hydatigena worm burdens found in dogs examined from Victoria, Australia (as described later in this discussion) (Jackson & Arundel, 1971). However, although individual susceptibilities of the puppies to infection with similar or different hydatid material varied considerably, no differences in the overall E. granulosus worm burdens were found between experimentally infected puppies purchased in Turkana, and similarly aged puppies bought in Nairobi (Table 12). Thus the overall susceptibilities of the puppies from the two areas to E. granulosus infection were found to be similar. Heavy Echinococcus infections in dogs are certainly not unique to Turkana, for infections of over 3×10^4 worms are reported to be not uncommon (Schwabe, 1969).

The biotic potential of large taeniids is high; for example, T. hydatigena sheds approximately two proglottids per day, each

containing upto 28,000 eggs (Featherston, 1969). T. saginata sheds upto 16 proglottids per day with approximately 200-82,430 eggs per proglottid (Rijpstra et al, 1961). In comparison, the biotic potential of Echinococcus spp., is low. Individual segments contain only 200-800 eggs (Rausch & Schiller, 1956; Arundel, 1972), and usually one segment is shed every two weeks (Gemmell, 1962). It seems that the low biotic potential of Echinococcus spp. is to some extent compensated for by the heavy worm burdens that infected dogs can harbour.

The high prevalence coupled with the heavy worm burdens of E. granulosus in the Turkana dogs would indicate that the infection pressure for man throughout Turkana District is considerable. This is especially so since the Turkana have such a close association with their dogs, particularly at night. At such times ingestion of freshly deposited eggs could easily occur.

The study undertaken in Lokichokio, provides information regarding the prevalence of infection in the dogs of this area, from a time before the drought (June and December, 1979) to a time when an estimated 70-90% of the domestic animals had died as a result of the drought conditions. The prevalence of infection in dogs rose from 57.4% to 71.0% and the proportion of heavily infected dogs from 27.5% to 55.1%. Since the methods for obtaining and examining the dogs were similar in all three surveys, it is likely that the observed increases in prevalence were due to the greater chances of the dogs becoming infected. This could have resulted from the increased scavenging opportunities provided by the animals that had succumbed to the drought.

The number of T. hydatigena found in the dogs, did not show any

increase between the three surveys at Lokichokio, but remained high, with an average of 9.4 worms per dog. Many of these tapeworms were identified by Professor J.H. Arundel (Veterinary Clinical Centre, University of Melbourne, Australia) during his sabattical stay in Kenya, Dr. P. Stevenson (Kenya Agricultural Research Institute, Muguga) and Dr. A. Jones (Commonwealth Institute of Helminthology, St. Albans). They confirmed that the tapeworms were T. hydatigena, although a single specimen of T. multiceps was also identified.

The high numbers of T. hydatigena in the Turkana dogs contrasts with T. hydatigena worm burdens reported in Victorian dogs in Australia (Jackson & Arundel, 1971). These workers found 95 of (12.1%) 1063 Victorian dogs harbouring T. hydatigena, with a mean worm burden of 1.76 worms. Their survey covered a high density sheep-grazing area where the poorer sheep in a group would be killed for dog food. Further-more, many of the farmers killed sheep for their own use and many fed the offal to dogs.

The high prevalence therefore of T. hydatigena in Turkana dogs provides evidence that the larva or cysticercus of the parasite is often available to the dogs. Indeed the practice of allowing the cysticerci of T. hydatigena to be eaten by dogs was observed on many occasions, and the dogs were often seen to eat cysticerci thrown to them. There seems little doubt that any hydatid cysts found in the livestock would also be fed to dogs.

It was evident from our surveys, that the Turkana keep many more dogs than they need, and that for a small payment (25-50p UK) they could easily be induced to part with them. The wholehearted cooperation we received from the Turkana during our surveys suggests that dog control measures could be readily implemented in this District.

The survey undertaken in Marsabit District showed that the number of dogs in this area were considerably fewer than in Turkana. The reason for the low numbers of dogs there was apparently due to them being killed by the Veterinary Department and the police in a rabies prevention campaign; several rabid dogs had been present in the area. In many townships in Marsabit District, not a single dog was to be seen anywhere. Consequently, only seven dogs were available for this survey, and all of these were obtained in and around Marsabit. These dogs were only obtained with the helpful cooperation of the Marsabit police and Veterinary Department. None of the seven dogs examined were infected with E. granulosus, but all harboured T. hydatigena with a mean worm burden of 5.9 per dog.

Kruuk (1980) reported that the Rendille tribesmen living in Marsabit District had few dogs, even prior to the rabies control programme as they regarded dogs as being useless for protecting livestock against predators. Gabra tribesmen, on the other hand, regard dogs as being useful in protecting their livestock and kept more dogs than the Rendille. The fewer dogs in the District coupled with the fact that the Rendille and Samburu (two largest tribes in the area) do not allow dogs into the houses, must considerably reduce the risk of hydatid infection to man in this area, in comparison to the situation in Turkana. The number of human hydatid cases seen in this District is certainly comparatively very low; only one surgical case was reported during the present study period.

4.5 Summary

1. The results of surveys to investigate the role of dogs in the transmission of E. granulosus in Turkana are presented.

2. One hundred and sixty-four dogs out of 281 (58%) dogs examined in Turkana were found to harbour E. granulosus infections. High prevalences of the parasite in dogs were found around the Kakuma, Lakonkai, Lokichokio and Nanam townships in northern Turkana and from around Lokori and Lotoboi townships in the south of the District. A comparatively lower prevalence was recorded in dogs examined in north-eastern Turkana around Lokitaung. The reasons for this are unknown.

3. None of seven dogs examined in Marsabit was infected with E. granulosus. The low number of dogs examined from this area reflects the low number of dogs actually present. The reason for the fewer dogs in this region is that they are continually destroyed by the Veterinary Department and the Police, in an effort to reduce the risk of rabies, which is endemic in the area. Rabies has not been recorded in Turkana, and no dog control programme exists. It was evident that the Turkana keep many more dogs than they need, and they could easily be induced to part with them.

4. Puppies purchased in Turkana and Nairobi showed no overall difference in their susceptibility to experimental infection with hydatid material from different intermediate hosts. However, great individual variations in susceptibility were observed between puppies fed the same or different material.

CHAPTER 5

THE SIGNIFICANCE OF WILDLIFE IN THE TRANSMISSION OF

E. GRANULOSUS IN TURKANA AND MASAILAND5. Introduction

The primary maintenance cycle of E. granulosus in nature occurs between wild carnivores and wild herbivores, but from the public health point of view the secondary cycle in dogs and domestic animals is more important. Other recognised species of Echinococcus are also maintained in nature in wildlife cycles, viz. E. oligarthrus (Diesing, 1863) uses wild felids, including the puma, jaguarundi and Geoffrey's cat as definitive hosts and the agouti as an intermediate host; E. multilocularis (Leuckart, 1863) is primarily perpetuated in a cycle involving foxes and microtine rodents, E. vogeli (Rausch and Bernstein, 1972) is maintained mainly in a bush dog/paca cycle. The last two species are known to cause accidental infections in man. Knowledge of the existence of wildlife cycles of some Echinococcus spp. is therefore of considerable public health importance; the epidemiological significance of wildlife E. granulosus cycles vary in different regions.

In North America and northern Eurasia the primary maintenance cycles of E. granulosus transmission occur between the wolf, moose and wild reindeer or caribou, these do not readily involve domestic ungulates (Cameron, 1960), and are independent of man and his livestock (Rausch, 1967b). Similar cycles involving jackals and deer may occur in Sri Lanka (Paramanathan & Dissanaiké, 1961), and between coyotes and deer in California (Romano et al, 1974).

Such cycles are of little public health significance. In Australia however, wildlife cycles occur between certain macropods and dingoes which may represent a continuous reservoir of infection for domestic livestock (Coman, 1972). Yet other wildlife cycles exist whose maintenance appears to be dependent on the existence of a domestic cycle, an example of this is the South American red fox and European hare cycle in Argentina (Schantz et al, 1972).

In Africa, numerous definitive and intermediate host species for Echinococcus have been reported from many countries, these are tabulated in Appendix 4, Tables 1 and 2.

Several authors have reported numerous species of wild herbivores and wild carnivores as hosts for E. granulosus in various parts of East Africa (Appendix 4, Tables 1 and 2); these are listed in a recent bibliography by Karstad (1979). Such findings lend support to the idea that in addition to the domestic cycles, a wildlife cycle must be considered in the epidemiology of E. granulosus in some regions of East Africa.

In certain parts of Kenya large concentrations of wild herbivores and carnivores are found. These form an essential part of the ecosystem providing ideal conditions for the transmission of E. granulosus amongst the wild animals. Knowledge of whether or not such wildlife cycles exist is an important factor before control programmes are contemplated.

The first definitive host of E. granulosus found in Kenya was a jackal, reported by Ginsberg (1958) and it was hypothesised that jackals and hyaenas might be the main hosts for the parasite in Kenya (Round, 1962). Nelson and Rausch (1963) found heavy infections of Echinococcus in domestic dogs examined in Kenya and although they found a few light infections in a silver-backed jackal, several

spotted hyaenas and Cape hunting dogs, plus hydatid cysts in a single wildebeest, they concluded that the main cycle of transmission in Kenya was between dogs and domestic livestock. Mango (1971) and Ng'ang'a (1974) supported this theory. But other workers including Sachs & Sachs (1968); Dinnik & Sachs (1969, 1972); Myers et al, (1970); Schiemann (1971); Fay (1972); Woodford & Sachs (1973); and Eugster (1978), have reported the parasite in wild animals, lending support to the idea that in addition to the domestic cycle, a wildlife cycle must be considered in the epidemiology of Echinococcus in some regions of East Africa. The main objective of this study was to examine the possible role of wildlife in the transmission of Echinococcus in Turkana, where there are no previous records of their involvement. A similar smaller survey to examine the role of wildlife in the parasite's transmission was also undertaken in Masailand, where many previous records of involvement of wild animals exist.

The abundance and species of wild animals found in Turkana and Masailand are given in Chapter 1. All the wild animals reported here were examined between February 1979 and June 1980.

The results obtained during this wildlife study and reported in this chapter have formed the basis of two publications: Macpherson & Karstad (1981) and Macpherson et al, (1981).

5.1 Materials and methods

5.1.1 Natural infections

The wild carnivores were usually shot at night using a spotlight, bait (usually the carcass of an animal examined earlier in the day) and a 'calling' procedure which consisted of playing a prerecorded tape of hyaenas over a loudspeaker which was attached to a landrover.

The recording attracted both hyaenas and jackals, sometimes from miles away, which on arrival would turn their attentions to the bait. Hyaenas are rarely seen or heard in Turkana and without such a 'calling' system it would have proved almost impossible to find any. As it is, 16 spotted hyaenas were shot and examined. The small intestines from the wild carnivores were removed in toto, placed into labelled plastic bags, and stored in a refrigerator overnight. The time interval from killing an animal to the examination of its intestine varied from eight to 10 hours.

The intestines were examined using the same methods as those employed for examining the dog intestines, and any worms found were recorded and processed in the same manner as described in Chapter 4. The length of a hyaena's small intestine is extremely long (Plate 13) and only the first two metres from the pylorus were examined carefully. A further metre was opened and examined by eye.

The lungs, liver, spleen, heart and kidneys of wild herbivorous animals were examined almost immediately after the animals were shot. Any hydatid cysts recovered were examined for the presence of protoscoleces and the whole preserved in Bouin's fixative. The fixed material was later sectioned and stained using Delafield's Haematoxylin and Eosin.

5.1.2 Experimental infections

Six spotted hyaenas and seven silver-backed jackals were captured from various locations in Masailand, and 11 puppies from three litters (approximately four weeks old) were purchased. To remove any naturally acquired infections each animal received 10 mg per Kg bodyweight of droncit (R) prior to infection.

The material used to infect the animals was obtained from

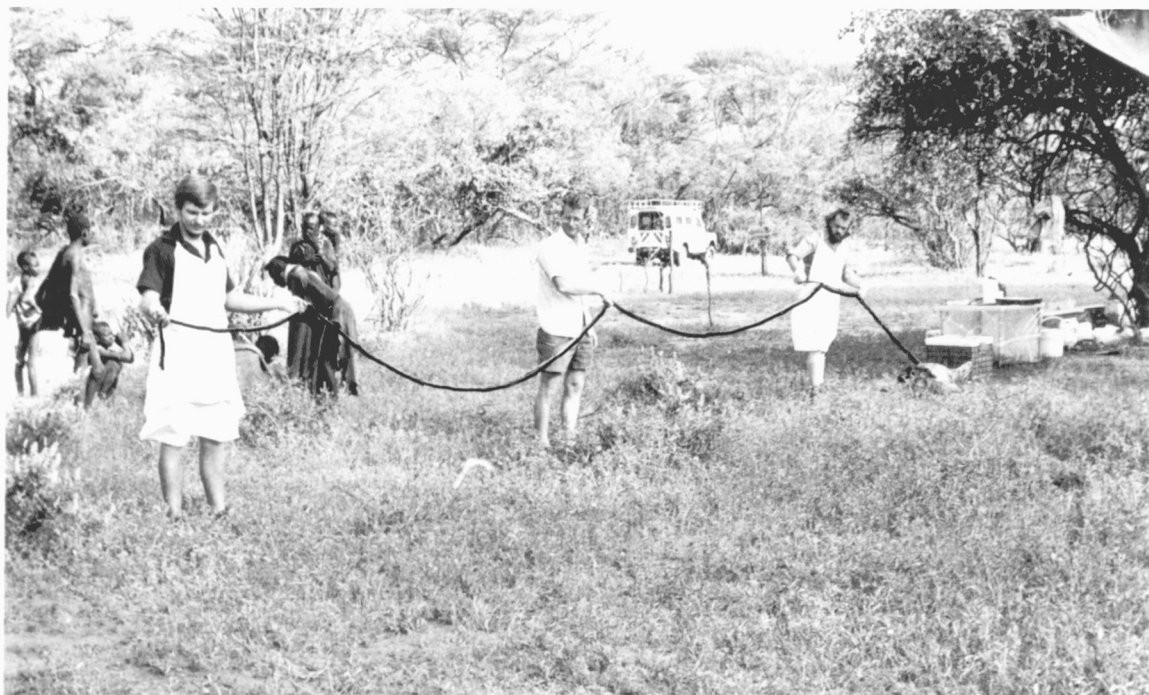


Plate 13. Small intestine from a spotted hyaena (on ground) being displayed to show its considerable length. Part of the field 'laboratory' set up in Lokichokio can be seen in the background.

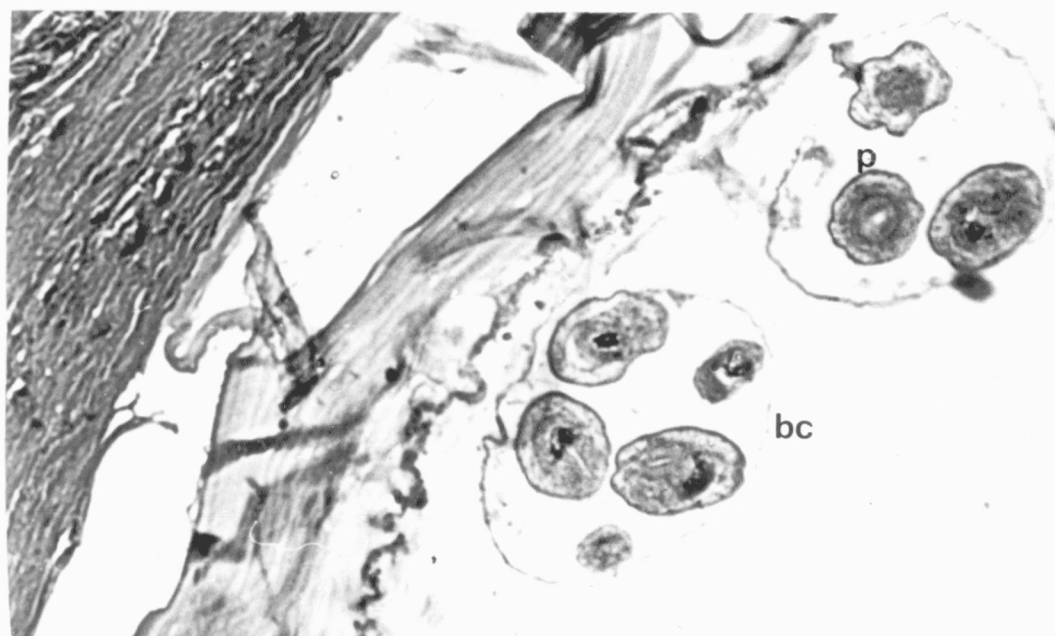


Plate 14. Transverse section through a fertile hydatid cyst from the lung of a wildebeest showing protoscoleces (p) contained in brood capsules (bc) (Haematoxylin and Eosin) (x160).

human hydatid cysts removed surgically from Masai and Turkana patients. The experimental design is shown in Table 14.

The infection and subsequent examination procedures followed those for the experimental puppies described in Chapter 4. The only difference was that the first 210 cm of the hyaena intestines only were processed thoroughly, and a further 250-300 cm of the intestine were opened and examined by eye.

5.2 Results

5.2.1 Natural infections

Turkana

Seventy-six wild carnivores of three species were examined from three areas in north-west Turkana, near Kakuma, Lokonkai and Lokichokio. These areas were selected because of the extraordinary high incidence of human hydatidosis recorded there (O'Leary, 1976; AMREF, 1979), and also because these were areas where some wild mammals were known to occur.

All 16 spotted hyaenas collected around Lokichokio township were uninfected. The 22 golden jackals were obtained from Kakuma (0/1 positive), Lokonkai (0/12 positive), Lokichokio (6/9 positive). The 38 silver-backed jackals were obtained from Kakuma (0/1 positive) and Lokichokio (11/37 positive). Thus of 76 wild carnivores, 17 (22.4%) were found infected with Echinococcus. The prevalence rate in all the jackals was 28.3%, or 29% in silver-backed jackals and 27.3% in golden jackals. Details of the infections are given in Table 13.

In the search for wild intermediate hosts 152 herbivores of five species were examined around the Lokichokio and Kakuma townships in Turkana. Thirty Grant's gazelle, 51 dik-dik, 10 warthogs, 34 hares (Lepus spp.), and 27 ground squirrels (Xerus spp.), were all found

to be negative for hydatid cysts.

Table 13. Wild carnivores examined for *Echinococcus* in Turkana:
February 1979 to June 1980

<u>Echinococcus</u> Infection	Species		
	Silver-backed jackal (<u><i>Canis mesomelas</i></u>)	Golden jackal (<u><i>Canis aureus</i></u>)	Spotted hyaena (<u><i>Crocuta crocuta</i></u>)
Light, < 200 worms	7	6	0
Medium, 200-1000 worms	1	0	0
Heavy, > 1000 worms	3	0	0
Total positive	11	6	0
Total examined	38	22	16

Masailand

One spotted hyaena and one silver-backed jackal examined from the Loita plains in Narok District were negative for *Echinococcus*. One silver-backed jackal examined in Tsavo West National Park was also negative. Twenty-six herbivores of six species were examined from the Loita plains, these included: 17 wildebeest, one impala, two Grant's gazelle, three Thompson's gazelle, and three topi. Hydatid cysts were found in the lungs of three wildebeest, of which two were fertile and contained a small number of protoscoleces in each cyst (Plate 14). The fertile cysts measured 2.5 and 5.5 cm in diameter whilst the sterile cyst was smaller having a diameter of only 2.0 cm.

5.2.2 Experimental infections

The results of the experimental infections are presented in

Table 14.

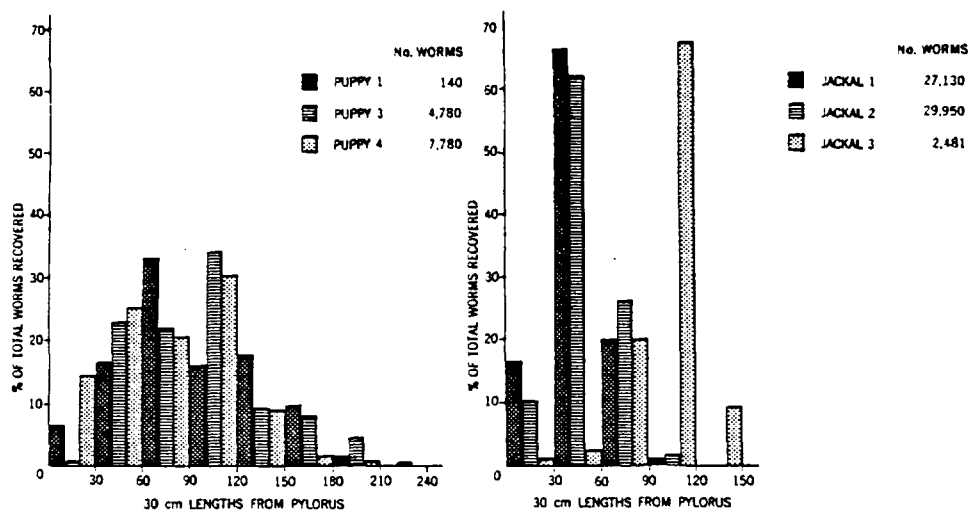
Table 14. Experimental design and results of experimental infections of spotted hyaenas, silver-backed jackals and dogs

Species infected	No.	Sex Male (M) Female (F)	Source of material	Number of worms recovered	Location of worms in the gut (cm) from pylorus
Hyaena	1	M	Human (Masai)	0	-
Hyaena	2	F	Human (Masai)	0	-
Hyaena	3	M	Human (Masai)	0	-
Puppy	1	M	Human (Masai)	0	-
Puppy	2	F	Human (Masai)	0	-
Jackal	1	F	Human (Turkana)	0	-
Jackal	2	M	Human (Turkana)	0	-
Puppy	3	M	Human (Turkana)	6875	36-76
Puppy	4	M	Human (Turkana)	0	-
Jackal	3	M	Human (Turkana)	0	-
Jackal	4	F	Human (Turkana)	4125	54-72
Puppy	5	F	Human (Turkana)	0	-
Puppy	6	F	Human (Turkana)	-	-
Puppy	7	F	Human (Turkana)	-	-
Hyaena	4	M	Human (Turkana)	0	-
Hyaena	5	F	Human (Turkana)	0	-
Hyaena	6	F	Human (Turkana)	0	-
Jackal	5	M	Human (Turkana)	27130	10-110
Jackal	6	M	Human (Turkana)	29950	10-110
Jackal	7	F	Human (Turkana)	2481	10-130
Puppy	8	F	Human (Turkana)	140	25-215
Puppy	9	F	Human (Turkana)	4780	30-200
Puppy	10	F	Human (Turkana)	7780	15-195
Puppy	11	M	Human (Turkana)	0	-

The first attempts to infect hyaenas (No. 1-3), jackals (No. 1-4) and puppies (No. 1-7) were not very successful (Table 14), and only one jackal and one puppy became infected. The reasons for this largely negative result, are unknown. Puppies six and seven died soon after exposure and were not examined.

The second attempt to experimentally infect further hyaenas (No. 4-6), jackals (No. 5-7) and puppies (No. 8-11) proved much more successful. Once again none of the hyaenas became infected, but heavy infections were found in the jackals and two of the puppies. The three jackals harboured some 27,130; 29,950 and 2,481 E. granulosus and the infected puppies 140; 4,780 and 7,780 E. granulosus. The distribution of the Echinococcus infections in the intestines is shown in Figure 6.

Figure 6. Distribution of E. granulosus in the small intestines of three puppies (left) and three silver-backed jackals (right) infected with E. granulosus of human origin



5.3 Discussion

This is the first time that golden jackals have been recorded as definitive hosts of E. granulosus in Kenya. The first record of these animals harbouring this parasite was made over one hundred years ago

by Panceri (1868) in Naples. Since then natural infections in C. aureus have been reported from Palestine (Witenburg, 1933), Pakistan (Lubinsky, 1959), Algeria (D'Arces, 1953), Sri Lanka (Dissanaike & Paramanathan, 1960), Lebanon (Dailey & Sweatman, 1965) Chad (Troncy & Graber, 1969) and Iran (Sadighian, 1969).

Although a high percentage (27.3%) of golden jackals harboured E. granulosus, the greatest number of worms recovered from any one infection was 44, and the number of worms from the other five infections totalled only 22. No gravid segments were seen in the golden jackal material, although the worms showed normal development and all possessed testes. No experimental infections were attempted with this species of jackal in Kenya, but Witenburg (1933) infected two of four young golden jackals in Palestine. After five weeks the worms were immature, possessed three segments and showed similar development to worms recovered from four of seven control dogs infected with the same source of material. Gravid segments were found in this species by Dailey & Sweatman (1965) from the single jackal they found infected in the Beka'a valley of Lebanon.

The silver-backed jackal appears to be a more important host in Kenya, and of the 11 found infected, three harboured in excess of 1000 parasites. The majority of the worms had gravid terminal segments, containing hundreds of shelled eggs.

The experimental infections revealed that the silver-backed jackal appears to be rather more susceptible than dogs for human hydatid material. Viljoen (1937) and Verster (1965) working in South Africa produced patent experimental infections in silver-backed jackals using protoscoleces from cattle, sheep or human hydatid cysts. In common with our experimental findings the infections Verster found in the jackals were usually much heavier than those

found in the dogs.

In our experimental animals the distribution of the worms along the intestine (Figure 6) revealed that the majority of the worms were located in the first 120 cm of intestine from the pylorus, most infections beginning some 10 cm posterior to the pyloric sphincter.

The first description of a natural infection in the silver-backed jackal was by Nelson & Rausch (1963) who found one of nine infected in Kenya. In an extensive survey in South Africa, Verster & Collins (1966) found 21 (9.7%) of 215 silver-backed jackals harbouring the parasite and recently Eugster (1978) reported five of (38.5%) 13 of the animals infected in Kajiado District. Three of the five positive animals reported by Eugster had worm burdens of greater than 20 individuals.

The high overall incidence of Echinococcus in the jackals is evidence that they may play a part in the perpetuation of the transmission cycle in Kenya. However, since no hydatid cysts were found in any of the 152 wild herbivores examined in Turkana it is unlikely that there is a purely wildlife cycle in this District.

The infections found in the jackals in Turkana were probably incidental to their scavenging on domestic livestock carcasses. There is also the possibility of the jackals becoming infected from the Turkana themselves, for burial of the dead is usually limited to respected old men and married women with children. If this is the case then the Turkana may not be dead-end hosts, as in most other regions of the world, especially since there is such a high prevalence of the disease in man and in view of our evidence that the human parasite from Turkana is highly infective to silver-backed jackals.

Where there is an unusually high prevalence rate of hydatidosis in a local population, certain socio-economic and cultural characteristics accentuate the risk for human infection. The very intimate association with dogs which are used as 'dog-nurses' to guard the children and to clean up when a child vomits or defaecates was thought to be a sufficient explanation by Nelson & Rausch (1963) who described this. They also suggested that the Turkana might become infected by eating the intestines of dogs. The Turkana are known to eat practically any kind of meat from both wild and domestic animals, but have previously been reported as denying eating carnivores (Gulliver & Gulliver, 1953). However, it has been my experience that most Turkana will eat wild carnivores, and they readily took the hyaenas and jackals we had killed. The Turkana regard the intestines of most animals as a great delicacy and eat them with only little cooking. They would have taken the small intestines of the carnivores we had examined, had they been permitted. The consumption of jackals must therefore, on occasion, represent a very real source of infection to some of the Turkana. They, however, emphatically denied eating dogs.

The absence of E. granulosus in the spotted hyaenas agrees with the findings of Eugster (1978), and also of Verster & Collins (1966) who found no Echinococcus in spotted and brown hyaenas (Hyaena brunnae), Troncy & Graber (1969) who did not find the parasite in striped hyaenas (Hyaena hyaena) and Graber & Thal (1980) who failed to find the parasite in two spotted hyaenas examined in the Central African Republic. However, E. granulosus infections in spotted hyaenas have been reported by Nelson & Rausch (1963) who found three infected animals of 19 examined in Kenya and also by Young (1975) in the Kruger National Park in South Africa. In both instances only a few adult E. granulosus were recovered. This fact,

coupled with our failure to infect hyaenas as reported here, with human protoscoleces, is evidence that hyaenas, which are phylogenetically widely separated from the Canidae, are not very suitable hosts for the parasite.

Smyth (1962b) and Smyth & Haslewood (1963) suggested that the physio-chemical properties of the host intestine, especially the composition of the host bile could determine host specificity. The small intestine of hyaenas is full of a creamy coloured fatty, calcareous mixture with many large pieces of undigested bone. The effect of this, if any, on the establishment of Echinococcus in the hyaena intestine is unknown. Smyth & Smyth (1968) further suggested that, in addition to physical and biochemical factors, certain morphological factors of the hosts intestine may play a role in the establishment and development of the cestode. Some preliminary studies indicate that the villi and crypts of Lieberkühn in the hyaena intestine are slightly different to those in dogs, but further work is required before this is confirmed. Whether or not these characteristics would effect the establishment of Echinococcus in the hyaena would be difficult to determine.

The very low density and sporadic distribution of the spotted hyaena in Turkana, where they are now probably limited in distribution mainly to the Mogilla and Songot Mountain ranges around Lokichokio, also indicates that they are of no significance in the transmission cycle of this disease in Turkana.

Other wild carnivores such as mongooses, the aardwolf and the bat-eared fox (Otocyon megalotis), are found in certain parts of Turkana but none of these are thought to be likely hosts of the adult worm due to their feeding habits (Nelson & Rausch, 1963). Mongooses feed primarily on rodents, but none of the 1,674 rodents

examined by Nelson & Rausch (1963) in Kenya were found to be infected with Echinococcus. Aardwolves and bat-eared foxes are mainly insectivorous.

Although members of the cat family are known hosts of other species of Echinococcus viz. E. oligarthrus and E. multilocularis, they are not regarded as being good hosts for E. granulosus, although, the lion appears to be an exception to this general rule. E. granulosus was recorded in lions by Ortlepp as early as 1937. He proposed a new species for the parasite found in the lion as E. felidis. This was subsequently reclassified at the subspecies level by Verster (1965) as E. granulosus felidis. However, due to the lack of evidence of ecological segregation or marked predator/prey specificity Rausch (1967b) regarded E. g. felidis as being synonymous with E. granulosus granulosus, the nominate subspecies. Other findings of infected lions have been made by Porter (1943) who found mature specimens in a captive animal. Verster & Collins (1966) found five of seven lions in South Africa positive, and Rodgers (1974) reported two of six lions (from the eastern Selous Game Reserve in southern Tanzania) harbouring E. granulosus.

Dinnik & Sachs (1972) found an infected lion in Narok District and also one of three lions infected in the Ruwenzori National Park, Uganda. These animals were found to harbour sexually mature worms with four to six segments, the most distal segment being gravid. Infected warthogs, 11 of (10.4%) 106, and buffaloes 25 of (17.2%) 145, have also been reported from this Park (Woodford & Sachs, 1973). These authors suggested that the warthog is probably the obligate intermediate host for the adult parasite found in lions in the Park. Recently a similar cycle between lions and warthogs has been suggested to occur in the Central African Republic (Graber & Thal, 1980).

Graber & Thal (1980) provided evidence that the parasite in the lion represents a different strain of the parasite from E. g. granulosus. They reported that apart from morphological differences seen in the parasites obtained from a lion, hydatid material from warthogs and bush pigs was not infective to two beagles. Thus the parasite found in warthogs and bush pigs may be infective to lions but not infective to dogs or other Canids. Further experimental evidence is now required to establish whether material from warthogs or bush pigs is infective to lions.

That lions are susceptible to experimental infection with material from wild intermediate hosts has been demonstrated by Young (1975). This author repeatedly obtained successful infections in lions with hydatid material obtained from liver cysts from Burchell's zebra. The same author reported that E. granulosus infections are common in lions in the Park and approximately 60 per cent of the zebras found in the Park harbour hydatid cysts. He considered therefore, that the zebra is most likely to be the most important intermediate host of E. granulosus for lions in this Park. Other wild intermediate hosts found in the Kruger National Park include, hippopotamus (McCully et al, 1967), buffaloes (Basson et al, 1970) and Impala (Young, 1975), which may all be susceptible to the strain of E. granulosus found in the lion.

In Kenya, lions may be important in a wildlife cycle in Masailand, since they and their wild prey species, particularly wildebeest, have both been found to harbour the parasite in this area (Dinnik & Sachs, 1972; Eugster, 1978). However, in Turkana, the number of lions is so low that it is unlikely that they play any important role in the transmission of the disease.

Cape hunting dogs, now very rare in Masailand and probably extinct in Turkana, appear to be very good hosts of the parasite. Nelson & Rausch (1963) were the first to report Echinococcus in this species, finding three out of four animals infected in Kenya. Verster (1965) reclassified Ortlepp's (1934) earlier classification of the parasite found in Cape hunting dogs from E. lycaontis, to E. granulosus lycaontis which like E. g. felidis was later regarded by Rausch (1967b) to be synonymous with E. g. granulosus. Unfortunately the number of Cape hunting dogs in the wild are now so few that it was not possible to determine experimentally the suitability of this carnivore as a definitive host for Echinococcus of Kenyan origin.

The findings presented here, that two of the three hydatid cysts obtained from three of the 17 infected wildebeest examined in Narok District were fertile, provides further evidence that the main food animals of the lion in this area harbour the larval stage of the parasite. Previous records include those of Nelson & Rausch (1963) who reported one out of 17 wildebeest harbouring fertile cysts, Schiemann (1971) two out of 450 (0.4%), Sachs (1976) three out of 70 positive (4.3%), and Eugster (1978) who found 69 out of 567 (12.2%) wildebeest positive. The lungs were found to be the usual predilection site of the cysts, of which most were fertile (Nelson & Rausch, 1963; Eugster, 1978).

Wildebeest, giraffe and warthog have been found to harbour fertile cysts in the Serengeti, a region adjacent to Narok District (Sachs & Sachs, 1968; Dinnik and Sachs, 1969). Myers et al (1970) reported hydatid cysts in two young captive baboons (Papio sp.) imported from Kenya. However, baboons probably play little part

in the transmission of the disease in nature as none of 180 baboons examined by Nelson & Rausch (1963) was found to be infected. One buffalo, one blue duiker, two of 24 impala, two of 26 Grant's gazelle, two of 38 hartebeest were found to harbour cysts in Kajiado District by Eugster (1978).

In Masailand it seems likely that lions, and to a much lesser extent Cape hunting dogs, are the true definitive hosts in a predator-prey relationship with the wild herbivores as intermediate hosts. The identification of the parasite found in these two definitive hosts must therefore remain open to further investigations, especially since now so many of their prey species have been found to harbour hydatid cysts and in view of the fact that hydatid material from warthogs and bush pigs may be non infective to dogs. Jackals as scavengers are important secondary hosts, capable of disseminating the parasite. The domestic cycle between dogs and domestic animals is likely to have evolved at a later date with possible differences in the infectivity of the parasite to man. This may readily arise due to the potential for genetic selection for new strains of the parasite in a single generation because of the mode of reproduction of the parasite (Smyth & Smyth, 1964). A succession of reintroductions must have also taken place during the more recent period of colonisation. The parasite in Turkana has probably escaped from a completely wildlife cycle and here the maintenance of the infection must depend on the scavenging of dogs and jackals, on infected domestic animals and possibly also on infected humans (Figure 7).

Since wild animals are found in many other areas in Kenya, it would be interesting to investigate whether wildlife cycles of E. granulosus exist in such regions. Further studies are now

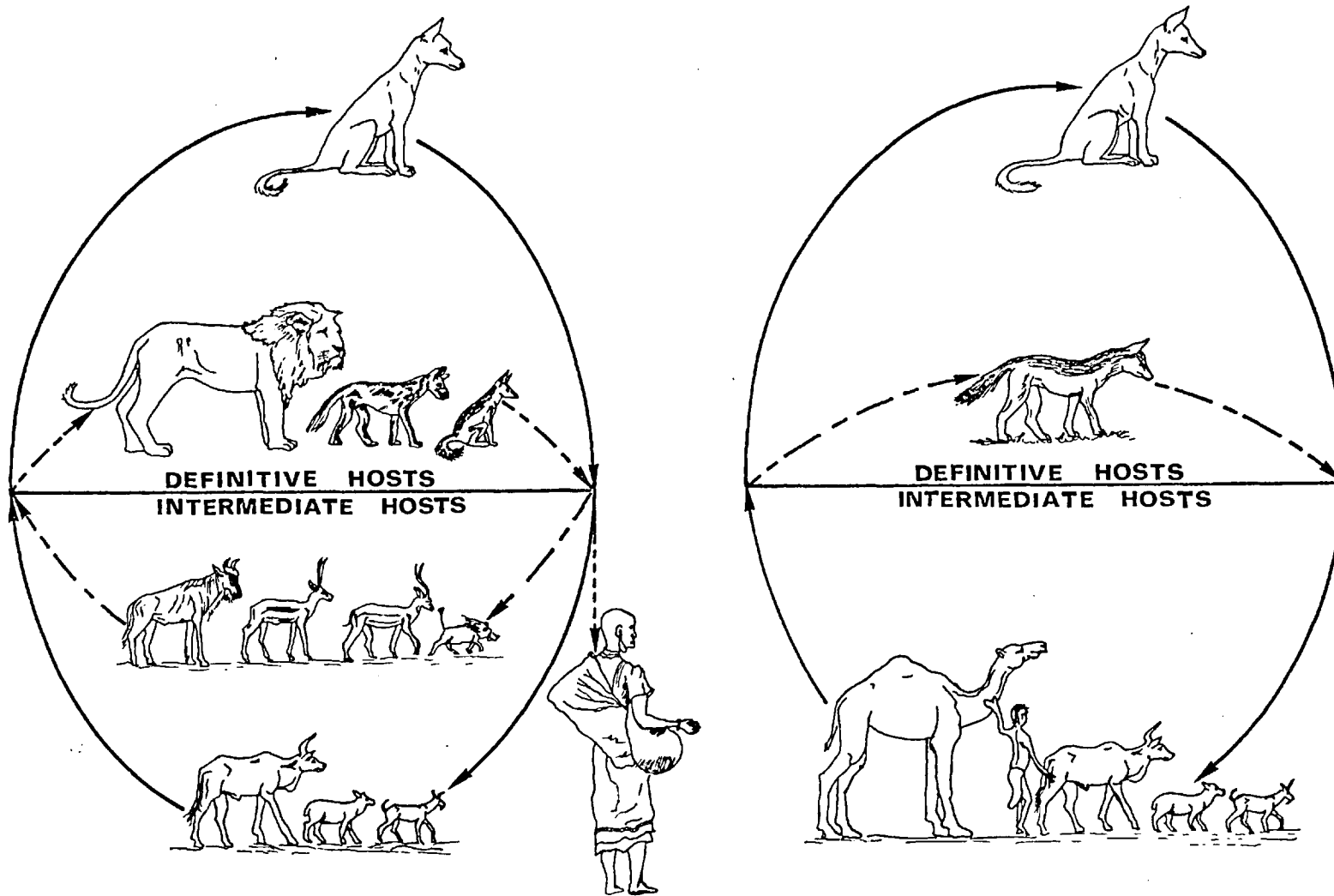


Figure 7. Possible life cycles of *E. granulosus* in Masailand (left) and Turkana (right)

required to examine the infectivity of the parasite found in wild animals, to man and his domestic animals and vice versa.

5.4 Summary

1. The results of a study to investigate the role of wildlife in the transmission of Echinococcus granulosus in the Turkana and Narok Districts of Kenya are presented.

2. A total of 76 wild carnivores belonging to three separate species were examined from Turkana District. Echinococcus adults were found in 11 of 38 silver-backed jackals and six of 22 golden jackals. This is the first record of golden jackals being infected with this parasite in Kenya. None of 16 spotted hyaenas harboured the parasite.

3. Four silver-backed jackals and four puppies were successfully infected with protoscoleces obtained from human hydatid cysts. Six spotted hyaenas fed the same material failed to become infected.

4. None of 152 wild herbivores of five species examined in Turkana harboured hydatid cysts. The natural jackal infections in this District are thought to be incidental and dependent on the continuance of the domestic cycle. The role of the Turkana themselves in the perpetuation of the cycle is discussed.

5. Twenty-six wild herbivores of six species in Narok District were examined for hydatid cysts, which were found in three wildebeest and a single topi.

6. The discovery of fertile cysts in wildebeest and the reported infections in lion, Cape hunting dogs and silver-backed jackals, support previous evidence of the existence of a wildlife cycle in Masailand. The relationship of this cycle to the domestic cycle operating in the same area is unclear and requires further investigation.

CHAPTER 6

MORPHOLOGICAL FEATURES OF E. GRANULOSUS IN KENYA6. Introduction

Speciation in the genus Echinococcus has long been a matter of controversy and the question has been the subject of a number of reviews (Rausch, 1953, 1967b; Rausch & Nelson, 1963; Smyth, 1964, 1969; Verster, 1965). It is now generally accepted that there are four species of the genus that are valid taxonomically, viz. E. granulosus, E. multilocularis, E. oligarthrus and E. vogeli (W.H.O., 1980). All these species are morphologically distinct from each other in both their adult and larval stages. The two main species responsible for hydatid disease are E. granulosus, which causes unilocular hydatid disease and E. multilocularis, which causes alveolar hydatid disease. The global distribution of these two species has been recently reviewed by Matossian et al, (1977). The other two species, E. oligarthrus and E. vogeli are only found in South and Central America (Rausch et al, 1978), where the latter is reported to cause polycystic hydatid disease in man.

Only one of these species, E. granulosus, has been reported in Kenya (Nelson & Rausch, 1963). Within this species, numerous subspecies have been described from various parts of the world, including five from South Africa: E. granulosus africanus, E. g. granulosus, E. g. felidis, E. g. lycaontis and E. g. ortleppi (Verster, 1965). In a recent review, Thompson (1979) lists no less than 10 subspecies that have been previously described for E. granulosus. However, the taxonomic validity of these subspecies remains controversial, particularly those which were described

primarily on morphological grounds. Rausch (1967b) in a critical review of the criteria used to determine subspecies, concluded that there was insufficient evidence to support more than two subspecies of E. granulosus, viz. E. g. granulosus, the nominate subspecies, which is thought to have originated in Europe and spread throughout the world by the early settlers, and E. g. canadensis which is the indigenous species in the arctic region of North America. The taxonomic reasoning behind rejecting the other subspecies related to whether the different populations were reproductively segregated, either by ecological or geographical factors (Rausch, 1967b).

Smyth & Smyth (1964) suggest that a 'continuum' of 'species' exists between E. granulosus and E. multilocularis, and point out that parasites of the genus Echinococcus possess two characteristics that particularly favour the expression of mutants, namely:-

(a) The adult tapeworm is a self-fertilising hermaphrodite. Thus if a mutation occurs, it could appear in both eggs and sperm and double recessives could develop; and

(b) The larval stage reproduces asexually, and may therefore give rise to a large population of genetically identical individuals from a single mutant egg.

Thus, as emphasised by Smyth (1977), E. granulosus has a mode of reproduction which favours the expression of mutants, with the result that new intraspecific variants can readily arise.

It is hardly surprising therefore that a large number of intraspecific 'variants' of E. granulosus have been reported, and that a complex speciation pattern exists within this species. Although the existence of intraspecific 'variants' is accepted by many authors, their taxonomic status is uncertain, and at present it is the practice

to refer to such intraspecific 'variants' as 'strains' (Smyth, 1977; W.H.O., 1979).

Studies on the possible existence of 'strains' of Echinococcus are very important epidemiologically, as each strain may vary in its infectivity to man and also to other intermediate and definitive hosts. A similar phenomenon of strain variation has been demonstrated among other helminths of medical importance. For example, Trichinella is transmitted to man from domestic pigs in Europe or America, whereas the parasite in Kenya is of very low infectivity to domestic pigs (Nelson et al, 1966). Another example is Schistosoma japonicum, in which at least four geographic strains exist; the Chinese mainland strain is highly infective to man but there are two strains in Taiwan, which are non infective to man (Hsü & Hsü, 1968). A similar situation appears to be found for E. granulosus; for example, there is evidence in Great Britain that the horse/dog strain is of low infectivity to man, whereas the sheep/dog strain is infective (Nelson, 1972; Smyth, 1977). The significance of other cycles of E. granulosus involving wild definitive and intermediate hosts has already been discussed (Chapter 5).

In Kenya, it has been hypothesised that the high prevalence of the disease amongst the Turkana may be due to a relatively high infectivity of the local strain to man (AMREF, 1978). Although it is unlikely that man is essential for the maintenance of the parasite in Turkana, it may be that since dogs and wild carnivores have access to human hydatid cysts a particularly virulent strain of the parasite for man may have been previously selected. The potential for strain differences to occur in the parasite found in Masailand and Turkana is good owing to the geographical separation of the two regions and also because of the possible additional existence

of a wildlife cycle operating in Masailand (Chapter 5).

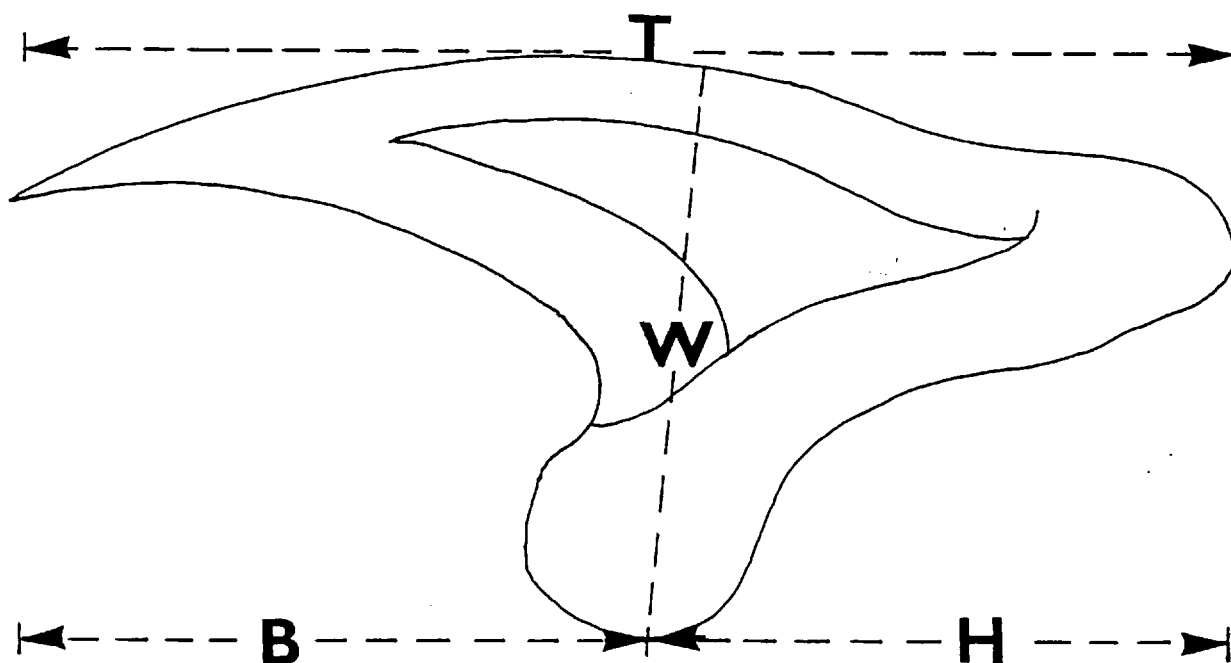
An attempt was therefore made to elucidate the speciation pattern of E. granulosus from these two geographically isolated areas, and also to examine whether the parasites found in all the different intermediate hosts from the two regions represent different strains of E. granulosus. The present chapter examines the morphological characteristics of the parasite found in many of the definitive and intermediate hosts from the two regions. Other isoenzymatic and physiological studies to examine the strain of E. granulosus from Kenya were also undertaken on material collected from the two regions. The results of these investigations are given in the subsequent two chapters.

6.1 Materials and methods

Protoscoleces from hydatid cysts removed from naturally infected camels, cattle, sheep, goats and man from Turkana, Masailand and Marsabit were preserved in 70% alcohol, for morphological examination later. Worms obtained from natural and experimental infections in dogs and jackals were also fixed in 70% alcohol or 10% formal saline. Morphological features of adult worms were examined from whole worms stained in either Gower's carmine or by the Performic acid-Schiff test for keratin (Pearse, 1968). The latter histochemical test proved to be a good method for examining reproductive structures in mature segments.

Hook measurements were made as described by Dailey & Sweatman (1965) (Figure 8). Usually, 10 large and 10 small hooks were measured from each scolex, selecting only those hooks seen in profile.

Figure 8. Rostellar hook measurements: T, total length; B, blade length; H, handle length; W, width



6.2 Results

6.2.1 Intermediate hosts

The cystic hooks of E. granulosus from all the intermediate hosts in Kenya appear to be similar. This applies to the total number of hooks (Table 15), the width, blade, handle and total length of the large and small cystic hooks (Figure 9) and their general shape (Figure 10).

Table 15. Number of hooks seen on protoscoleces from different intermediate hosts from Kenya

Host	Source	No. larvae Examined	Total no. larval hooks		Third row of tiny hooks seen
			Range	Mean	
Human	Masai	20	28-33	30.4	-
Human	Turkana	23	25-40	32.8	-
Human	Samburu	24	30-42	34.9	-
Camel	Turkana	52	28-43	33.6	+
Cattle	Masai	70	26-39	33.5	-
Goat	Turkana	35	25-38	32.7	-
Goat	Masai	51	29-41	34.8	-
Sheep	Masai	148	24-41	33.5	-

An incomplete third row of tiny hooks was seen in two camel protoscoleces only. These were much smaller than the small hooks and only five and eight hooks were seen in two protoscoleces. These tiny hooks were not included in the total number of rostellar hooks recorded (Table 15).

The total length of the large and small cystic hooks did not vary considerably between hosts, but varied within material from the same host, and even within individual protoscoleces (Figure 9).

In addition to variations in the measurements of the larval hooks shown in Figure 9, variations seen in the shape of the larval hooks are shown in Figure 10.

Figure 9. Total length, blade length, handle length and width of large and small cystic hooks from naturally infected intermediate hosts in Kenya. The vertical line represents the range of measurements, and the horizontal bar the mean. The rectangle around the mean denotes the standard deviation. T = Turkana, M = Masai, S = Samburu

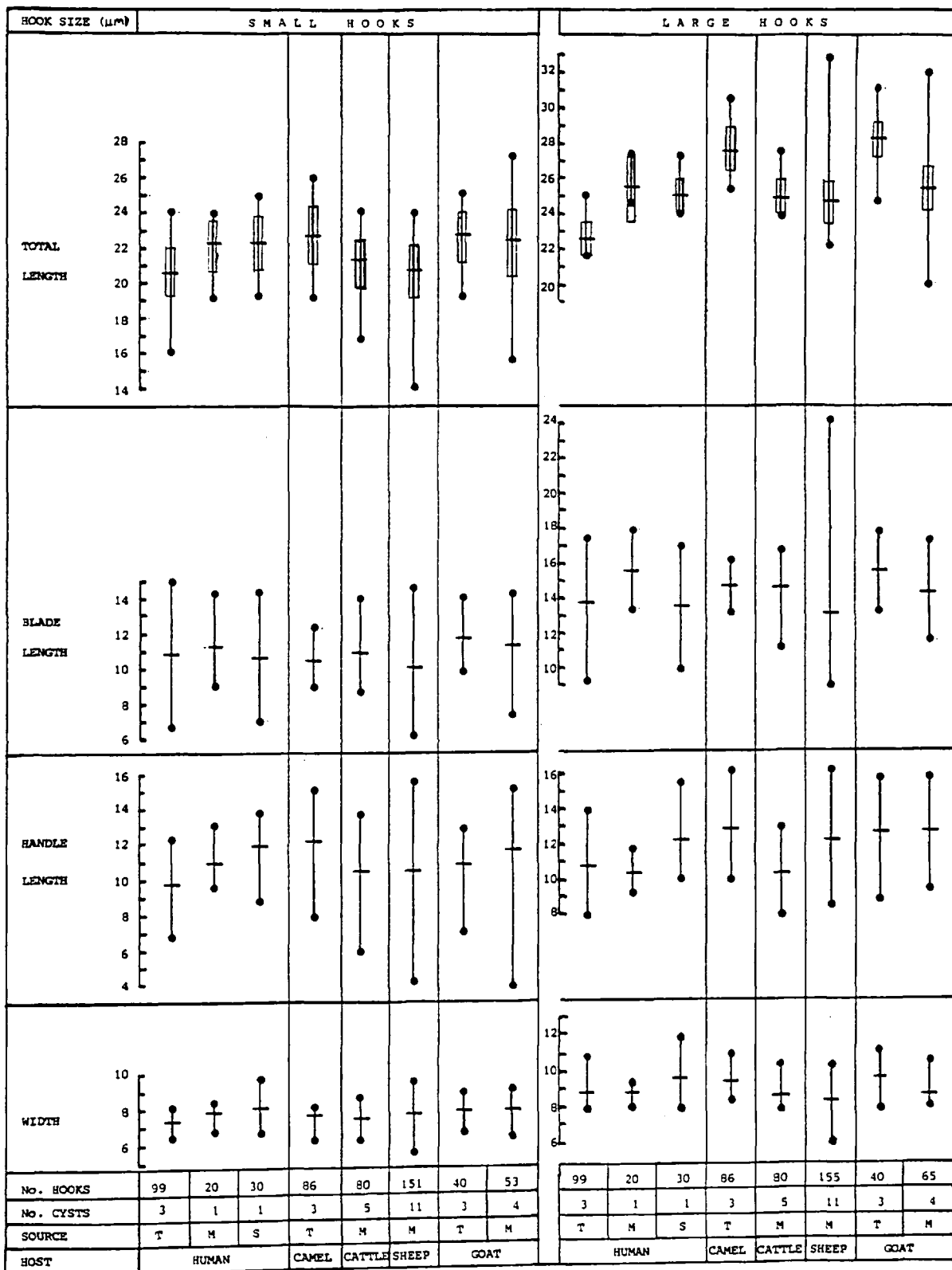
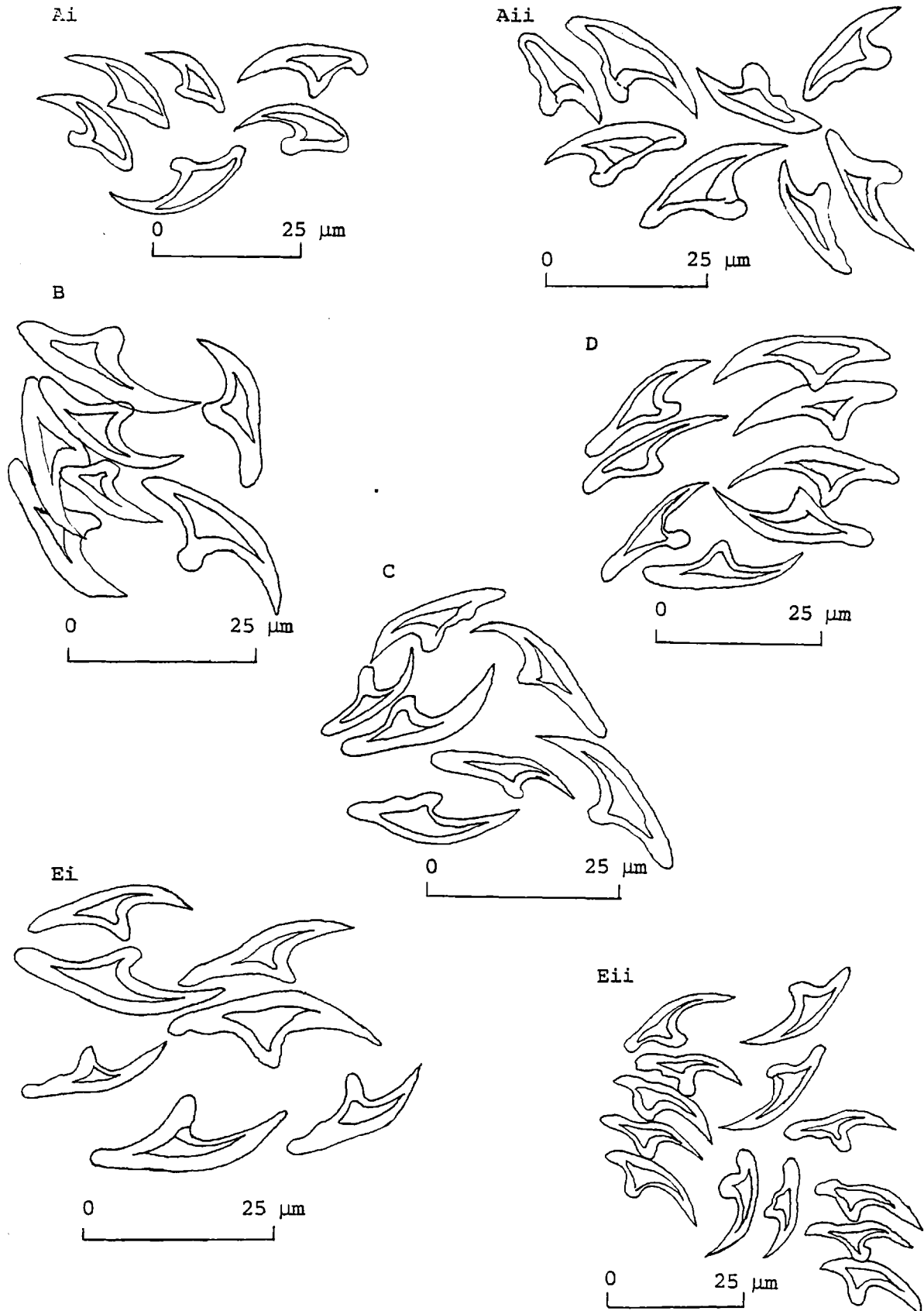


Figure 10. Morphology of cystic large and small hooks from man
(Ai - Turkana, Aii - Masai), camels (B), cattle (C), sheep (D) and
goats (Ei - Turkana, Eii - Masai)



6.2.2 Experimental dog and jackal infections

At least 10 worms from each experimental infection were studied, and the following is a brief description of the morphological features of the worms recovered.

Morphologically, the experimental worms were similar to each other. Usually three segments were observed, but occasionally there were only two. The anterior segment was immature containing the initial genital rudiment or anlagen, the second usually mature, and the third segment usually contained a well developed uterus containing either free cells, or juvenile eggs (Plate 15). The genital pore opened near the middle of the segmental margin in the mature segments, and posterior to middle in the third more developed segments. The cirrus sac was pear shaped, extended into the centre of the segment in mature segments, and lay either horizontally to the long axis of the segment or slightly tilted anteriorly from the genital pore. The uterus in the third terminal segments were characteristic in having well developed lateral sacculations.

Testes were found distributed in mature segments from below the ovary and vitelline gland at the posterior end of the segment and forwards to near the anterior end of the segment (Plate 16). The number of testes ranged from 30-75, and had an almost equal distribution both anterior and posterior to the genital pore in material of cattle/dog, goat/dog, sheep/dog and human/dog and human/jackal origin. Slightly more testes were found posterior to the genital pore in camel/dog worms, and more testes were found anterior to the genital pore in naturally infected dogs (Table 16). In the latter two cases, the observed mean distribution was never constant and in some specimens the opposite distribution of testes was seen. The total number of testes from all the different host species over-



Plate 15. Whole mount of E. granulosus adult worms of experimental Turkana goat/dog origin (36 days old). Note number and arrangement of segments, position of genital pores in mature and terminal segments (arrows) and shape and position of cirrus sac. (70% Alcohol; Gower's carmine) (x40).



Plate 16. Mature segment of E. granulosus of experimental Turkana goat/dog origin (36 days old). Note distribution of testes (te), shape and position of cirrus sac (cs). ov = ovary, vg = vitelline gland, ut = uterus (70% Alcohol; Performic acid - Schiff) (x60)

lapped, and it therefore was not possible to differentiate between worms by testes number and distribution (Table 16).

Table 16. Number and distribution of testes in mature segments of *E. granulosus* from experimentally and naturally infected carnivores

Material	No. Examined	Anterior		Posterior		Total	
		Range	Mean	Range	Mean	Range	Mean±SD
Turkana Camel/dog	40	13-26	19.9	18-34	23.9	33-60	44±5.3
Masai Cattle/dog	10	15-28	22.3	20-28	23.1	36-56	45±7.0
Turkana Goat/dog	17	11-25	19.0	15-30	20.0	30-53	39±7.0
Masai Sheep/dog	20	17-29	24.4	17-31	23.6	36-58	48±6.4
Turkana & Masai Human/dog	20	19-36	25.6	15-39	26.1	35-75	51±8.9
Turkana Human/jackal	11	17-25	20.7	17-30	21.8	36-54	44±4.2
Turkana Dog (Natural)	20	19-45	29.8	18-35	25.7	39-75	55±10.7

6.2.3 Natural dog infections

Numerous worms from several dogs naturally infected with *E. granulosus* in Turkana were examined morphologically.

The worms consisted of either three or four segments. In three segmented worms, the arrangement of segments was: immature, mature and gravid. In four segmented worms, the arrangement was either two immature, one mature and one gravid segment or, one immature, two mature and one gravid segment. The genital pore was always near the middle of the segmental margin in mature segments and posterior to

middle in gravid segments. The cirrus sac was either horizontal or tilted slightly anteriorly. The cirrus sac remained in some of the gravid segments. The uterus in a mature segment was a thin tube; in gravid segments it had well developed lateral sacculations. The length of the terminal segment and the number and size of the rostellar hooks are given in Table 17. The testes number and distribution in mature segments is given in Table 16.

6.2.4 Natural jackal infections

None of the golden jackal worms were gravid but many of the silver-backed jackal worms had gravid terminal segments. Usually three segments were present, comprising one immature and two mature segments or one immature, one mature and one gravid segment. Occasionally two or four segments were present. The position of the genital pore and the shape and position of the cirrus sac, were similar to that found in the dog material. The length of the terminal segments and number and size of the rostellar hooks is given in Table 17. Unfortunately, it proved impossible to count the testes in the jackal material due to poor staining of the specimens.

6.3 Discussion

The number, size and shape of the cystic hooks examined from hydatid material obtained from numerous Kenyan hosts exhibited little variation. Moreover, the number and size of the large and small cystic hooks from the Kenyan material is comparable with the findings of most previous workers. However, they are generally smaller than those reported for the British horse strain of E. granulosus (Williams & Sweatman, 1963; Thompson, 1975) and larger than material examined from camels, from Syria (Dailey & Sweatman, 1965).

Table 17. Comparative measurements (in μm) of the rostellar hooks and terminal segments of *E. granulosus* from Kenya

Source (and host)	Terminal segment			Rostellar hooks							
	No. Examined	Length of segment		Total No. of rostellar hooks		Large hooks			Small hooks		
		Range	Mean	Range	Mean	No. Examined	Range	Mean	No. Examined	Range	Mean
Lokichokio (<i>C. mesomelas</i>)	27	607-1200	902	27-34	30.1	90	26-34	29.4	90	19-26	22.8
Lokichokio ⁺ (<i>C. aureus</i>)	21	610-720	694	28-35	31.0	45	26-35	30.0	45	18-24	20.7
Lokichokio (<i>C. familiaris</i>)	40	519-1725	1273	26-37	31.8	89	28-37	31.8	87	20-27	23.9
Lodwar* (<i>C. familiaris</i>)	93	1371-2378	1773	28-36		287	32-40	36.0	221	19-31	26.0
Ngong* (<i>C. familiaris</i>)	24	1285-2363	1813			61	31-45	40.0	52	21-39	29.0

⁺ Mature segments only

* From Nelson & Rausch (1963)

The larger number of hooks and the smaller size of the large and small hooks of camel material from Syria, found by Dailey & Sweatman (1965), was not observed in the camel material from Kenya. However, the tiny accessory hooks seen in the Syrian camel material (Dailey & Sweatman, 1965) were observed in two protoscoleces in the Kenyan camel material. These tiny accessory hooks have also been described in material from many other hosts (Vogel, 1957; Sweatman & Williams, 1963; Williams & Sweatman, 1963; Rausch & Nelson, 1963; Dailey & Sweatman, 1965; Verster, 1965; Pandey, 1972).

The size of the adult rostellar hooks from the naturally infected dogs and jackals were smaller than those reported by Nelson & Rausch (1963), for material obtained from naturally infected dogs from Turkana and Masailand. The smallness of the hooks may have been due to the age of worms examined, for Yamashita et al, (1956) found that the large and small hooks of E. granulosus continue increasing in length up to the 375th day p.i. That the worms examined from the dogs and jackals were young infections, would reasonably only apply to the golden jackal material, for no gravid segments were seen in any of the worms recovered from this host.

The value of the size of rostellar hooks for taxonomic purposes is questionable. Rausch (1953) showed that the size of rostellar hooks showed geographical variation. Verster (1965) found that the identity of the definitive host affected hook size and also that hook size was not constant in successive generations. She also found, in addition to variations in size, variations in the number and shape of the rostellar hooks and concluded that the number, size and shape of rostellar hooks were not reliable criteria for taxonomic

purposes. Morphological variations may merely reflect phenotypic adaptations of the parasite to different environments and may therefore not reflect genotypic differences (Smyth, 1968; Thompson, 1979). Thus, as stressed by Rausch (1967b), morphological features should not be considered as the sole criteria for discrimination between strains but may be used in supporting evidence for strain differences.

The terminal segment of the adult worms from the present study were smaller than those reported by Nelson & Rausch (1963). Again, for the golden jackal material, this may have been due to the fact that the worms were not gravid. Verster (1965) found extreme variations in the length of the terminal segment, not only in different strains, but also in a single host. Schantz et al, (1976) also found that the gravid segments from worms from foxes experimentally infected with E. granulosus of sheep origin, were significantly smaller than those obtained from experimentally infected dogs. The taxonomic significance of the small terminal segments seen in the Turkana material may therefore be of little value in species determination.

The shape and position of the cirrus sac in adults of E. granulosus of British horse/dog origin has been shown to differ from that of sheep/dog material (Williams & Sweatman, 1963; Thompson, 1975). The cirrus sac in the horse strain has a round appearance and is tilted acutely anteriorly, whereas in the sheep strain it is pearshaped and not tilted acutely anteriorly. The material examined from Kenya generally exhibited the latter form.

The gravid uterus seen in all worms examined here had lateral sacculations which is a characteristic feature of E. granulosus (Rausch, 1953; Verster, 1965).

The number of testes in all worms ranged from 30 to 75; however the mean number of testes from each source was fairly similar, although generally fewer testes were found in goat/dog material and more testes were found in the naturally infected dogs than that seen in the human/sheep/cattle/camel/dog material (Table 16).

Williams & Sweatman (1963) gave the range of testes as 32-42 in E. granulosus equinus, whereas the average number in New Zealand sheep/dog (E. g. granulosus) material was about 50. Dailey & Sweatman (1965) found fewer (21-33) testes in mature segments of camel/dog worms which they assigned to E. g. granulosus. Verster (1965) in the study of seven 'subspecies' of E. granulosus in South Africa reported a range of 25 to 80 testes, although the numbers varied within relatively narrow limits for any given 'subspecies'. The average number of testes seen in these 'subspecies' varied between 32 to 68. She concluded that testes distribution was a reliable character for both species and subspecies differentiation. She laid stress on the character of the distribution of testes posterior to vitellaria to distinguish between some members of the species E. granulosus at the 'subspecies' level. The same character was used by Williams & Sweatman (1963) to help distinguish between horse/dog and sheep/dog worms. Pandey (1972) however, found this feature to vary in the goat/dog material from India, some mature segments having testes which only reached the anterior border of the ovary, whereas in others they extended below the vitellaria. Similar observations were seen in the present material although in most mature segments testes were confluent below the vitellaria.

Gill & Rao (1967) reported a range of 20 to 40 testes from mature segments of buffalo/dog origin, with more than half of them lying in the posterior half of the segment. Pandey (1972) gave the range of testes as 29 to 68 (average 44) in Indian goat/dog material,

the majority lying anterior to the genital pore. Both Gill & Rao (1967) and Pandey (1972) agree with the 'continuum' of 'species' theory proposed by Smyth & Smyth (1964) and state that the buffalo/dog and goat/dog material may be considered as separate strains of E. granulosus which differ from the 'classical' descriptions of this species.

Nelson & Rausch (1963) found the testes numbered from 45 to 59 (incorrectly printed as 45 to 49) with an average of 53 in adult E. granulosus from various species of carnivore in Kenya. This number is similar to the present findings in naturally infected dogs from Turkana. Other features found in the present material from the naturally infected dogs and jackals, viz. position of genital pore, number of segments, shape of cirrus sac and form of the gravid uterus are all consistent with the findings of Nelson & Rausch (1963), for their material from Kenya. They concluded that the species of Echinococcus occurring in East Africa is E. granulosus. Although the rostellar hooks and terminal segments examined here were smaller than those reported by these authors, the morphological findings from the present material support this view.

No firm conclusions based on morphological observations can be made regarding the strains of E. granulosus found in Turkana and Masailand except that the morphological features of the material from the various hosts from these two areas exhibit no marked differences. This finding will be considered with the results from the isoelectric focusing and in vitro cultivation experiments, described in chapters 7 and 8.

6.4 Summary

1. Morphological features of cystic hooks from camels, cattle, sheep, goats and man from Turkana, Masailand and Marsabit were all found to be similar.

2. Adult hook measurements from golden and silver-backed jackals and dogs from Turkana were similar to each other but generally smaller than that of material described by other authors for E. granulosus. The terminal segment from these worms were also smaller than those reported elsewhere. The possible significance of these findings is discussed.

3. Only slight morphological differences were found in worms from experimental dog and silver-backed jackal infections and natural dog infections.

4. The results indicate that the species of Echinococcus in Kenya is E. granulosus. No significant morphological differences were encountered to predict strain variations in the Kenyan material examined.

CHAPTER 7

ISOELECTRIC FOCUSING OF GPI AND PGM ENZYMES FROM ADULT AND
CYSTIC STAGES OF E. GRANULOSUS OF HUMAN AND ANIMAL ORIGIN7. Introduction

Recent biochemical, electrophoretic and isoenzyme studies have provided further support for the existence of strains within the species E. granulosus (Le Riche & Sewell, 1978; McManus & Smyth, 1978, 1979; Kumeratilake et al, 1979; McManus & Macpherson, 1980; McManus, 1981). Previous workers have suggested that the UK sheep and horse strains vary in their infectivity to man (Nelson, 1972; Smyth, 1977), but nothing is known regarding the potential infectivity to man of camel, cattle, sheep and goat 'strains' of Kenyan origin. This study was undertaken to examine whether cystic material from the numerous intermediate hosts in Kenya vary isoenzymatically from each other. The Kenyan samples were also compared with lyophilised material of horse and sheep origin from the UK and a single lyophilised sheep sample from Argentina.

The electrophoretic mobility profiles of two enzymes, glucosephosphate isomerase (GPI) (E.C.5.3.1.9) and phosphoglucomutase (PGM) (E.C.2.7.5.1) were compared after isoelectric focusing (IEF). This particular technique was selected because it is relatively simple, produces reproducible results and has a greater resolution and sensitivity when compared with other electrophoretic methods for protein separation. Moreover, this technique, and the two enzymes used, have proved successful in helping to differentiate between the UK horse and sheep strains of E. granulosus (McManus & Smyth, 1979).

An attempt was also made to investigate the possible occurrence of qualitative and quantitative differences in the GPI and PGM isoenzymes in larvae and adults of E. granulosus of the same origin.

In an attempt to discover the larval source of natural infections around Lokichiokio township, adult GPI isoenzyme patterns obtained from worms from naturally infected dogs and a silver-backed jackal were compared to the patterns obtained from experimental infections.

7.1 Materials and methods

7.1.1 Parasite material and preparation of enzyme extracts

Hydatid cysts from camels and goats were obtained from Lodwar abattoir, and also from cattle, sheep and goats from the KMC (Athi River) and Ongata Rongai (Nairobi) abattoirs. Human hydatid material was obtained from surgical operations performed on Turkana, Masai and a Samburu patient. Adult worms of E. granulosus were obtained from naturally infected dogs and a jackal at Lokichokio and from experimentally infected puppies and a silver-backed jackal. The experimental carnivores were sacrificed some 36-40 days p.i.

All isoelectric focusing procedures, with only minor modifications, were as described in detail by McManus & Smyth (1979). Protoscoleces were freed from brood capsules and purified by treatment in pepsin/Hank's medium as described previously (McManus & Smyth, 1978). After isolation, the protoscoleces were rinsed several times in HBSS and finally once in distilled water. Adult worms were treated similarly, but without the pepsin/Hank's treatment. Material was then transferred to a chilled 1 ml glass homogenizer (Jenkons) and hand homogenized on ice with a minimum of either distilled water or alternatively, with a solution of

freshly prepared enzyme stabilisers. The enzyme stabilisers consisted of 2 mM each of, dl-dithiothreitol (Cleland's reagent); e-Amino-n-Caproic acid, and ethylenediaminetetraacetic acid (EDTA), made up in distilled water (Kilgour & Godfrey, 1973). All homogenates were centrifuged at 8,000 r.p.m. for 5 min in a M.S.E. bench centrifuge operated in a coldroom at 4°C. The final supernatants were then beaded into 15µl droplets in liquid N₂ and stored in liquid N₂. Some of the supernatants were also lyophilised and stored at -20°C for comparison.

Protoscolecocytes from individual hydatid cysts only were used for the preparation of each enzyme extract. A number of extracts from each host species was thus prepared to investigate the possibility of polymorphic variants and also to examine whether the tissue location of a cyst within a particular host, had any effect on the isoenzyme pattern produced.

7.1.2 Isoelectric focusing

All experiments were performed using LKB ampholine polyacrylamide gel (PAG) plates, pH range 3.5 to 9.5, on the LKB 2117 multiphor with LKB 2103 power supply (LKB - Produkter, Bromma, Sweden). Beaded samples (15µl of supernatant, containing approximately 250µg protein, (Lowry et al, 1951)) were thawed on supplied (5x10 mm) filter paper application pieces, and applied to the gel. These applicators were removed 45 minutes after commencing the isofocusing run in order to prevent tailing of absorbed proteins. Human haemoglobin controls were applied at intervals along the gel. The PAG plates were transferred to the pre-chilled cooling plate of the multiphor which was kept at 2 to 4°C by circulating ice-cooled water from a Churchill Chiller thermocirculator. The cathode

wick was soaked in 1M sodium hydroxide and the anode wick in 1M phosphoric acid, the wicks were trimmed and positioned on the gel. The power pack was set to deliver 1.4 kV, 30W, and maximum current, and electrofocusing was completed after two hours.

After the electrofocusing was complete the electrofocusing wicks were removed with their underlying gel and the pH gradient was measured across the gel using an Ingold membrane electrode (Pye Unicam); pH readings were taken every 5 mm. The gel was then removed, and either cut into portions or left whole and placed into a staining tray(s). When cut into portions, it was possible to stain for GPI and PGM using the same gel

The staining methods for GPI and PGM were based on the procedures described in Harris & Hopkinson (1976). The reaction mixtures contained:

GPI - 50 ml 0.1M Tris - HCl buffer, pH 8.0;

26 mg D-fructose-6-phosphate (2Na);

8 mg nicotinamide adenine dinucleotide phosphate (NADP);

8 mg 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium
bromide (MTT);

3 mg phenazine methosulphate (PMS);

70 mg MgCl₂;

8 U glucose-6-phosphate dehydrogenase (G6PDH);

500 mg agar.

PGM - 50 ml 0.1M Tris - HCl buffer, pH 8.0;

40 mg D-glucose-1-phosphate (2Na) with 1% D-glucose-1,6-diphosphate;

8 mg NADP;

8 mg MTT;

3 mg PMS;

70 mg $MgCl_2$;

8 U G6PDH;

500 mg agar.

All chemicals, including the enzyme stabilisers were obtained from Sigma Ltd., Poole Dorset.

The gel portions were stained in the dark at room temperature for 45 mins., after which time the results were photographed.

7.2 Results

During the course of this investigation, a total of 23 hydatid cysts from humans, five from camels, 22 from cattle, 40 from sheep and 24 from goats were examined isoenzymatically. The tissue distribution of these cysts, and the geographical origin of the hosts are presented in Appendix 5, Table 1. Additionally, photographs of the zymograms produced by the larvae and also by adult worms from naturally and experimentally infected dogs and silver-backed jackals are shown in Plate 17 (i) and (ii) to Plate 21 (i) and (ii). The pI values of all the stained bands of larval material for both GPI and PGM are shown in Appendix 5, Tables 2 and 3.

There were no differences in the GPI or PGM patterns of any one sample regardless of whether larvae were treated with or without enzyme stabilisers, lyophilised, stored in liquid N_2 or used freshly. However, additional isoenzyme bands were observed in material which had been stored in liquid N_2 and thawed two or more times (Plate 17 (i) (arrow)). Similar additional isoenzyme bands are common in stored enzyme extracts from other organisms and tissues (Harris & Hopkinson, 1976), and are probably a result of oxidation of these enzymes (Kilgour, V., personal communication).

The isoenzyme patterns produced for each enzyme were unaffected

by the tissue location of the cyst in the host (Plates 17 (i) (ii), 18 (i) (ii), 19 (i), 21 (i) (ii)).

No polymorphic variations in the isoenzyme profiles of GPI or PGM were observed between E. granulosus of human, camel, cattle or sheep origin (Plates 17 (i) (ii), 18 (ii), 19 (i) (ii) (iii), 21 (i) (ii)). However, two distinct banding patterns were apparent in the goat material, for both GPI and PGM (Plates 18 (i), 19 (ii) (iii), 21 (i) (ii)). One of these patterns (type A) was evident in 20 samples, while the other (type B), was found in only four samples. Of the four type B samples, three originated in Turkana goats and one from a Masai goat.

The major banding patterns obtained for E. granulosus of human, cattle, sheep and goat (type A) origin were very similar for both GPI and PGM (Plates 19 (ii) (iii), 21 (i) (ii)). A number of samples of lyophilised protoscoleces from UK sheep and a single sample from an Argentinian sheep produced GPI and PGM patterns which were very similar to those obtained from Kenyan sheep.

In the cattle material, however, an additional characteristic cathodic band ($pI = 7.4$) was evident in the GPI isoenzyme profile (Plate 19 (i) (ii) (iii) (arrow)). Although this extra band was present in many of the cattle samples, it was not visible in all extracts examined (Plate 19 (i)). This might have been due to less protein (and hence enzyme activity) being applied to the gel.

The banding patterns obtained for both GPI and PGM from camel and goat (type B) material were very similar (Plates 19 (ii) (iii), 21 (i) (ii)). In contrast these patterns were different from those obtained for the human, cattle, sheep and goat (type A) material (Plates 19 (ii) (iii), 21 (i) (ii)). With GPI, the major difference between E. granulosus of camel or goat (type B) origin and the other

hosts, was the absence of the two lower cathodic bands, at pI 6.8 and 6.9. With PGM, the differences were much more pronounced.

The isoenzyme patterns for GPI and PGM from experimental adult worms of E. granulosus, were very similar to those produced by the larval material used to infect the puppies (Plates 18 (i) (ii), 19 (iii)). The only difference between the adult and larval patterns for each particular intermediate host, are the extra cathodic bands seen in adult patterns for GPI. No extra cathodic bands were evident in the PGM isoenzyme profiles for the adult worms.

The larval isoenzyme pattern differences which occur between the camel or goat (type B) forms and the other intermediate host forms, are also reflected in the corresponding adult worm patterns.

The GPI isoenzyme pattern of adults produced from an experimentally infected silver-backed jackal (human material) was identical to that of adults obtained from similarly infected puppies (Plate 20 (iii)).

Interestingly, the GPI (Plate 20 (iii)) isoenzyme patterns of adults from 13 out of 16 naturally infected dog samples and one silver-backed jackal sample (Plate 19 (iii) - J11) examined from Lokichokio, were very similar to those produced from the experimental human and sheep infections. Two out of the 16 patterns were very similar to the experimental cattle infection (Plate 20 (iii)), and one out of the 16 naturally infected dog patterns had a camel/goat (type B) pattern (Plate 19 (iii) - DC).

Plate 17 (i) and (ii)

Electrophoretic patterns obtained with soluble extracts of protoscoleces removed from hydatid cysts from (i) humans and (ii) camels, and stained for the enzyme GPI.

Key:-

(i)	(ii)
HT1 - Human (Turkana) - mesenteric	TC1 - Camel (Turkana) - spleen
HT2 - Human (Turkana) - liver	TC2 - Camel (Turkana) - lung
HT3 - Human (Turkana) - mesenteric	TC3 - Camel (Turkana) - lung
HT4 - Human (Turkana) - liver	TC4 - Camel (Turkana) - spleen
WH1 - Human (Samburu) - mesenteric	TC5 - Camel (Turkana) - spleen
HT5 - Human (Turkana) - liver	
HM1 - Human (Masai) - liver	
HM2 - Human (Masai) - liver	
HT6 - Human (Turkana) - retrouterine	
HT7 - Human (Turkana) - liver	
HT8 - Human (Turkana) - liver	
HT9 - Human (Turkana) - retroperitoneal	
HT10 - Human (Turkana) - liver	
HT11 - Human (Turkana) - liver	
HT12 - Human (Turkana) - liver	

Eb = Haemoglobin control

Plate 17 (i)

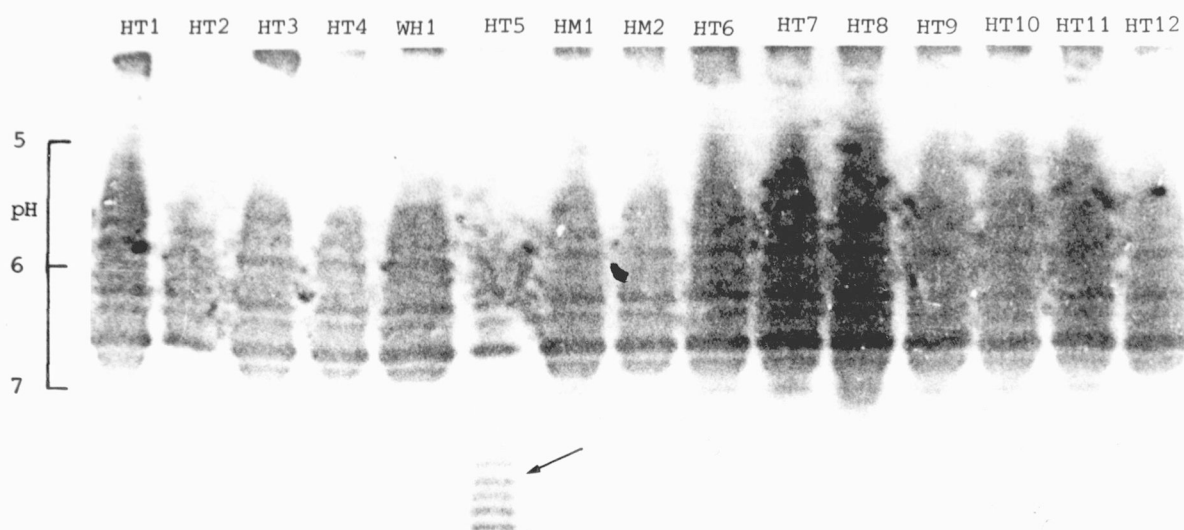


Plate 17 (ii)

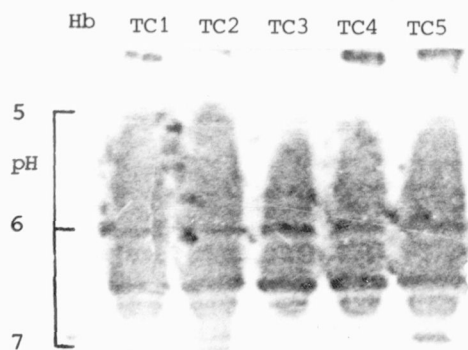


Plate 18 (i) and (ii)

Electrophoretic patterns obtained with soluble extracts of protoscoleces removed from hydatid cysts from (i) goats and (ii) sheep; also included are patterns from adult worms obtained by experimental infection with larval material from these two intermediate hosts. Both gels were stained for the enzyme GPI.

Key:-

(i)	<u>Type</u>	(ii)
MG1 - Goat (Marsabit) - lung	A	MS2 - Sheep (Masai) - lung
MG3 - Goat (Masai) - lung	A	MS4 - Sheep (Masai) - liver
MG1 - Goat (Marsabit) - lung	A	TD5 - Dog infected with material from MS4
MG4 - Goat (Masai) - lung	A	MS6 - Sheep (Masai) - liver
MG5 - Goat (Masai) - liver	A	ND9 - Dog infected with material from MS6
MG6 - Goat (Masai) - lung	A	ND10 - Dog infected with material from MS7
MG8 - Goat (Masai) - liver	A	MS7 - Sheep (Masai) - lung
MG9 - Goat (Masai) - lung	A	J11 - Jackal - natural infection
MG10 - Goat (Masai) - lung	B	MS11 - Sheep (Masai) - lung
MG11 - Goat (Masai) - lung	A	MS12 - Sheep (Masai) - liver
MG12 - Goat (Masai) - lung	A	MS13 - Sheep (Masai) - spleen
MG13 - Goat (Masai) - liver	A	MS15 - Sheep (Masai) - lung
MG14 - Goat (Masai) - liver	A	MS16 - Sheep (Masai) - liver
MG15 - Goat (Masai) - liver	A	MS17 - Sheep (Masai) - lung
TG2 - Goat (Turkana) - liver	B	S - Sheep (UK)
TD7 - Dog infected with material from TG4		MS8 - Sheep (Masai) - liver
TG4 - Goat (Turkana) - spleen	B	MS9 - Sheep (Masai) - liver
TD7 - Dog infected with material from TG4		MS10 - Sheep (Masai) - lung
Hb = Haemoglobin control		MS5 - Sheep (Masai) - lung

Plate 18 (i)

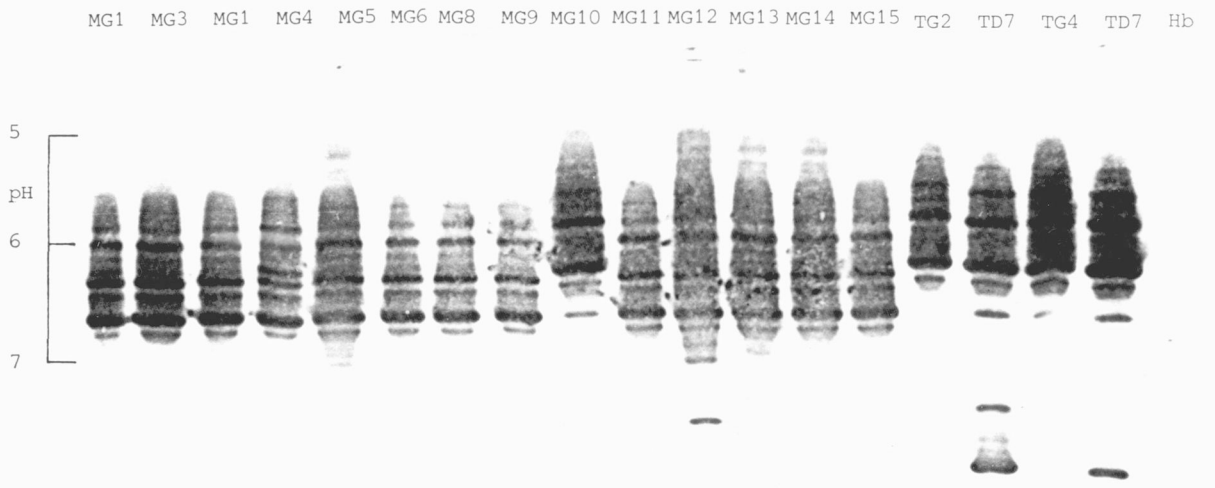


Plate 18 (ii)

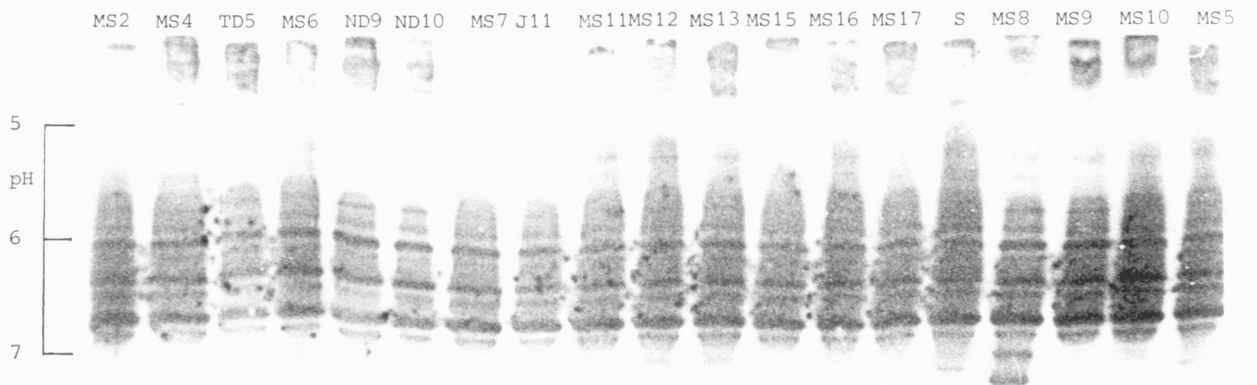


Plate 19 (i), (ii) and (iii)

Electrophoretic patterns obtained with soluble extracts of protoscoleces removed from hydatid cysts from (i) cattle (ii) camels, cattle, sheep and goats and (iii) humans, camels, cattle, horse (UK), sheep (UK and Kenya) and goats; also included are patterns from naturally and experimentally infected dogs and a natural jackal infection. All three gels were stained for GPI.

Key:-

(i)	TG1 - Goat (Turkana) (B)
MC1-MC6, MC8-MC22 - Cattle (Masai) - lung	MC1 - Cattle (Masai)
MC7 - Cattle (Masai) - heart	H - Horse (UK)
(ii)	S - Sheep (UK)
MS2 - Sheep (Masai)	DC - Dog - natural infection
MS3 - Sheep (Masai)	TC2 - Camel (Turkana)
TC1 - Camel (Turkana)	MS2 - Sheep (Masai)
TC2 - Camel (Turkana)	ND4 - Dog - infected with human material
TC3 - Camel (Turkana)	HT3 - Human (Turkana)
TG1 - Goat (Turkana) (B)	HT2 - Human (Turkana)
MG1 - Goat (Masai) (A)	J11 - Jackal - natural infection
MG2 - Goat (Masai) (A)	DN - Dog - natural infection
MG3 - Goat (Masai) (A)	TD1 - Dog - infected with human material
MC3 - Cattle (Masai)	HT4 - Human (Turkana)
MC4 - Cattle (Masai)	WH1 - Human (Samburu)
(iii)	MG1 Goat (Masai) (A)
S - Sheep (UK)	MC2 - Cattle (Masai)
MS1 - Sheep (Masai)	
HT1 - Human (Turkana)	
TC1 - Camel (Turkana)	Hb = Haemoglobin control

Plate 19 (i)

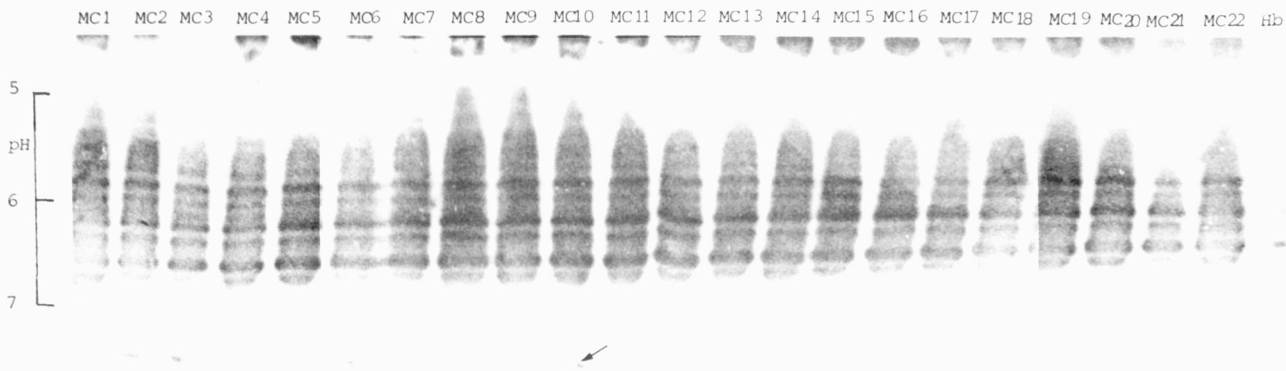


Plate 19 (ii)

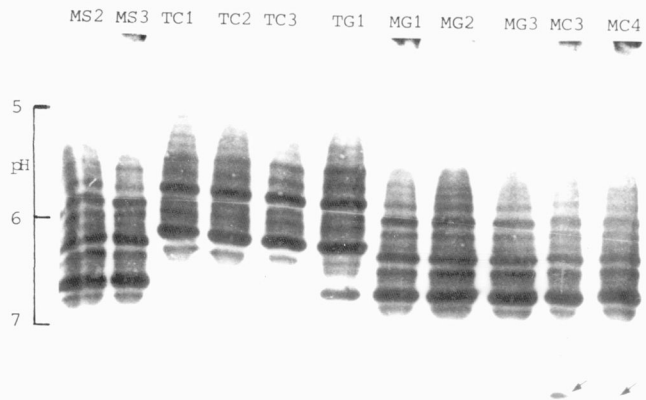


Plate 19 (iii)

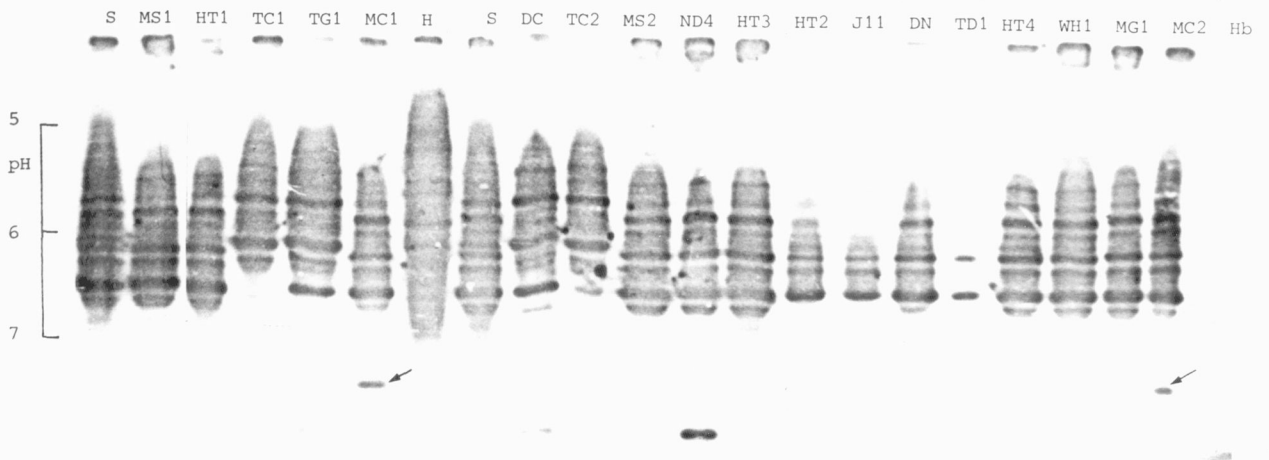


Plate 20 (i), (ii) and (iii)

Electrophoretic patterns obtained with soluble extracts of adult E. granulosus worms obtained from experimental dog infections and stained for enzymes GPI(i) and PGM(ii). Plate 20 (iii) shows the GPI electrophoretic patterns obtained from 15 naturally infected dogs examined around Lokichokio and from an experimental dog and a silver-backed jackal fed human material.

Key:-

(i) and (ii)

- B - dog infected with cattle protoscoleces
- C - dogs infected with camel protoscoleces
- G - dogs infected with goat protoscoleces (type B)
- H - dogs infected with human protoscoleces
- S - dogs infected with human protoscoleces

(iii)

- DH - naturally infected dogs with human, sheep or goat, type A pattern
- DB - naturally infected dogs with cattle type pattern - extra cathodic band (arrowed) was seen on gel but is very faint in photograph
- EJ - Experimental jackal infected with human material
- ED - Experimental dog infected with human material

Plate 20 (i)

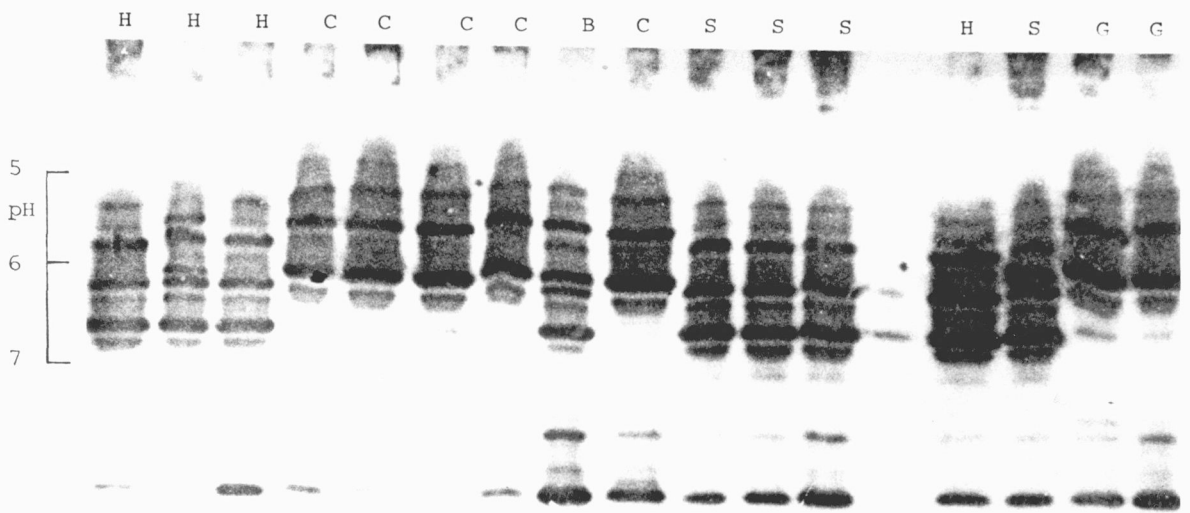


Plate 20 (ii)

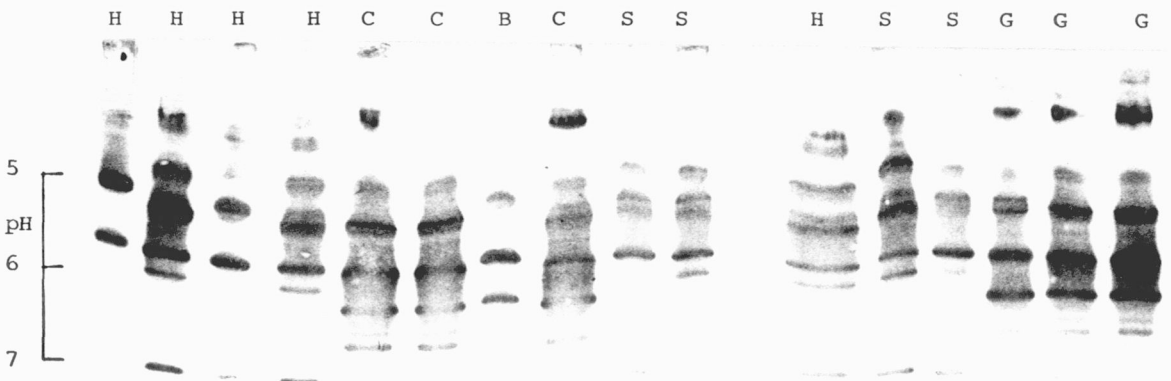


Plate 20 (iii)

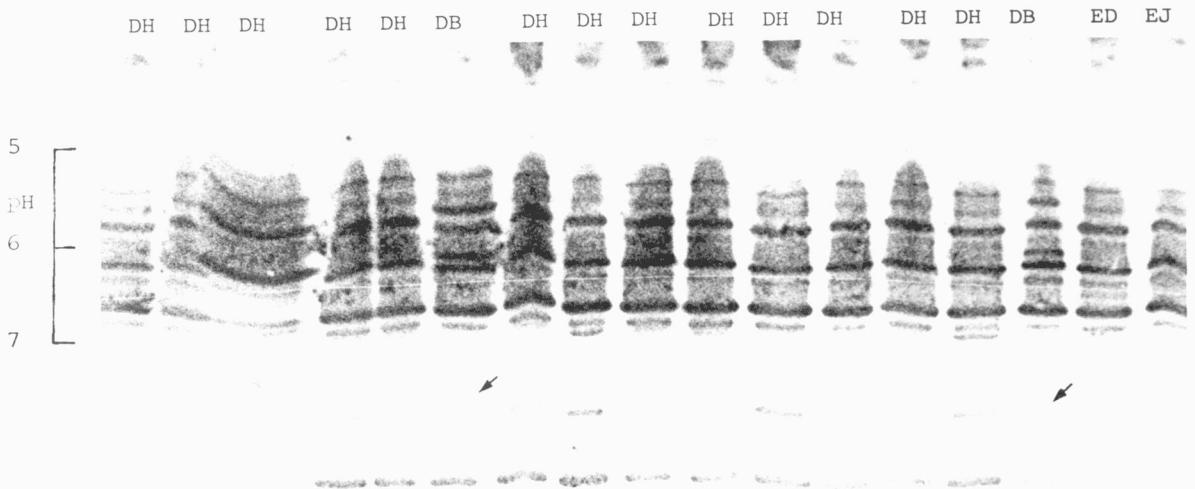


Plate 21 (i) and (ii)

Electrophoretic patterns obtained with soluble extracts of protoscoleces removed from hydatid cysts from: (i) Camels, cattle, sheep and goats and (ii) humans, camels, sheep and goats. Both gels were stained for the enzyme PGM.

Key:

(i)	(iii)
MS1 - Sheep (Masai) - liver	TG1 - Goat (Turkana) - liver (B)
MS2 - Sheep (Masai) - lung	TC1 - Camel (Turkana) - spleen
MS3 - Sheep (Masai) - liver	MS1 - Sheep (Masai) - liver
TC1 - Camel (Turkana) - spleen	TC2 - Camel (Turkana) - lung
TC2 - Camel (Turkana) - lung	DC - Naturally infected dog
TC3 - Camel (Turkana) - lung	MS2 - Sheep (Masai) - lung
TG1 - Goat (Turkana) - liver (B)	HT3 - Human (Turkana) - mesenteric
MG1 - Goat (Marsabit) - lung (A)	HT4 - Human (Turkana) - liver
MG2 - Goat (Masai) - lung (A)	WH1 - Human (Samburu) - mesenteric
MG3 - Goat (Masai) - lung (A)	MG1 - Goat (Masai) - lung (A)
MC4 - Cattle (Masai) - lung	MG2 - Goat (Masai) - lung (A)

Plate 21 (i)

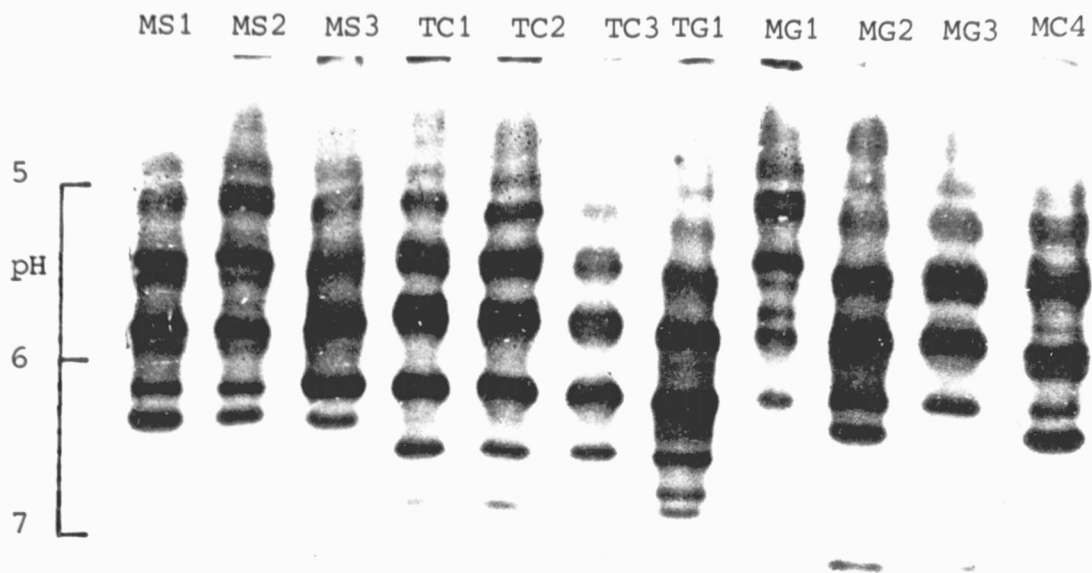
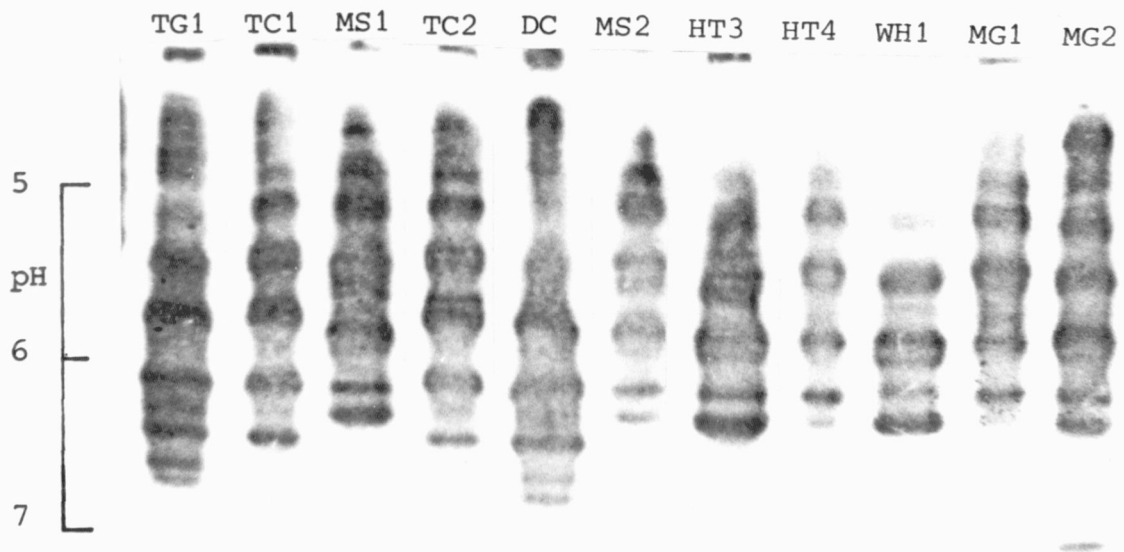


Plate 21 (ii)



7.3 Discussion

In a recent electrophoretic study, Le Riche & Sewell (1978) showed that the GPI isoenzyme profiles for E. granulosus of UK sheep and cattle origin were very similar, but that these patterns were different to those produced by extracts of the parasite obtained from two infected Nigerian camels. The findings presented here, using the more sensitive method of IEF, support those earlier observations. However, the cattle material from Kenya differed from the human and sheep material in having one extra cathodic band in the GPI isoenzyme pattern.

McManus (1981), using biochemical criteria, suggested that the human and sheep forms of E. granulosus from Kenya have a very close affinity, but that the cattle, goat and camel forms are distinct, both from each other and from the human and sheep types. Again, the present investigation substantially corroborates that earlier study, but indicates that the strain picture in Kenya may be more complex than was previously suggested. The uniformly consistent GPI and PGM isoenzyme patterns obtained for the human, sheep, cattle and camel forms suggest that each may exist, although not necessarily separately, as monomorphic 'strains' in Kenya. In contrast, however, the two distinct isoenzyme profiles for both GPI and PGM strongly suggests that E. granulosus of goat origin exists there as two 'sub-strains'. As far as I am aware, this is the first time that sub-strains of E. granulosus have been detected in the same country. There is evidence, however, from biochemical work that the sheep forms of E. granulosus from the UK, Kenya and South America may represent geographically distinct sub-strains (McManus & Smyth, 1978; McManus, 1981). It is somewhat surprising, therefore, that the GPI and PGM isoenzyme profiles for E. granulosus

of sheep origin from these three areas are so similar. This highlights the importance of using several different criteria, including ecological, immunological, morphological, biochemical, isoenzymatic and in vitro developmental studies when attempting to fully characterise a particular strain or even sub-strain of E. granulosus in any geographical locality.

An interesting and important aspect of the current study is that the GPI and PGM isoenzyme patterns exhibited by larvae from all the intermediate hosts investigated are reflected in the homologous adult patterns produced by experimental infections. Thus the basic pattern of both enzymes in all forms in the larva to adult transition, does not appear to be influenced by the change in host environments. The extra cathodic bands evident in the GPI isoenzyme profiles for the adults, may represent new isoenzymes synthesised as an adaptation to the differing physiological conditions in the intestine of the definitive host, or, they may be host enzymes adsorbed onto the surface of the worms. It is more likely, however, that these extra bands are of parasite origin and reflect the metabolic changes which occur in the parasite during its development from larva to egg-producing adult (McManus, 1981).

Kumaratilake et al (1979) found no differences between the soluble protein profiles, after IEF, of E. granulosus from sheep and E. granulosus of sheep origin which had been serially passaged through several generations of mice. Similarly, McManus (personal communication) has found no difference in the GPI isoenzyme profiles of E. granulosus from horses and E. granulosus of horse origin which had been passaged through gerbils. Thus, it appears

that the isoenzyme patterns are not influenced unduly by the intermediate host. The major bands are similarly not altered in the definitive host; this has been shown by the experimental dog infections.

Of epidemiological significance, is the fact that adult E. granulosus worms from 13 out of 16 naturally infected dogs examined in Lokichokio, gave a similar GPI isoenzyme pattern to that of adult worms from dogs experimentally infected with human and sheep hydatid material. It is likely, therefore, that these naturally infected dogs became parasitised through ingesting hydatid cyst material from one or either of these host species. Additionally, although goat (type A) material was not experimentally fed to dogs, it is probable that similar GPI and PGM isoenzyme patterns to the experimental human/dog and sheep/dog forms would have been obtained. Therefore, by analogy the naturally infected dogs, with the human/sheep isoenzyme pattern could also have ingested hydatid material from goats with the type A pattern. Two of the 16 naturally infected dogs had experimental cattle/dog GPI isoenzyme patterns and a single naturally infected dog had a camel or goat (type B) GPI isoenzyme pattern.

In the light of these findings, this study should be repeated to investigate whether the results reported here could also be detected with other enzymes. It is suggested that all future material for isoelectric focusing experiments should be prepared using enzyme stabilisers and stored in a beaded form in liquid N₂.

7.4 Summary

1. Soluble enzyme extracts from protoscoleces obtained from human, camel, cattle, sheep and goat hydatid cysts were compared on

the basis of their isoenzyme patterns for GPI and PGM, using isoelectric focusing in polyacrylamide gels. Similarly this method was used to compare the Kenyan material with many lyophilised UK sheep and horse samples and a single lyophilised sheep sample from Argentina.

2. The GPI and PGM isoenzyme patterns for larvae of human, camel, cattle and sheep origin were consistent, suggesting that each may exist, although not necessarily separately, as monomorphic strains in Kenya. Two different isoenzyme patterns were evident in the goat material which suggests that 'sub-strains' exist in this host. One of these goat patterns (type A) was similar to those of human, cattle and sheep (Kenya, UK and Argentina) material, which were similar to each other. The second goat pattern (type B) was similar to the pattern obtained for the camel material. Cattle material differed to the human and sheep material in having an additional cathodic band in the GPI enzyme pattern.

3. The results obtained indicate that the location of the cyst in the intermediate host, has no affect on the isoenzyme patterns produced for GPI and PGM.

4. The GPI and PGM isoenzyme patterns from larvae of all the intermediate hosts investigated are repeated in the homologous adult worm patterns produced by experimental infections in puppies. The human larval GPI and PGM patterns also remained constant in the adult patterns produced by experimental infection in a silver-backed jackal.

5. The only change observed in the larval and adult patterns was the existence of additional cathodic bands in the GPI isoenzyme pattern. These additional bands are thought to be of parasitic origin, possibly reflecting metabolic changes between the larval and adult

forms.

6. Of 16 naturally infected dogs examined around Lokichokio, 13 had human/sheep/(goat type A), type GPI patterns, two had cattle GPI patterns and one had a camel/goat (type B) pattern.

7. It is suggested that these investigations should be extended to include other enzymes, using material prepared with enzyme stabilisers and stored in a beaded form in liquid N₂.

CHAPTER 8

IN VITRO CULTIVATION OF E. GRANULOSUS8. Introduction

It is now well accepted that, as with other parasites, in vitro cultivation of cestodes is an important technique employed in the study of their physiology, biochemistry and immunology. Within the last decade, substantial progress has been made in in vitro cultivation techniques and recently it has proved possible to collect excretory/secretory antigens from in vitro cultured worms of Taenia pisiformis (Rickard & Outteridge, 1974) and T. saginata (Rickard & Adolph, 1976) and use them as successful vaccines. The commercial viability of such vaccines however, cannot be fully exploited until the parasites can be grown repeatedly in a continuous culture system. The in vitro system is also advantageous in that it dispenses with the requirement of keeping laboratory hosts for the purposes of maintaining parasites for experimental use. With hydatidosis, this latter advantage of the system is especially relevant, for studies on this disease are inhibited by the lack of facilities, in most laboratories, for maintaining infected dogs. Since the strobilate stage in dogs is dangerously infective to man, the advantages of being able to study the sexually mature, gravid worms in vitro, are considerable. The importance of the in vitro approach for research into hydatidosis has been emphasised at several WHO/FAO working groups (Munich, 1974, Nairobi, 1976 and Geneva, 1981).

Early attempts to culture the metacestode stage of E. granulosus resulted in cystic development only (Smyth, 1962a). This raised

questions regarding the nature of the factor(s) controlling strobilisation. It was subsequently discovered that a diphasic medium with a solid nutritive substrate (e.g. coagulated serum) was necessary for strobilate differentiation to be induced (Smyth, 1967; Smyth et al, 1966). The nature of the 'stimulus' for strobilisation to take place is, at present, still unknown, although many factors have been suggested (Smyth, 1972).

The first success in obtaining strobilate worms in vitro from the protoscolex stage, was with hydatid material of sheep origin using a diphasic medium consisting of a coagulated serum base overlaid with a complex liquid medium (Smyth, 1967; Smyth, 1972; Smyth & Davies, 1974b). In later experiments, when protoscoleces of horse hydatid cyst origin were cultured, no segmentation or further development took place in vitro. In such cultures, larvae grew a little and then ceased to develop further although remaining active and seemingly healthy for several months (Smyth & Davies, 1974a). This result led the authors to conclude that protoscoleces from horse and sheep hydatid cysts represent different strains of E. granulosus with perhaps different nutritional and physiological requirements. Clearly, the culture system employed satisfied the requirements of the sheep strain, but not the horse strain, to develop to sexual maturity in vitro. Thus, the in vitro technique provided a new method for differentiating between these two and possibly other strains. It has not yet been possible to culture the whole life cycle of the parasite in vitro, as fertilization has not yet been achieved.

Recently, human hydatid material obtained surgically from a Saudi Arabian was cultured in vitro to the two-segment worm stage (Smyth et al, 1980). The developmental pattern of human material

thus corresponds to that of the sheep strain, and not to that of the horse strain.

Recent attempts to culture cattle material obtained from Ireland, has suggested that, like the horse strain, hydatid material from this host does not grow in vitro (Smyth, 1979). In Spain, Osuna (Quoted by Smyth, 1979) found that Echinococcus from pigs failed to segment in vitro. Nothing is known about the growth characteristics of protoscoleces from other intermediate hosts of E. granulosus, and no previous in vitro culture studies have been performed on hydatid material of Kenyan origin.

The aim of the present study was to examine the growth characteristics of larval hydatid material of human, camel, cattle, sheep and goat origin from Kenya. This study was undertaken to compliment the morphological and isoenzyme studies, reported in chapters 6 and 7, in an attempt to identify the particular 'strain(s)' of E. granulosus that exist in Kenya.

8.1 Materials and methods

Many of the basic problems encountered in the setting up of E. granulosus cultures have already been reviewed in detail by Smyth & Davies (1974b) so that only the essential procedures are summarised here.

8.1.1 Culture technique

(a) Culture vessels: Two types of containers were used:

(i) disposable, plastic, tissue culture flasks (Sterilin) with a volume capacity of 200 ml and (ii) screw-capped glass milk dilution (M.D.) bottles (Kimax, USA). Both these vessels have been found previously to be satisfactory for the culture of E. granulosus.

(b) Agitation: The culture vessels were gently and continuously

agitated with a simple horizontal orbital motion, at about 60 revolutions per minute, in a constant temperature orbital shaker (Gallenkamp).

(c) Gas phase: All fresh media were gassed with sterile 5% carbon dioxide, 10% oxygen and 85% nitrogen.

(d) Temperature: The orbital shaker was set and maintained at 38.5°C (dog body temperature).

(e) pH: The pH of the cultures was maintained at about 7.2, using the colour change of the phenol red, in the basic media and salt solutions, as an indicator.

(f) Routine replacement of media: Media replacement was necessary to remove toxic wastes and to replenish nutrients and vitamins. The frequency of such replacements varied from culture to culture, but normally 5-10 ml of media were changed every three days.

8.1.2 Culture media

In all experiments, a diphasic medium was used. This was made up of a liquid phase of culture media above a coagulated serum base.

(a) Preparation of solid phase

The coagulated serum bases were prepared by adding 20 ml newborn calf serum (Gibco, Flow) to each culture container and coagulated by heating in an oven at 76°C for 30-60 minutes. After coagulating and cooling, the bases were incubated for 48 hours at 38°C to check sterility and stored at 4°C until required.

(b) Preparation of liquid phase

The liquid phase was made up from commercially available synthetic basic media supplemented by serum, glucose, yeast extract, dog bile and antibiotics. The final composition of the liquid phase is given in Table 18.

Table 18. Composition of the liquid phase of the diphasic media used for *in vitro* cultivation of *E. granulosus*

	ml
NCTC 135*	130.0
Foetal calf serum (inactivated)	50.0
5% Yeast extract	18.0
30% Glucose	2.8
5% Dog bile	0.7
	<hr/>
	201.5

* Can be replaced by RPMI 1640

The above constituents of the liquid phase were prepared using the following methods.

(i) Synthetic basic media. Two types of media were used: RPMI 1640 (Flow) and NCTC 135 (Gibco). The method of reconstitution of these media from commercially available powder was completed according to instructions provided by the manufacturers. All media were sterilised by positive pressure filtration through a 0.2 μ m millipore filter (Millipore) and stored in sterile containers. After incubation at 38 $^{\circ}$ C for 48 hours, antibiotics were added to sterile media, which were then stored until required at 4 $^{\circ}$ C.

(ii) Foetal calf serum, FCS (Gibco, Flow). This serum is supplied in sterile 100 ml bottles and was stored at -20 $^{\circ}$ C until required. After thawing, the serum may be kept at 4 $^{\circ}$ C for short periods. FCS was always inactivated before use, by heating in a water bath at 56 $^{\circ}$ C for one hour.

(iii) Glucose. A stock of 30% glucose (Analar, BDH) solution was made up in triple distilled water, sterilised by filtration through 0.2 μ m millipore filters and stored at 4 $^{\circ}$ C until required.

(iv) Yeast extract. A 5% solution (w/v) of yeast extract powder (Oxoid) was made up in NCTC 135. It was sterilised by filtration through 0.2 μm millipore filters and incubated at 38°C for 48 hours. All sterile solutions were stored at 4°C until required.

(v) Dog bile. Bile was aseptically aspirated from the gall-bladders of several dogs and diluted with sterile HBSS to give a 5% solution. The bile solution was sterilised by filtration through a 0.2 μm pore size filter and stored at -20°C until required.

(vi) Antibiotics. Penicillin and streptomycin (BDH, Flow), available as a mixed solution of concentration 5000 I.U./ml and 500 $\mu\text{g}/\text{ml}$ were stored at -20°C until required. Antibiotics were routinely added to the basic media and HBSS at a level of 2 ml/100 ml giving a final concentration of 100 I.U./ml penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin.

Origin of parasite

Details of parasite origins and the sterile procedures used to extract protoscoleces from hydatid cysts have been given earlier in the materials and methods section of chapter 2. Once protoscoleces had been aseptically removed from a cyst, they were then either processed immediately, or stored in hydatid fluid for one or two days at 4°C.

8.1.3 Condition and preparation of culture material

Before setting up cultures, each sample of protoscoleces was examined for (a) the presence of bacteria and (b) viability. Infected material was discarded. Only those protoscoleces with a mean viability of 60% or more were used for culture.

Sterile, viable protoscoleces were pepsinised by incubating the

larvae for 15-45 minutes at 38°C in about 20 ml of 0.2% pepsin made up in HBSS and maintained at pH 2.0 with 5.0 N hydrochloric acid. The larvae were then rinsed in four or five changes of warm sterile HBSS, on a roller at 38°C, allowing 15 minutes for each rinse. After the final rinse larvae were transferred to a culture vessel containing 50 ml warm evaginating medium. The evaginating medium contained 50 ml NCTC 135 and 1 ml 5% dog bile. This was then gassed with sterile 5% CO₂, 10% O₂ and 85% N₂, and finally placed into the orbital shaker and left overnight at 38°C.

Setting up of cultures

After 18-24 hours in the evaginating medium, most of the medium was removed and an aliquot of 0.1 ml protoscoleces was transferred to each culture vessel, containing the solid serum base and 20 ml liquid media. The cultures were gassed for approximately 30 seconds before being placed into the orbital shaker.

Assessment of early development

Worms in the culture vessels were examined daily using an inverted microscope. Because of the extended length of time for segmentation to occur in E. granulosus (14 days), as compared to other cestodes, e.g. Hymenolepis (3 days), Smyth & Davies (1974b) describe several presegmentation growth phases. These criteria allow an early evaluation of the culture system under test. Their developmental criteria and the earliest time normally taken to observe similar changes in the dog host are listed below.

It should be emphasised that the time taken for these stages to be reached in vitro is found to be longer than in the natural definitive host (Smyth & Davies, 1974b).

<u>Stage</u>		<u>Days</u>
P.S.1	Evagination	0
P.S.2	Excretory canals just visible	3
P.S.3	Excretory bladder clear; calcareous corpuscles gone	6
P.S.4	'Banding'; pinching off of 1st segment seen	11
S.5	Segmentation. 1st segment clearly formed	14
S.6	2nd segment appears	17
S.7	Testes appear	22

P.S. = Presegmentation S = Segmentation

8.2 Results

The results of the 'successful' cultures are presented in Table 19.

Table 19. In vitro cultivation of *E. granulosus* 'strains' from Kenya

Culture 'strain'	Days taken to reach indicated stage						Maximum No. of days cultured
	P.S.1	P.S.2	P.S.3	P.S.4	S.5	S.6	
Human	0	6	9	-	-	-	16
Cattle	0	6	8	-	-	-	37
Camel	0	6	-	-	-	-	6
Sheep	0	6	8	-	-	-	16
Goat	0	7	9	21	42	59	65

From the numerous attempts to culture the various 'strains' in vitro, only one goat and a single cattle culture were maintained in culture for longer than 16 days. All other cultures were unfortunately lost earlier because of fungal infections. The preliminary culture work was performed in a flow hood in the Botany Department of Nairobi University, where fungal cultures were

regularly examined. It was not surprising therefore that such infections occurred! All the 'successful' results reported here were obtained from later experiments performed at the Wellcome Trust laboratories, where sterile in vitro techniques were routinely performed.

The single 'successful' goat culture material was obtained from four individual unilocular pulmonary cysts from an infected animal at Ongata Rongai. Isoelectric focusing studies revealed that the material belonged to the type A banding pattern found in the majority of goat specimens examined (Chapter 7).

The goat material had a 100% evagination, even before being placed into the evaginating media. The growth of the material was initially similar to that reported by Smyth & Davies (1974b) for the sheep strain. However, after the P.S.3 stage was reached (Plate 22), the worms grew more slowly and segmentation was not evident until day 42 of culture (Plate 23).

Protoscoleces from cattle (as with the human and sheep forms) reached the P.S.3 stage in approximately 6-9 days (variations in development were seen in worms in all cultures, figures in Table 19 represent mean times when 50% or more had reached that particular stage). No further growth was observed even after 37 days in culture (Plate 24).

8.3 Discussion

The results of the in vitro culture investigations with E. granulosus indicate that goat (type A) material of Kenyan origin grows and segments in vitro like the human and sheep strains of the parasite from other countries. However, material of cattle origin does not appear to segment in vitro and therefore behaves

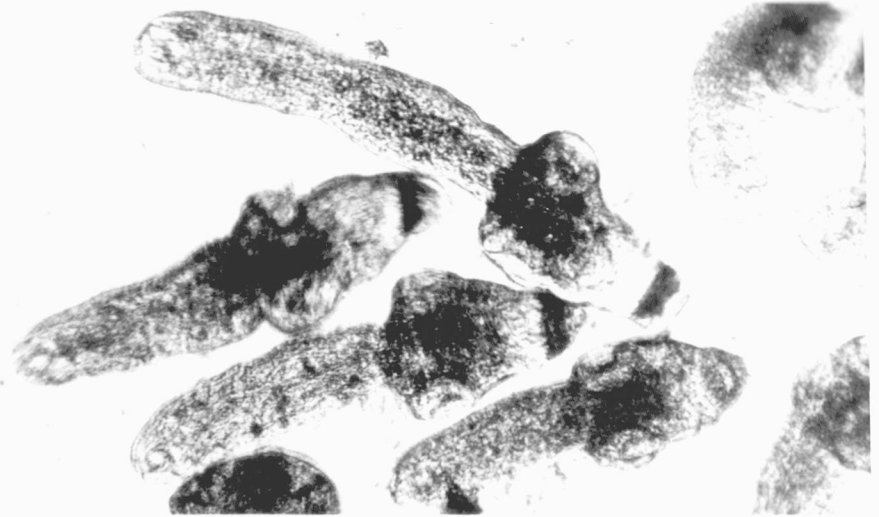


Plate 22. 31 day old goat culture showing P.S.3 stage of development (x88).



Plate 23. 42 day old goat culture showing S.5 stage of development. After a further 17 days in culture, two segmented worms (S.6 stage) were seen (x88).

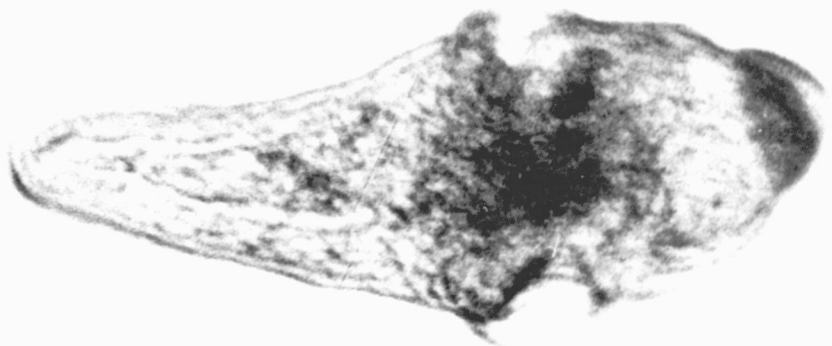
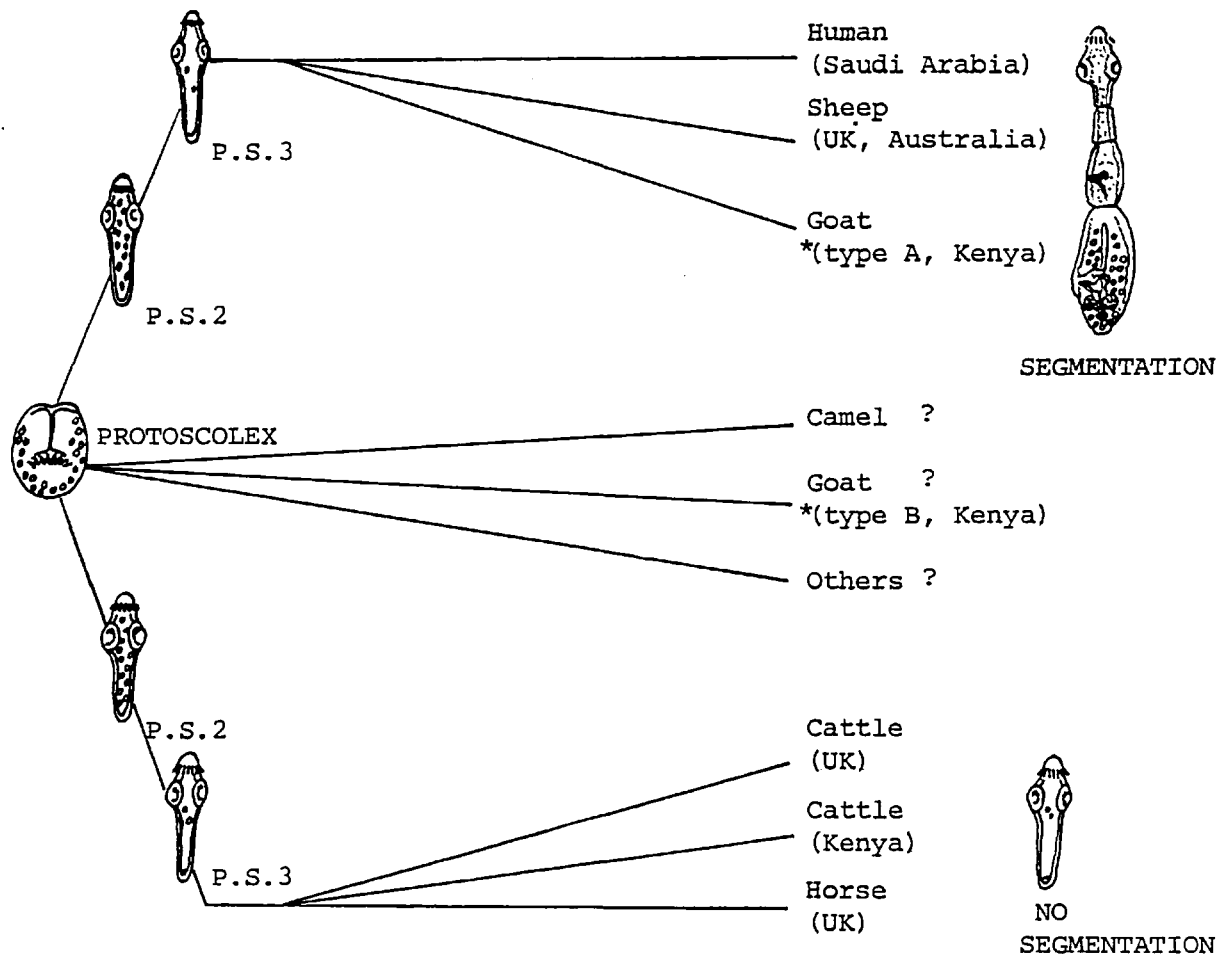


Plate 24. Eight day old cattle culture showing P.S.3 stage of development. No further development was seen even after 37 days in culture (x211).

rather like the horse and cattle strains found in the UK (Figure 11).

The growth and segmentation period for the goat material was slower than that reported for even the slow developing sheep strain cultures (Smyth & Davies 1974b). However, as Smyth & Davies (1974b) point out, considerable variation in development times occurs between cultures, and even within the same culture. Thus, this preliminary result should be repeated before any significance can be attached to this observation.

Figure 11. In vitro pattern of differentiation of the larval stage of E. granulosus from various intermediate hosts



* Type A and B refer to the two goat forms found during isoelectric focusing studies (Chapter 7).

The results obtained here, support the isoelectric focusing

results recorded in chapter 7. These showed that the human/sheep and goat (type A) isoenzyme patterns for GPI and PGM were identical to each other, but were slightly different to the cattle form. The human material cultured in vitro from the strobilar stage by Smyth et al, (1980) also had identical GPI and PGM isoenzyme patterns to that of the sheep strain. Material from these three hosts therefore, appear to be very similar to each other, in their isoenzyme patterns for GPI and PGM, their growth characteristics in vitro culture and their morphological features (Chapter 6). Cattle material differs slightly from these three forms in its GPI isoenzyme patterns and also in its behaviour during in vitro culture. Cattle hydatid cysts in Kenya also differ from human, sheep and goat cysts in that they have a very low fertility rate (Chapter 2).

Unfortunately, nothing is yet known regarding the growth characteristics of camel or goat (type B) material. Although they differ isoenzymatically (GPI and PGM) from the human, sheep and goat (type A) forms, this does not mean that they will fail to grow and segment in vitro.

Further studies are now urgently required to investigate the growth characteristics of camel material in vitro, especially as camels are particularly important intermediate hosts in many regions of the world where human hydatidosis is also a serious problem. Additional in vitro culture studies are also required with the Kenyan human, sheep and goat material to both confirm the preliminary results reported here and to examine the growth characteristics of all these forms.

8.4 Summary

1. The results of numerous attempts to culture in vitro, hydatid material from the many different hosts of E. granulosus found in Kenya, from the protoscolex to the stobilate adult stage are presented.

2. Only one goat and one cattle culture were maintained for longer than 16 days. The goat material was found to segment after 42 days in culture, whereas the cattle material grew a little initially and then stopped and no further growth was observed even after 37 days in culture.

3. The results of the above observations are discussed in relation to in vitro culture work with E. granulosus by others, and the need for further research in this field is stressed.

GENERAL DISCUSSION

The previous eight chapters have dealt with studies on the epidemiology and strain differentiation of E. granulosus in Turkana, Masailand and to a lesser extent Marsabit District in Kenya. The results from each chapter have been fully discussed and it is intended in this general discussion to examine only the overall findings of the study and to relate them to the work published by previous researchers in this field. The areas in which further research is required are also presented.

Numerous hypotheses have been proposed to explain the extraordinarily high incidence of hydatidosis reported in the Turkana tribe. One of these considered in the present work, is the suggestion that the parasite in Turkana had evolved into a strain with an unusually high infectivity in man. It is known that various strains of E. granulosus are host specific; for example there is strong evidence that the horse strain in the UK may not be infective to sheep (Hatch & Smyth, 1975). In Russia, Zen'kov (1971) found that E. granulosus of pig origin was not infective to sheep, but that from sheep was sometimes infective to pigs.

From the present morphological and isoenzyme studies, no differences have been found between human and goat (type A) material examined from Turkana and that of the human, sheep or goat (type A) forms in Masailand. The results from the in vitro culture studies indicate that goat (type A) material has similar growth characteristics to that of human (Saudi Arabia) and sheep (UK and Australia) strains. The human (Saudi Arabia) and sheep (UK) strains had identical GPI and PGM isoenzyme profiles (Smyth et al, 1980), which have also been found to be the same as the human and sheep

forms found in Kenya (this study). Further supporting evidence that human and sheep material is identical comes from the biochemical studies by McManus (1981). He found that the biochemical compositions of larvae from sheep (Kenya) and sheep (UK) were identical and those from sheep (Masai) and human (Turkana) were similar. McManus (1981) also found that material from human (Turkana) and sheep (Masai) were metabolically very similar. All this evidence suggests that sheep and human material is very similar. This is further supported by epidemiological evidence, which has shown that, in countries with a sheep/dog cycle, the strain of E. granulosus involved is infective to man (Smyth, 1979).

The present isoenzyme, morphological and in vitro culture studies, suggest that in Kenya at least, that goat (type A) material is identical to the human and sheep material. McManus (1981) found that the goat material he examined from Masailand was different in its biochemical composition and in its metabolism to human and sheep material. However, it is possible that the goat material he examined contained a mixture of the two types (A & B) of goat material, which may explain the differences found. This work should now be repeated to examine whether the two goat types (A & B) evident from their distinct GPI and PGM isoenzyme patterns are also found using biochemical analysis.

The results obtained for the camel and goat (type B) material indicate that they differ from human, sheep and goat (type A) material in their GPI and PGM isoenzyme patterns. Material from cattle (Masai) is very similar isoenzymatically to the human, sheep and goat (type A) material, but differs isoenzymatically from camel and goat (type B) material. However, protoscoleces of cattle origin appear to fall into the same developmental pattern as protoscoleces of UK cattle and horse origin. Cattle

material also differs from the human and sheep material biochemically (McManus, 1981). Larval material of camel and goat (type B) appears to be very similar isoenzymatically, but differs from that of human, cattle, sheep and goat (type A). Camel material also differs from human, sheep, cattle and goat material in its biochemical composition and in its metabolism (McManus, 1981). Unfortunately, the growth pattern in vitro of material from camels has not yet been elucidated.

There is now a need for detailed information on the cross-infectivity of E. granulosus of different host origins in Kenya and especially of their potential infectivity to man. Dailey & Sweatman (1965) found that E. granulosus of camel/dog and cattle/dog origin was infective to sheep, camels and long-tailed macaque monkeys (Macaca irus) but not to donkeys. These authors concluded that in Lebanon and Syria the camel and cattle forms were identical to each other and resembled E. granulosus granulosus, whereas material from donkeys was closer to E. g. equinus, the horse strain of the parasite. Unfortunately, although numerous donkeys are kept by the Turkana and Masai, nothing is known about their role in the disease pattern in the two areas.

There is little supportive evidence from the present study that there is a unique strain of E. granulosus in Turkana, which is especially infective to man. It is likely, however, that the parasite occurring in sheep and goats is very similar to that found in man. Epidemiological evidence indicates that camels and goats are the principal hosts perpetuating the domestic cycle, with cattle, sheep and also man playing a subsidiary role. Whether or not camel/dog, goat/dog, sheep/dog or cattle/dog material are infective to man, or whether they represent non-

infective 'strains' in this area, is unknown and requires further investigation. It seems likely that, since the goat (type A)/dog and sheep/dog parasites are so similar to the human/dog material, they would all be infective to one another. The cross-infection investigations performed by Dailey & Sweatman (1965) further suggest that camel/dog and cattle/dog material is infective to sheep and non-human primates. Therefore, it is possible that material from all the domestic intermediate hosts in Turkana and Masailand is infective to man. This being the case, then any of the infections found in the naturally infected dogs, from whatever intermediate host source, may be capable of infecting man.

The extremely high incidence of hydatidosis seen in the Turkana and not in the Masai, may therefore be related to other factors and not be due to a particularly virulent strain of the parasite for man in Turkana.

There is no evidence for the existence of a wildlife cycle in Turkana, although a high prevalence of E. granulosus was found in jackals.

The importance of jackals in the epidemiology of E. granulosus in Turkana is unknown. It is very unlikely that they are involved in the perpetuation of the cycle in this District, but they may cause some infections through contaminating water-holes, to which they have easy access. Another source of infection for some of the Turkana would be the consumption of jackal intestines. However, it is improbable that infected jackals are the source of many or indeed any of the infections found in man in Turkana.

In Masailand, there is strong evidence for the existence of a wildlife cycle, but whether or not the parasite found in the wild carnivores is infective to man or his livestock is unknown at

present. Cross-infectivity studies are required to determine this factor. It is unlikely that a wildlife cycle would exist in Marsabit as the game population in this region of Kenya, as in Turkana, is sparse.

Epidemiological studies on hydatid disease, in countries where an unusually high prevalence rate of hydatidosis has been found in a local population, have identified, at least tentatively, certain socio-economic and cultural characteristics which accentuate the risk for human infection. The Maori population in New Zealand, for example, has a higher rate of infection than the rest of the population (Burridge & Schwabe, 1977). In Scandinavia, the reindeer-herding Lapps appear to be the population most affected (Huldt et al, 1973), as do the sheep farming Basque population in California (Araujo et al, 1975). Similarly, in Lebanon, Christians have been found to be at greater risk than the Moslem population (Schwabe & Daoud, 1961). These latter authors suggested that the lower incidence of hydatid disease found amongst the Moslems, may be related directly to Moslem beliefs about the uncleanliness of the dog.

Many behavioural differences have been suggested to account for the unusually high prevalence of hydatid disease amongst the Turkana and the low prevalence seen in the Masai (Nelson & Rausch, 1963; Nelson, 1972; Schwabe, 1969; AMREF, 1978, 1979, 1980; Eugster, 1978). However, as most of these have been previously discussed in the relevant chapters they will not be considered in detail here. It has been suggested, that the observed prevalence patterns of the disease in the two areas, can be attributed to the fact that the Turkana keep many more dogs than the Masai and have a very much closer association with them. The Turkana dogs are used as 'dog-nurses' to clean up

after a child vomits or defaecates and are permitted to sleep in the huts at night. Dogs are not allowed in the Masai huts at night. However, most Masai families keep a dog to act as a guard to warn of wild animals or stock thieves. Masai children have a fairly close contact with their dogs, but the elders are fairly strict about avoiding contact with them.

The very much lower prevalence of the disease found in the nomadic pastoralists living on the east side of Lake Turkana may be due to the very much smaller dog population there rather than to any behavioural differences.

Occupational associations have also been suggested to account for increased levels of infection found amongst particular groups. For example, in Lebanon an unexpectedly high prevalence was found amongst shoemakers, who at one time used dog faeces for tanning leather (Schwabe & Daoud, 1961).

In Turkana, the use of dog faeces for dressing cuts and septic sores, the use of lubricants which may contain dog faeces, to prevent the large numbers of necklaces which are worn by the women from rubbing their necks and the clearing of faecal matter from the manyatta by the women, have all been suggested to be occupational hazards which may contribute to a higher prevalence of the disease amongst these groups (AMREF, 1978, 1979, 1980).

Another suggestion for the high prevalence of the disease amongst the Turkana has come from an unusual serological finding. Serological tests on surgically confirmed Turkana patients, have revealed an abnormally high number of false negative results, when using the fairly accurate test of immunoelectrophoresis (Chemtai, personal communication). These results were recently confirmed by three independent studies on the same positive serum (AMREF, 1979).

The explanation for these false negative results is still being sought. One suggestion is that some of the Turkana fail to mount an immune response against the parasite. This theory is also supported by the rarity of anaphylactic shock following rupture of cysts in patients undergoing surgery. Whatever the cause of these false negative results, further work on this aspect must be carried out in the hope that a reliable technique may be perfected which could help with epidemiological studies on the distribution and prevalence of the disease.

The reasons for the higher human incidence of hydatidosis in northern Turkana compared with the south of the District, is unknown and requires further investigation, particularly since a high prevalence of the disease in dogs from both areas has been recorded. A recent study has suggested that the number of dogs per Manyatta in the north is greater than the number per manyatta in the south (Patton, personal communication).

The extremely high prevalence of E. granulosus found in the domestic dogs in many areas throughout Turkana District and the previously reported high prevalence in Masai dogs (Nelson & Rausch; 1963; Eugster, 1978) gives a reliable indication of the relative degree of risk of infection to man and his domestic animals in these two regions. The observed low prevalence found in the cattle, sheep and goats in Turkana, is therefore rather surprising, particularly as a much higher prevalence was found in these animals in Masailand where the dogs appear to have a lower prevalence of the disease. It is not understood why this was found and further investigation is necessary. In particular, studies are needed to examine the extent of environmental contamination with Echinococcus

eggs and the survival and availability of such eggs to animals under the climatic and pastoral conditions of Turkana and Masailand. Such studies on natural contamination of the environment are hindered at present by the inability to differentiate Echinococcus eggs from those of other Taeniid species. Recently, however, Smyth (1979) devised a tentative 'physiological key' which may be used for the identification of the eggs of Echinococcus with some degree of certainty. The technique involves hatching and culturing the 'unknown' eggs in vitro; whilst this would probably prove an impossible task in Turkana, it may be possible to perform the work in a laboratory elsewhere. Other investigations are required to examine the methods of egg dispersal. At present, little is known about the dispersion of eggs in the environment. Obviously, the movements and defaecation habits of the definitive host determine the primary site of egg deposition but recent evidence suggests that considerable secondary dispersion occurs almost immediately afterwards. Gemmell & Johnstone (1977) found that the eggs of Taenia hydatigena spread upto 80 m within 10 days. There is yet other evidence which suggests that small numbers of eggs may be dispersed much further (Gemmell, 1978b). Although various possible agents have been suggested, the mechanisms responsible for disseminating the eggs are still uncertain. It is at present assumed that animals are involved and birds (Silverman & Griffiths, 1955), flies (Heinz & Brauns, 1955) and beetles (Bily et al, 1978) are all known disseminators of eggs. Other mechanisms, such as dust born or wind dispersal are now thought to be unlikely methods of dissemination, as E. granulosus eggs have been shown to be very susceptible to dessication (Laws, 1968). This latter factor would be particularly pertinent in the hot,

dry Turkana environment.

It is very unlikely that E. granulosus eggs would survive very long in Turkana as ground temperatures are often as high as 60°C, and the relative humidity (RH) drops from between 60-70% at 6.00 am to 30-40% by mid afternoon. In contrast, Masailand temperatures are much lower and the relative humidity is generally a lot higher being around 90% in the early morning and falling to about 40% by 3.00 pm (National Atlas of Kenya, 1970).

Meymerian & Schwabe (1962) were unable to activate eggs of E. granulosus after exposure to moist heat at 60°C for 10 minutes and Nosik (1952), observed that exposure of E. granulosus eggs to 50°C for 1 hour rendered them uninfected to cattle. Laws (1968) showed that T. pisiformis, T. ovis and E. granulosus eggs were unusually susceptible to desiccation, which he considered would dominate all other natural restrictions on the survival of Taeniid eggs in nature. He showed that eggs of E. granulosus perished after being maintained at an RH of 50% at 25°C for 24 hours. However, the eggs of T. hydatigena maintained under similar conditions were still viable after five days.

It would appear therefore, that E. granulosus eggs in the Turkana environment would not survive very long and ingestion of freshly deposited eggs by the intermediate host would be an important factor in the maintenance of the cycle. Hence, infections in Turkana are unlikely to be wind borne, or obtained from such foods as wild berries, nuts, roots, wild plant leaves or the dried milk, edodo, eaten by the Turkana. The high prevalence of the disease is likely to be due primarily to the close contact between the Turkana and their dogs in the manyatta. This contact is probably much more common for the females who spend most of the time at the

manyattas, than for the males who are usually out in the field during the day.

The suspected poor survival of the E. granulosus eggs in the Turkana environment, compared with their survival in the Masai environment, may provide some indication for the low prevalence of the parasite seen in sheep, goats and cattle in Turkana and the much higher prevalence of the disease amongst the Masai livestock. The observed higher prevalence in Turkana camels, may be due to the more advanced age at which camels are slaughtered compared to that of the other livestock. In other countries, the prevalence of the disease in camels has been shown to increase with age (Mobedi et al, 1970; Afshar et al, 1971; Hamdy et al, 1980). The increase in prevalence of infection is thought to be related to the opportunity for infection, rather than any increased susceptibility dependent on age.

Taenia hydatigena was found to be very prevalent in the dogs examined in Turkana and the larval stage Cysticercus tenuicollis has been reported in approximately 60% of goats examined (AMREF, 1980). Goats must therefore come into frequent contact with viable T. hydatigena eggs. The greater survival of T. hydatigena eggs vis à vis E. granulosus eggs reported by Laws (1968), may account for the higher Cysticercus tenuicollis prevalence found in the goats. A similar situation to the one found in Turkana has been reported in Australia (Gemmell, 1958). This author discovered that the incidence of T. hydatigena in sheep did not vary between different climatic regions, but interestingly, the incidence of E. granulosus in these animals was markedly lower in the hot dry districts compared with the cooler, wetter districts. He concluded that the limiting factor for E. granulosus survival was high temperatures but that this

did not appear to affect the prevalence of T. hydatigena.

Other suggested sources of infection (AMREF, 1978, 1979, 1980), are infected water-holes. These may be an important source of infection especially for the Turkana. Free flowing rivers are more numerous in Masailand and southern Turkana than in northern Turkana. In northern Turkana water is obtained for most of the year from water-holes dug in dry river beds (Plate 25). The Turkana and their animals are dependent on these water-holes which are also used by dogs and jackals. The conditions for the survival of Echinococcus eggs in these water-holes are probably much more favourable than in the surrounding soil. Gemmell (1958) found that E. granulosus eggs kept in water, were still viable after storage at 70°F (21°C) for 10 weeks but were not viable after 10 days when stored under similar conditions at 90°F (32°C). The temperature of two water-holes measured in Kakuma on a single day per month over a three month period varied from 21°C in the early morning to 28°C by mid afternoon.

Recently, water samples examined from water-holes from many areas in Turkana have shown that such water-holes are contaminated with large numbers of Taeniid eggs (Stevenson, personal communication). The identification of these eggs has so far not been attempted.

There is an urgent need for a control programme to be introduced in Turkana, but a greater understanding of the mode of transmission of the parasite is required first. Control programmes in other regions of the world such as those in Cyprus, Tasmania, Iceland, New Zealand and the Falkland Islands, have shown that reduction of transmission or even total eradication of the parasite is possible. However, effective control measures are expensive and funds are generally limited. It is essential therefore, to



Plate 25. Turkana women collecting water from a water-hole dug in a dry river bed near Nanam. Note presence of dog.



Plate 26. Turkana women holding their dogs shortly before they dosed them with droncit (R).

establish priorities before any control programme is initiated, so that the strategies selected are cost effective.

In Turkana, with the absence of a wildlife cycle, the problem of controlling the disease is much simplified and initial efforts for control should be aimed at the dog-livestock cycle. This could involve the destruction of the numerous feral and stray dogs and also the many surplus dogs kept by the Turkana. During this study it was obvious that the Turkana kept many more dogs than they required and were quite willing to part with some of them for a small fee. This fee could probably be dispensed with by gaining the collaboration of the local chief in the control programme. Indeed any control effort is only likely to succeed if the people are properly informed and consulted, prior to any programme being initiated. Those dogs that the Turkana want to keep should be dosed with an effective taenicide such as droncit (R). Since reinfestation can occur almost immediately, the treatment should be repeated at not less than six week intervals.

A small survey was undertaken around Lokitaung to assess the response of the people to having their dogs treated with a taenicide. The people were first informed about the disease and how they could contract it from their dogs; this was done through a Turkana interpreter. They were then asked if they would like their dogs to be treated to remove the parasite. Positive responses were always obtained. Tablets of droncit (R) were then enclosed in small pieces of meat and handed to the Turkana, who then fed them to their dogs (Plate 26). It is my opinion therefore, that with careful collaboration and 'involvement' of the people in dosing their dogs, such a programme would be both successful and acceptable to the people.

Other measures should also be implemented, for example, the banning of dogs from the area immediately surrounding the main 'abattoir' and the disposal of infected offal down deep pits constructed at the site. All these measures are aimed at the control of the parasite in its definitive host, for if this was possible it would result in a decrease in the incidence of hydatidosis in the domestic livestock and also hopefully in man.

A decrease in the incidence of hydatidosis in man and his domestic livestock would also decrease the incidence of echinococcosis in the dogs and jackals.

Control programmes in other countries have shown that substantial reductions in the prevalence of echinococcosis in man and his domestic animals can occur within relatively short periods of time (Reviewed by Gemmell, 1978a, 1979). There is no reason to suppose that under skilful supervision, a similar reduction in the disease should not occur with the implementation of a suitable control programme in Turkana.

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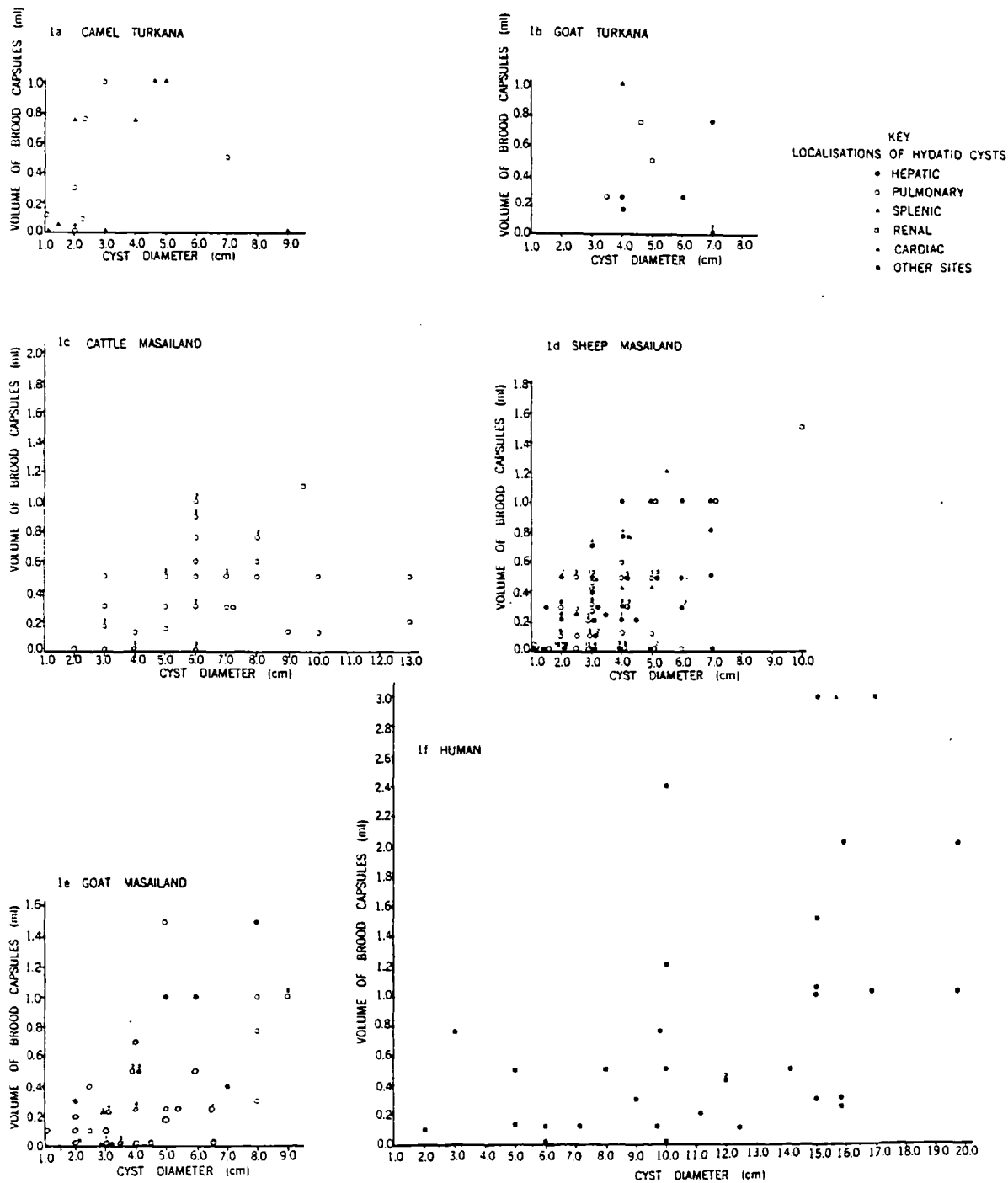
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APPENDIX I
FIGURE 1

VOLUME OF BROOD CAPSULES OBTAINED FROM HYDATID CYSTS OF VARYING SIZES AND DIFFERENT HOSTS



APPENDIX 1

Table 1. Occurrence and Prevalence of Hydatidosis in Camels

<u>COUNTRY</u>	<u>REFERENCE</u>	<u>PERIOD</u>	<u>OCCURRENCE OR PREVALENCE</u>
<u>Africa</u>			
CHAD	Graber <i>et al</i> , 1969	1954-1968	59 (37.3%) of 158 camels infected
DJIBOUTI	FAO-WHO-OIE, 1967	1967	Reported in camels
ETHIOPIA	FAO-WHO-OIE, 1979	1979	Reported in camels
EGYPT	El-Kordy, 1946	1946	31.0% of 200 camels infected
	El-Garhy & Selim, 1958	1955-1956	7.3% of camels infected
	Cahill <i>et al</i> , 1965	1963	7(18.4%) of 31 camel sera positive
	Moch <i>et al</i> , 1974	1973	94 (32.6%) of 288 camels infected
	Sedik <i>et al</i> , 1977	1976	1.25% of 2000 camels infected
	Hamdy <i>et al</i> , 1980	1977-1978	144 (7.95%) of 1811 camels infected
KENYA	Leese, 1915	1914	Reported in camels
LIBYA	FAO-WHO-OIE, 1967	1967	Widespread in camels
MAURITANIA	FAO-WHO-OIE, 1967	1967	Widespread in camels
MOROCCO	Faure, 1949	1949	44% of camels infected
	Senevet, 1951	1951	6-19% of camels infected
NIGERIA	Dada, 1977	1971-1975	41 (0.01%) of 31,782 camels infected
	Dada & Belino, 1979	1979	1460 (57.5%) of 2539 camels infected
	Dada <i>et al</i> , 1979b	1977-1979	22 (70.9%) of 31 camels infected
	Dada <i>et al</i> , 1980	1977-1978	1987 (55.5%) of 3580 camels infected
SOMALIA	FAO-WHO-OIE, 1979	1979	Moderate incidence in camels
SUDAN	Steward, 1950	1950	Infection present in most camels examined
	Malek, 1959	1959	9 (52.9%) of 17 camels infected
	El-Badawi <i>et al</i> , 1979	1974-1975	35.3% of 17 camels infected
TUNISIA	Dévé, 1923	1923	30% of camels infected
	Cousi, 1951	1951	30% of camels infected
	Ben Osman, 1965	1965	45% of camels infected
WESTERN SAHARA	FAO-WHO-OIE, 1979	1979	Moderate incidence in camels
<u>Asia</u>			
AFGHANISTAN	FAO-WHO-OIE, 1979	1979	High incidence in camels
INDIA	Gupta, 1979	1978	Single case in a camel reported
IRAN	Alavi & Maghami, 1964	1963	6 (10.4%) of 58 camels infected
	Mobedi <i>et al</i> , 1970	1968-1969	612 (64.0%) of 955 camels infected
	Afshar <i>et al</i> , 1971	1970	15 (42.8%) of 35 camels infected
	Motakef <i>et al</i> , 1976	1969-1974	378 (11.0%) of 3380 cattle and camels infected
IRAQ	Senekjie & Beattie, 1940	1940	Single case in a camel reported
	Imari, 1962	1959	75% of 68 camels infected
	Babero <i>et al</i> , 1963	1963	27 (49.1%) of 55 camels infected
	Altaif, 1974	1971-1972	14 (56.0%) of 25 camels infected
	Al-Abbassy <i>et al</i> , 1980	1975-1976	31 (20.4%) of 152 camels infected
JORDAN	FAO-WHO-OIE, 1979	1979	Moderate incidence in camels
*KUWAIT	Hassounah & Behbehani, 1976	1975	15 (22.0%) of 68 camels infected
LEBANON	Pipkin <i>et al</i> , 1951	1948	67.4% of 34 camels infected
SYRIA	Turner <i>et al</i> , 1936	1934-1935	100% of 15 camels infected
	Dailey & Sweatman, 1965	1965	31 (100%) of 31 camels infected
SAUDI ARABIA	FAO-WHO-OIE, 1967	1967	Widespread in camels
YEMEN ARAB REPUBLIC	FAO-WHO-OIE, 1979	1979	Reported in camels

*Camels imported from Iraq 35.4% of 31 infected, and Somalia 10.8% of 37 infected

APPENDIX 1

Table 2. Occurrence and prevalence of hydatidosis in domestic livestock in Africa

COUNTRY	REFERENCE	PERIOD	CATTLE		SHEEP		GOATS		OTHER INTERMEDIATE HOSTS REPORTED
			No SLAUGHTERED	% INFECTED	No SLAUGHTERED	% INFECTED	No SLAUGHTERED	% INFECTED	
ALGERIA	Jore d'Arces, 1953	1953	-	30.5	-	21.8			
ANGOLA	FAO-WHO-OIE, 1979	1979		++		+			
BOTSWANA	FAO-WHO-OIE, 1979	1979		+		+			
BURUNDI	FAO-WHO-OIE, 1979	1979		(+)		+			
CAPE VERDE ISLANDS	FAO-WHO-OIE, 1979	1979		(+)		(+)			(+)
CENTRAL AFRICA	Graber <i>et al.</i> , 1969	1954-1968	7,465	1.08	5,533	0.19			
CONGO, P.R.	FAO-WHO-OIE, 1979	1979		++		(-)			
EGYPT	El-Kordy, 1946	1946	200	10.0	200	1.5			Buffaloes, pigs
	Abdou, 1965	1960-1964		10.0		5.0			
	Sedik <i>et al.</i> , 1977		4,000	0.1	5,000	0.2			Buffaloes, pigs
	Hamdy <i>et al.</i> , 1980	1977-1978	1,114	0.27	2,200	0.27			
EQUATORIAL GUINEA	FAO-WHO-OIE, 1979	1979		+-					
ETHIOPIA	Graber <i>et al.</i> , 1978	1976		R					
GUINEA (BISSAU)	FAO-WHO-OIE, 1979	1979		+					Pigs
GUINEA	FAO-WHO-OIE, 1979	1979		++		++			
IVORY COAST	Mishra & N'Depo, 1978	1976-1978	1,520	0.13-2.3					
LESOTHO	FAO-WHO-OIE, 1979	1979		+		+			
LIBYA	Dar & Taguri, 1979	1979		R		R			R
MALAWI	FAO-WHO-OIE, 1979	1979		(+)					
	Senevet, 1951	1951		43.0		2.3-80.0			
	Vaysse, 1955	1955		40.0		20.0			
	Pandey, 1980	1977-1978							Donkeys
MOZAMBIQUE	De Castro Amaro, 1960	1960	>400,000	1-4.5	90,000	0-6.5			Pigs
	Buck & Courdurier, 1962			+		+			
NAMIBIA	FAO-WHO-OIE, 1979	1979		(+)		(+)			(+)
NIGERIA	Dada, 1977	1971-1975	1,439,437	0.009	305,984	0.008	821,625	0.003	
	Dada & Bellino, 1979	1979	3,555	16.0	1,177	20.9	1,195	22.8	
	Dada <i>et al.</i> , 1979b	1977-1979	2,085	1.5	310	7.1	190	18.4	Pigs
	Dada <i>et al.</i> , 1980	1977-1978	4,844	14.7	1,800	11.4	1,260	26.5	
	Fabiyi, 1979	1972-1973	-	-	-	-	-	-	
NIGER	FAO-WHO-OIE, 1979	1979		++		+			
RWANDA	FAO-WHO-OIE, 1979	1979		(+)		+			
SIERRA LEONE	FAO-WHO-OIE, 1979	1979		+		++			++
SOMALIA	FAO-WHO-OIE, 1979	1979		++		+			
SOUTH AFRICA	Verster, 1962	1945-1960	4,886,441	0.7	12,936,392	1.8			Pigs
	Verster & Collins, 1966	1963-1965	1,706,420	1.1	5,571,224*	0.9			Pigs
SUDAN	Eisa <i>et al.</i> , 1962	1962	87	25.0	31	19.4	6	33.3	
	El-Badawi <i>et al.</i> , 1979	1979	770	4.3	1,898	8.1	346	3.2	
SWAZILAND	FAO-WHO-OIE, 1979	1979		+++		++			
TANZANIA	Masaba <i>et al.</i> , 1977	1975	12,408	4.3					
TUNISIA	Senevet, 1951	1951		to100.0		20-60.0			
	Menchari, 1965	1965		8.0-86.0		7.0			Pigs
UGANDA	Mitchell, 1968	1968		6.6					
	Owor & Bitakaramire, 1975	1967-1972	11,319	13.0					
UPPER VOLTA	FAO-WHO-OIE, 1979	1979		+		+			
ZAIRE	FAO-WHO-OIE, 1979	1979		++		+			Pigs ++
ZAMBIA	FAO-WHO-OIE, 1979	1979		+		(+)			
ZIMBABWE	Chambers, 1978	1978		R					

* includes goats (+) exceptional occurrence + low sporadic occurrence ++ moderate occurrence
 +++ high occurrence +- disease exists Syl Infection in wild animals R disease reported

APPENDIX 1

Table 3. Occurrence and Prevalence of Hydatidosis in Domestic Livestock in Kenya

REFERENCE	PERIOD	ABATTOIR	ANIMAL ORIGIN	CATTLE		SHEEP		GOATS		OTHER INTERMEDIATE HOSTS REPORTED
				No. SLAUGHTERED	% INFECTED	No. SLAUGHTERED	% INFECTED	No. SLAUGHTERED	% INFECTED	
Leese, 1915	1915	-	-	-	-	-	-	-	-	Camels
Walker, 1925	1924	-	-	-	-	Rare	-	-	-	
Daubney, 1926	1925	-	-	Present	-	Present	-	-	-	
Hudson, 1934	1934	KABETE	-	Found Occasionally	-	Found Occasionally	-	-	-	Pigs
Ginsberg, 1956	1954	KMC	African stock	-	46.7	-	41.9	-	18.0	
	1954	KMC	European stock	-	17.1	-	17.1	-	-	
	1955	KMC	African stock	-	41.1	-	53.0	-	15.2	
	1955	KMC	European stock	-	17.6	-	28.4	-	-	
Froyd, 1960a	1958	KMC	Whole country	1,000	25.5	-	-	-	-	
Froyd, 1960b	1959	KMC	Whole country	1,000	30.2	-	-	-	-	
Vet. Serv. Report, 1962	1961	-	Northern Kenya	-	-	-	-	-	-	Camels
Nelson & Rausch, 1963	1961	-	Turkana	-	Common	-	Common	-	Common	Camels
Vet. Serv. Report, 1967	1966	-	Turkana	-	-	-	-	-	Widespread	
Mango, 1971	1970-1971	-	Rift Valley	1,766	29.2	1,834	32.3	172	33.1	
			Central	215	23.7	-	-	-	-	
			Eastern	282	17.7	406	9.1	777	11.1	
			Coast	31	16.1	-	-	-	-	
			North Eastern	622	16.8	-	-	279	4.3	
Ng'ang'a, 1974	1961-1970	KMC	Whole country	1,162,237	12.8*	489,702	20.3-37.2 ^x	245,231	14.7-23.6 ^x	Pigs (U.B.F.)
Vet. Serv. Report, 1972	1971	KMC	Whole country	56,975	28.0	-	-	-	-	
Vet. Serv. Report, 1973	1972	-	Whole country	44,895	13.3	-	-	-	-	
Eugster, 1978	1973-1975	KMC	Kajiado	1,446	46.7	44	29.5	100	9.0	
		ONGATA RONGAI								
Vet. Serv. Report*, 1978	1977	KMC	Whole country	51,140	6.1	-	-	-	-	
Vet. Serv. Report*, 1980	1978	LOCAL SLAUGHTERHOUSES	Whole country	154,341	1.3	-	-	-	-	-
		ONGATA RONGAI ^{ox}	Mainly Kajiado	21,283	6.2-11.5	12,423	7.0-13.1	26,388	4.5-7.9	
		KMC	Whole country	50,809	7.4	-	-	1,874	1.12	
		KMC MOMBASA	Whole country	12,859	2.6	-	-	-	-	
		LOCAL SLAUGHTERHOUSES	Whole country	199,563	4.4	208,191 ⁺	1.9	-	-	
ONGATA RONGAI ^{ox}	Mainly Kajiado	22,441	8.7-16.6	16,403	8.4-14.4	36,090	5.0-9.1			

U.B.F. Uplands Bacon Factory * liver condemnations only + sheep and goats

^x based on liver and lung condemnations only, with the lower figure showing liver condemnations only

^o these figures are included in the Local slaughterhouse data supplied

APPENDIX 1

Table 4. Location and condition of hydatid cysts in Masai cattle, sheep and goats

HOST C = CATTLE G = GOAT S = SHEEP	ANIMALS POSITIVE	% OF TOTAL ANIMALS POSITIVE	FERTILE	% OF CYSTS FERTILE	AV. % VIABILITY OF FERTILE CYSTS	STERILE	% OF CYSTS STERILE	CALCIFIED	% OF CYSTS CALCIFIED	TOTAL NO. OF CYSTS EXAMINED	AVERAGE NO. OF CYSTS PER ANIMAL	
LIVER ONLY	C	19	15.1	2	2.6	58	40	52.6	34	44.7	76	4.0
	G	5	8.9	1	16.7	90	4	66.7	1	16.7	6	1.2
	S	16	28.1	24	46.2	76	19	48.1	9	5.8	52	3.3
LUNGS ONLY	C	90	71.0	42	14.5	72	236	81.7	11	3.8	289	3.2
	G	41	73.2	34	41.5	81	43	52.4	5	6.1	82	2.0
	S	8	14.0	14	77.8	83	2	11.1	2	11.1	18	2.3
SPLEEN ONLY	C	1	0.8				1			1	1.0	
HEART ONLY	C	1	0.8	1	-	52				1	1.0	
LIVER & LUNGS	C	13	10.3	21	18.1	70	77	66.4	18	15.5	116	8.9
	G	8	14.3	25	58.1	87	16	37.2	6	3.8	43	5.4
	S	30	52.6	224	72.0	89	69	22.2	18	5.8	311	10.4
LIVER & LUNGS & SPLEEN	C	2	1.6				47	45.2	57	54.8	104	52.0
	G	1	1.8	15	75.0	85	5	20.0			20	20.0
	S	1	1.8	105	100.0	96					105	105.0
LIVER & SPLEEN	S	1	1.8	4	80.0	82			1	20.0	5	5.0
LIVER & LUNGS & KIDNEY	S	1	1.8	3	60.0				2	40.0	5	5.0
LIVER & LUNGS & SPLEEN & KIDNEY	G	1	1.8	2	33.3	98	3	50.0	1	16.7	6	6.0
TOTAL	C	126	100.0	66	11.2	63	401	68.3	120	20.4	587	4.7
	G	56	100.0	77	47.8	86	71	44.1	13	8.1	161	2.9
	S	57	100.0	374	75.4	85	90	18.1	32	6.5	496	8.7

APPENDIX 1

Table 5. Analysis of liver and lung hydatid cysts found in cattle from Masailand: February to November 1979 and April to September 1980

INFECTION IN LIVER AND LUNG LOCALIZATIONS		LIVER AND LUNGS OF SAME ANIMAL								ONLY LIVER				ONLY LUNGS				TOTAL LIVER				TOTAL LUNGS											
		LIVER				LUNGS				LIVER		LUNGS		LIVER		LUNGS		LIVER		LUNGS													
		LIVER	F	AV. % VIA	S	C	LUNGS	F	AV. % VIA	S	C	LIVER	F	AV. % VIA	S	C	LUNGS	F	AV. % VIA	S	C	LIVER	F	AV. % VIA	S	C	LUNGS	F	AV. % VIA	S	C		
SIZE OF CYST	Small < 2.0 cm	19			4	15	38			36	2			36		2	34	29			23	6	55			6	49	67			59	8	
	Medium 2-6 cm	1			1		5			4	1			38	2	58	36			217	27	69	185	5	39	2	58	37	222	27	69	189	6
	Large 7-10 cm						15	5		10				2			2			38	13	80	25		2			2	53	18	80	35	
	Extra Large > 10 cm																			5	2	74	3					5	2	74	3		
	Total	20			5	15	58	5		50	3			76	2	58	40	34	289	42	72	236	11	96	2	58	45	49	347	47	72	286	14
NUMBER OF CYSTS PER ANIMAL	1	6				6								8					41					14				47					
	2-4	6				5								7					34					13				39					
	5-10													2					11					2				11					
	10	1				2								2					4					3				6					
GROWTH OF CYST	Unilocular	20				58								76					288					96				346					
	Multilocular																																
	Lobulated																		1								1						

APPENDIX 1

Table 6. Analysis of all liver and lung hydatid cysts found in sheep from Masailand: February to November, 1979 and April to September, 1980

INFECTION IN LIVER AND LUNG LOCALISATIONS		LIVER AND LUNGS OF SAME ANIMAL										ONLY LIVER					ONLY LUNGS					TOTAL LIVER					TOTAL LUNGS				
		LIVER					LUNGS																								
		LIVER	F	AV. % VIA	S	C	LUNGS	F	AV. % VIA	S	C	LIVER	F	AV. % VIA	S	C	LUNGS	F	AV. % VIA	S	C	LIVER	F	AV. % VIA	S	C	LUNGS	F	AV. % VIA	S	C
SIZE OF CYST	Small < 2.0 cm	26	2	81	12	12	33	7	80	24	2	15			6	9	5	1		2	2	41	2	81	18	21	38	8	80	26	4
	Medium 2-6 cm	120	107	91	12	1	127	105	88	17	5	35	23	77	12		13	13	83			155	130	84	24	1	140	118	86	17	5
	Large 7-10 cm	3	3	79			4	3	85	1		2	1	66	1							5	4	72	1		4	3	85	1	
	Extra Large > 10 cm																														
	Total	149	112	91	24	13	164	115	88	42	7	52	24	76	19	9	18	14	83	2	2	201	136	84	43	22	182	129	86	44	9
NUMBER OF CYSTS PER ANIMAL	1	5				4					6					5					11					9					
	2-4	10				12					5					2					15					14					
	5-10	12				9					5					1					17					10					
	10	3				5															3					5					
GROWTH OF CYST	Unilocular	131				84					42					18					173					102					
	Multilocular					19																				19					
	Lobulated	18				61					10										28					61					

APPENDIX 1

Table 7. Analysis of all liver and lung hydatid cysts found in goats from Masailand: February to November, 1979 and April to September, 1980

INFECTION IN LIVER AND LUNG LOCALISATIONS		LIVER AND LUNGS OF SAME ANIMAL												ONLY LIVER				ONLY LUNGS				TOTAL LIVER					TOTAL LUNGS				
		LIVER						LUNGS																							
		LIVER	F	AV. % VIA	S	C	LUNGS	F	AV. % VIA	S	C	LIVER	F	AV. % VIA	S	C	LUNGS	F	AV. % VIA	S	C	LIVER	F	AV. % VIA	S	C	LUNGS	F	AV. % VIA	S	C
SIZE OF CYST	Small < 2.0 cm	10	1	45	3	6	1			1	1				1	14	1	98	9	4	11	1	45	3	7	15	1	98	10	4	
	Medium 2-6 cm	17	12	65	5		11	8	87	3	3			3	62	28	81	33	1		20	12	65	8		73	36	84	36	1	
	Large 7-10 cm	1	1	99			2	2	91		1	1	90			6	5	94	1		3	2	95	1		8	7	92	1		
	Extra Large > 10 cm										1			1																	
	Total	28	14	70	8	6	14	10	89	4	6	1	90	4	1	82	34	81	43	5	34	15	68	12	7	96	44	86	47	5	
NUMBER OF CYSTS PER ANIMAL	1	3				5				4					22					7					27						
	2-4	3				2				1					17					4					19						
	5-10	2				1									2					2					3						
	10																														
GROWTH OF CYST	Unilocular	28				13				5					78					33					91						
	Multilocular					1									2										3						
	Lobulated									1					2						1				2						

Table 1. Reported human hydatid cases in Africa

<u>COUNTRY</u>	<u>REFERENCE</u>	<u>PERIOD</u>	<u>CASES</u>
ALGERIA	Paniglionne, 1965a	1965	5.1-6.1 per 100,000 p.a.
	Cherid & Nosny, 1972	-	500 cases
	Bazyak, 1973	1968-1970	142 Pulmonary cases
	Mentouri <u>et al</u> , 1974	1963-1973	349 Hepatic cases
	Abada <u>et al</u> , 1977	1962-1975	100 Cerebral cases
	Benhamla <u>et al</u> , 1974	1968-1970	2 Hepatic cases
CHAD	Fourre & Imbert, 1965	1963	1 Pulmonary case
	Sirol & Lefevre, 1971	1968-1971	3 Surgical cases
EGYPT	Khalil Bey, 1934	1934	1 Cardiac case
	Bishr, 1947	1946	1 Pulmonary case
	Abdine, 1949	1947	1 Hepatic case
	Botros <u>et al</u> , 1975	1972-1973	1 Pulmonary case
ETHIOPIA	Fuller, 1976	1976	Clinically high in southern Ethiopia
IVORY COAST	Schmidt <u>et al</u> , 1978	1978	3 Pulmonary cases
LIBYA	Fossati, 1970	1959-1968	147 Thoracic cases
	Dar & Taguri, 1978	1971-1976	180 Surgical cases
MADAGASCAR	Brygoo <u>et al</u> , 1971	1971	1 Oral case
MALI	Dembele & Sangare, 1974	1968	1 Pulmonary case
MOROCCO	Chenebault, 1967	-	200 Pulmonary cases
	de Tienda & Wemean, 1973	-	96 Surgical cases
	Galindo <u>et al</u> , 1975	1965-1974	22 Chest cases
	Imani <u>et al</u> , 1977	-	10 Renal cases
NIGERIA	Duncan, 1961	1961	1 Abdominal case
	Alabi & Cruz, 1970	1970	1 Pulmonary case
	Afonja <u>et al</u> , 1972	1971	1 Pulmonary case
SENEGAL	Clerc, 1947	1947	1 Sub-Lingual Case
	Chabal <u>et al</u> , 1971	1965	1 Pulmonary case
	Diop <u>et al</u> , 1973	1971	1 Pulmonary case
SOUTH AFRICA	Schrire, 1938	1937	4 Thoracic cases
	Haqberg, 1954	1954	1 Intrauterine case
	Hewitson, 1962	-	24 Pulmonary cases
	Proctor, 1964	1954-1961	6 Intracranial cases
	Van der Heever <u>et al</u> , 1970	1968-1970	2 Intracranial cases
	Perl <u>et al</u> , 1972	1972	1 Tongue case
	Rubin, 1973	1973	1 Pelvic case
	Blumsohn <u>et al</u> , 1977	1977	1 Multiple case
	Gajjar & Sinclair-Smith, 1978	1961	2 Renal cases
	Kaysner, 1980	1978-1980	19 Diagnosed cases
SUDAN	Eisa <u>et al</u> , 1962	1962	High in Humans in southern Sudan
TANZANIA	Foley, 1944	1943	1 Abdominal case
TUNISIA	Wolcott <u>et al</u> , 1971	1971	37 Pulmonary cases
	Czerucki, 1972	1966-1970	48 Hepatic cases
	Haddad <u>et al</u> , 1976	1961-1976	30 Surgical cases
	Najah & Sawicz Birkowska, 1976	1956-1975	211 Chest cases
	Ben Ismail <u>et al</u> , 1977	-	9 Cardiac cases
	Fourati <u>et al</u> , 1977	1970-1975	125 Hepatic cases
	Fourati <u>et al</u> , 1978	1970-1975	280 Pulmonary cases
	Bettaieb <u>et al</u> , 1978	1963-1977	32 Spinal cases
	Khalfallah <u>et al</u> , 1978	1973-1977	107 Pulmonary cases
	Delleur <u>et al</u> , 1980	1980	1 Mammary case
	Hassine <u>et al</u> , 1980	-	42 Confirmed cases
UGANDA	Snell & Mukasa, 1948	1948	1 Hepatic case
	Owor & Bitakaramire, 1975	1967-1972	23 Surgical cases
ZAMBIA	Patel, 1969	1969	1 Hepatic and splenic case
ZAIRE	Nogue, 1923	1919	1 Peritoneal cyst
ZIMBABWE	Holmgren <u>et al</u> , 1971	1971	1 Pulmonary case

APPENDIX 2

Table 2. Reported human hydatid cases from Kenya, 1952-1980

<u>REFERENCE</u>	<u>PERIOD</u>	<u>No. OPERATIONS</u>	<u>TRIBE</u>
Wray, 1958	1952	29	} More than half Turkana
	1953	22	
	1954	20	
	1955	46	
	1955-1958	25	
Nelson & Rausch, ⁺ 1963	1957-1961	64	'Reasonably certain all Turkana'
McClatchie & Rajpal, 1965	1961	60	-
	1962	78	-
McClatchie & Manku, 1967	1967	3	2 Turkana, 1 Masai
Clifford, 1968	1968	3	2 Turkana, 1 Pokot
Warambo, 1970	1965	1	-
Röttcher, 1973	1968-1972	163	142 Turkana, 17 Masai
Irvin, 1974	1964-1970	34	All Turkana
O'Leary, 1976	1971-1975	789	776 Turkana, 10 Merille and 3 West Pokot
Eugster, 1978	1962-1976	78	76 Masai
O'Leary <u>et al.</u> , 1979	1976-1978	241	All Turkana
AMREF, 1980 *	1976-1980	457	All Turkana
Mirza <u>et al.</u> , 1979	1979	1	Case Report - 1 Turkana
Okelo <u>et al.</u> , 1980	1980	1	Case Report - 1 Turkana
French, 1980 ^o	1976-1979	355	All Turkana

+ From Cummins (Personal Communication)

* Includes all 241 cases reported by O'Leary (1979)

o All included in AMREF (1980.)

APPENDIX 2

Table 3. Location of hydatid cysts from Turkana patients February 1979 to September 1980

<u>LOCATION</u>	<u>No. OF PRIMARY CASES</u>	<u>No. OF RECURRENT CASES</u>	<u>% OF OPERATIONS PERFORMED</u>
Hepatic only	22	3	39.0
Mesenteric	11	3	21.9
Hepatic and Mesenteric	3	-	4.7
Retroperitoneal	3	-	4.7
Submandibular	3	-	4.7
Abdominal Wall (Superficial)	2	-	3.1
Behind Knee (Superficial)	1	-	
Behind Left Ear (Superficial)	1	-	
Chest Wall (Superficial)	1	-	
Hepatic, Mesenteric and Splenic	-	1	
Hepatic, Mesenteric and Uterine	1	-	
Left Breast	1	-	
Lumbar (Superficial)	1	-	21.9
Neck (Superficial)	1	-	
Outside Peritoneal Cavity	1	-	
Retro-Orbital	1	-	
Rectrouterine and Retroperitoneal	-	1	
Spleen	1	-	
Uterus	1	-	
Uterus and Urinary Bladder	-	1	

APPENDIX 3

Table 1. Reports of Echinococcus infections in dogs in Africa

<u>Country</u>	<u>Region</u>	<u>Reference</u>	<u>Number Examined</u>	<u>Number Infected</u>	<u>Percent Infected</u>
Algeria	Algier	Senevet, 1951			4-20
		Pampiglione, 1965a	163	26	15.9
Chad	All Regions	Troncy & Graber, 1969	117	4	3.4
Egypt	Cairo	Abdel Azim, 1938	-	-	3.0
	Alexandria	Abdel Azim, 1938	-	-	2.0
	Upper Egypt	Abdel Azim, 1938	-	-	10.0
	Abbasieh	El-Garhy & Selim, 1958	28	2	7.1
	Cairo	Mock <u>et al</u> , 1974	570	22	3.9
Kenya	Nairobi	Nelson & Rausch, 1963	16	8	50.0
	Turkana	Nelson & Rausch, 1963	27	19	70.4
	Kajiado	Eugster, 1978	165	45	27.3
Morocco	Marakech	Faure, 1949	-	-	15.0
	Fès	Faure, 1949	-	-	70.0
	Rabat	Robert, 1927	-	-	1.2
Nigeria	Kaduna	Dada <u>et al</u> , 1979b	150	3	2.0
	Zaria	Dada <u>et al</u> , 1979a	180	1	0.6
	Kano	Dada <u>et al</u> , 1980	145	9	6.2
South Africa	Pretoria	Ortlepp, 1934	25	5	20.0
	All Regions	Verster, 1979	1063	10	0.9
Sudan	Khartoum	Malek, 1959			20.8
	Kapoeta District	Eisa <u>et al</u> , 1962	36	32	86.5
	Torit District	Eisa <u>et al</u> , 1962	30	8	26.6
	Khartoum	El-Badawi <u>et al</u> , 1979	33	1	3.0
Tunisia	Thaba	Dévé, 1923	-	-	42.8

APPENDIX 3

Table 2. Regional differences of Echinococcus infection in dogs in Turkana (April 1979 - July 1980)

1. Kakuma - April, 1979

				INTENSITY OF INFECTION		
SEX	No. EXAM	No. INF	% INF	HEAVY	MEDIUM	LIGHT
MALE	13	7	53.8	1	0	6
FEMALE	16	10	62.5	1	0	9
TOTAL	29	17	58.6	2	0	15

2. Lakonkai - April, 1979

				INTENSITY OF INFECTION		
SEX	No. EXAM	No. INF	% INF	HEAVY	MEDIUM	LIGHT
MALE	13	9	69.2	5	0	4
FEMALE	21	13	61.9	3	1	9
TOTAL	34	22	64.7	8	1	13

3. Lokichokio - June and December, 1979

				INTENSITY OF INFECTION		
SEX	No. EXAM	No. INF	% INF	HEAVY	MEDIUM	LIGHT
MALE	28	15	53.6	6	1	8
FEMALE	33	20	60.6	3	8	9
TOTAL	61	35	57.4	9	9	17

Lokichokio - June, 1980

				INTENSITY OF INFECTION		
SEX	No. EXAM	No. INF	% INF	HEAVY	MEDIUM	LIGHT
MALE	26	19	73.1	8	3	8
FEMALE	43	30	69.8	19	4	7
TOTAL	69	49	71.0	27	7	15

Lokichokio - Total

				INTENSITY OF INFECTION		
SEX	No. EXAM	No. INF	% INF	HEAVY	MEDIUM	LIGHT
MALE	54	34	63.0	14	4	16
FEMALE	76	50	65.8	22	12	16
TOTAL	130	84	64.6	36	16	32

4. Lokitaung (Incl. Kaling, Koenech Palin and Loitanit) -
August, 1979

				INTENSITY OF INFECTION		
SEX	No. EXAM	No. INF	% INF	HEAVY	MEDIUM	LIGHT
MALE	17	5	29.4	2	1	2
FEMALE	19	1	5.3	0	0	1
TOTAL	36	6	16.7	2	1	3

5. Lokori (Incl. Lotoboi) - September 1979 and July 1980

				INTENSITY OF INFECTION		
SEX	No. EXAM	No. INF	% INF	HEAVY	MEDIUM	LIGHT
MALE	7	2	28.6	2	0	0
FEMALE	8	6	75.0	3	1	2
TOTAL	15	8	53.3	5	1	2

6. Nanam - June, 1980

				INTENSITY OF INFECTION		
SEX	No. EXAM	No. INF	% INF	HEAVY	MEDIUM	LIGHT
MALE	17	11	64.7	5	3	3
FEMALE	20	16	80.0	8	5	3
TOTAL	37	27	73.0	13	8	6

APPENDIX 4

Table 1. Occurrence and Prevalence of Echinococcus granulosus in wild carnivores in Africa

<u>Species</u>	<u>No. Examined</u>	<u>No. Infected</u>	<u>% Infected</u>	<u>Country</u>	<u>References</u>
<u>CANIDAE</u>					
Jackal (<u>Canis</u> sp.)	1	1		Kenya	Ginsberg, 1958
Silver-backed jackal (<u>Canis mesomelas</u>)	9 215	1 21	11.1 9.7	Kenya South Africa	Nelson & Rausch, 1963 Verster & Collins, 1966
	13	5	38.5	Kenya	Eugster, 1978
Golden jackal (<u>Canis aureus</u>)	- 81	- 1	? 1.2	Algeria Chad	Jore d'Arces, 1953 Troncy & Graber, 1969
Cape silver fox (<u>Vulpes chama</u>)	24	1	4.1	South Africa	Verster & Collins, 1966
Cape hunting dog (<u>Lycaon pictus</u>)	1 4	1 3		South Africa Kenya	Ortlepp, 1934 Nelson & Rausch, 1963
	1	1		South Africa	Verster & Collins, 1966
<u>HYAENIDAE</u>					
Spotted hyaena (<u>Crocuta crocuta</u>)	19 -	3 -	15.8 REPORTED	Kenya South Africa	Nelson & Rausch, 1963 Young, 1975
<u>FELIDAE</u>					
African wild cat (<u>Felis lybica</u>)	15	1	6.7	South Africa	Verster & Collins, 1966
Lion (<u>Panthera leo</u>)	1 7 13 6	1 5 2 2		South Africa South Africa East Africa Tanzania	Ortlepp, 1934 Verster & Collins, 1966 Dinnik & Sachs, 1972 Rodgers, 1974
			'COMMON'	South Africa	Young, 1975
	1 3	1 1		Kenya Central African Republic	Eugster, 1978 Graber & Thal, 1980

APPENDIX 4

Table 2. Occurrence and prevalence of hydatid cysts in wild animals in Africa

<u>Species</u>	<u>No. Examined</u>	<u>No. Infected</u>	<u>% Infected</u>	<u>Country</u>	<u>Reference</u>
Baboon (<u>Papio</u> sp.)	1	1		Mozambique	De Castro Amaro, 1960
	150	2	1.3	Kenya	Myers <u>et al</u> , 1970
Blue duiker (<u>Cephalophus monticola</u>)	1	1		Kenya	Eugster, 1978
Buffalo (<u>Syncerus caffer</u>)	-	-	3.0	South Africa	Young & Van den Heever, 1969
	97	5	6.0	South Africa	Basson <u>et al</u> , 1970
	145	25	17.2	Uganda	Woodford & Sachs, 1973
	1	1		Kenya	Eugster, 1978
Bushpig (<u>Potamochoerus porcus</u>)	2	-	REPORTED	Central African Republic	Graber & Thal, 1980
Cape molerat (<u>Georhynchus capensis</u>)	-	-	-	South Africa	Verster, 1962
Colobus monkey (<u>Colobus</u> sp.)	1	1		Tanzania	Myers <u>et al</u> , 1965
Giraffe (<u>Giraffa</u> sp.)	-	-	REPORTED	Tanzania	Sachs & Sachs, 1968
	44	-	REPORTED	Kenya	Fay, 1972
Grant's gazelle (<u>Gazella granti</u>)	26	2	7.7	Kenya	Eugster, 1978
Hartebeest (<u>Alcelaphus buselaphus cokii</u>)	65	-	REPORTED	Kenya	Fay, 1972
	38	2	5.3	Kenya	Eugster, 1978
Hippopotamus (<u>Hippopotamus amphibius</u>)	97	17	16.0	South Africa	McCully <u>et al</u> , 1967
Impala (<u>Aepyceros melampus</u>)	600	3	0.5	South Africa	Young, 1975
	24	2	8.3	Kenya	Eugster, 1978
Oryx (<u>Oryx algazel</u>)	9	1		Chad	Graber <u>et al</u> , 1969
Puku (<u>Adenota wardonii</u>)	5	1		Tanzania	Dinnik & Sachs, 1969
Waterbuck (<u>Kobus</u> spp.)	34	-	REPORTED	Kenya	Fay, 1972
Warthog (<u>Phacochoerus aethiopicus</u>)	-	-	REPORTED	Zambia	Verster, 1962
	-	-	REPORTED	Tanzania	Sachs & Sachs, 1968
	14	1	7.1	Central Africa	Graber <u>et al</u> , 1969
	17	7	41.2	East Africa	Dinnik & Sachs, 1969
	80	-	60	Tanzania	Schiemann, 1971*
	106	11	10.4	Uganda	Woodford & Sachs, 1973
	73	-	FREQUENT	Central African Republic	Graber & Thal, 1980
Wildebeest (<u>Connochaetes taurinus</u>)	-	-	REPORTED	Namibia	Verster, 1962
	17	1	5.9	Kenya	Nelson & Rausch, 1963
	450	2	0.4	Tanzania	Schiemann, 1971*
	70	3	4.3	East Africa	Sachs, 1976*
	567	69	12.2	Kenya	Eugster, 1978
Zebra (<u>Equus burchelli</u>)	-	-	60.0	South Africa	Young, 1975

* Quoted from Eugster, 1978

APPENDIX 5

Table 1. Number of cysts examined isoenzymatically from each intermediate host, the tissue site of each cyst and the geographical origin of the host.

<u>Host</u>	<u>Tissue distribution</u>	<u>No. cysts examined</u>	<u>Geographical origin</u>
Human	Liver	14	Turkana
	Liver	1	Samburu
	Mesenteric	2	Turkana
	Mesenteric	2	Masai
	Spleen	2	Turkana
	Retroperitoneal	1	Turkana
	Retrouterine	1	Turkana
Camel	Spleen	3	Turkana
	Lung	2	Turkana
Cattle	Lung	21	Masai
	Heart	1	Masai
Sheep	Liver	23	Masai
	Lung	15	Masai
	Spleen	2	Masai
Goat	Lung	10	Masai
	Lung	1	Marsabit
	Lung	1	Turkana
	Liver	7	Masai
	Liver	1	Turkana
	Spleen	1	Masai
	Spleen	3	Turkana

APPENDIX 5

Table 2. pI values for the major isoenzymes detectable in plates 17, 18 and 19 stained for the enzyme GPI

<u>Human</u>	<u>Cattle</u>	<u>Sheep</u>	<u>Goat (type A)</u>	<u>Goat (type B)</u>	<u>Camel</u>
5.7	5.7	5.8	5.9	5.6	5.6
5.9	5.8	5.9	6.0	5.8	6.1
6.0	6.0	6.1	6.3	6.0	6.2
6.2	6.1	6.3	6.4	6.3	6.3
6.3	6.3	6.5	6.5	6.4	6.4
6.5	6.5	6.7	6.7	6.5	6.5
6.6	6.6	6.8	6.8	6.7	
6.7	6.8	6.9	6.9		
6.8	6.9				
6.9	7.4				

Table 3. pI values for the major isoenzymes detectable in plate 21 stained for the enzyme PGM

<u>Human</u>	<u>Cattle</u>	<u>Sheep</u>	<u>Goat (type A)</u>	<u>Goat (type B)</u>	<u>Camel</u>
5.1	5.0	4.8	4.9	5.0	4.9
5.3	5.2	5.0	5.1	5.2	5.0
5.4	5.4	5.1	5.4	5.5	5.3
5.8	5.5	5.4	5.5	5.8	5.4
5.9	5.7	5.5	5.6	6.1	5.6
6.1	5.9	5.8	5.8	6.3	5.7
6.3	6.0	5.9	6.1	6.4	6.1
7.1	6.1	6.1	6.3	6.7	6.4
	6.3	6.2	7.1	6.8	6.8
	7.1	7.1			