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A COMPARATIVE STUDY OF *ECHINOCOCCUS GRANULOSUS* FROM HUMAN AND ANIMAL HOSTS IN KENYA USING ISOELECTRIC FOCUSING AND ISOENZYME ANALYSIS

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Abstract—MACPHERSON C. N. L. and MCMANUS D. P. 1982). A comparative study of *Echinococcus granulosus* from human and animal hosts in Kenya using isoelectric focusing and isoenzyme analysis. *International Journal for Parasitology* 12: 515–521. The soluble enzyme extracts from protoscoleces obtained from hydatid cysts of human, camel, cattle, sheep and goat origin were compared on the basis of their isoenzyme patterns for GPI and PGM using isoelectric focusing. Consistent GPI and PGM isoenzyme patterns were obtained for larvae of human, camel and sheep material. Cattle material varied occasionally in having an additional cathodic band in some of the GPI patterns. Two distinct isoenzyme patterns were evident in the goat material for both enzymes. The more common goat patterns were similar to those of human, cattle and sheep (Kenya, U.K. and Argentina) material, which were similar to each other. The rare goat patterns were similar to those obtained for camel material. Cyst location in the various intermediate hosts had no effect on the zymograms obtained. Additionally, no alteration in the major banding patterns was observed between the larvae and homologous adults produced by experimental infections. Of 26 naturally infected dogs, 19 produced adult GPI zymograms resembling human/sheep/goat (common form) experimental infection patterns, three were similar to experimental cattle infections and four had camel/goat (rare form) patterns.

INDEX KEY WORDS: *Echinococcus granulosus;* hydatid cysts; protoscolex; human; camel; cattle; goat; sheep; Turkana; Masailand; isoelectric focusing; strain differentiation; Kenya.

INTRODUCTION

THERE IS increasing evidence from various areas of the world that biologically distinct intraspecific variants or strains of *Echinococcus granulosus* exist in different vertebrate hosts (Smyth, 1977, 1979; Thompson, 1979; World Health Organisation, 1980). The recognition of intraspecific variants was based primarily on morphological criteria, but this has subsequently been questioned (Rausch, 1967). However, recent *in vitro* culture, biochemical and electrophoretic studies have provided further support for the existence of strains of *E. granulosus* (Smyth & Davies, 1974; Le Riche & Sewell, 1978a; McManus & Smyth, 1978, 1979; Kumaratilake, Thompson & Dunsmore, 1979; McManus, 1981) and there is circumstantial evidence to suggest that these may vary in their infectivity to their definitive and intermediate hosts including man (Nelson, 1972; Thompson & Smyth, 1976).

Speciation in *E. granulosus* has been most extensively studied in the United Kingdom where substantial evidence has been accumulated to indicate that there are distinct horse and sheep strains of the parasite (Smyth, 1979). However, relatively little is known concerning the biological characteristics of hydatid material from other intermediate hosts, such as camels, cattle, goats and the numerous wild herbivores from which hydatid cysts have been reported. One country in which strain characterisation of *E. granulosus* can be examined in a number of intermediate hosts is Kenya. Recent preliminary studies (McManus & Macpherson, *Parasitology* 81: xxxi-xxxiii, 1981; McManus, 1981) suggest the existence of an unusually complex strain picture there. In Masailand (southern Kenya), hydatid cysts have been reported in numerous wild herbivores and are common in cattle, sheep and goats, but the incidence of the disease in the human population is only approximately 2·0 per 100,000 per annum (Eugster, unpublished DVM thesis, University of Zurich, 1978). In contrast, the disease has a surprisingly low prevalence in cattle, sheep and goats in Turkana (Macpherson, unpublished PhD thesis, University of London 1981) and has not been found in wild herbivores in this region (Macpherson, Karstad, Stevenson & Arundel, in press). Paradoxically, the incidence of hydatid disease in the Turkana tribesmen (numbering some 169,000 individuals, 1969 census) is extremely high and on average, 10 operations for the removal of hydatid cysts from Turkana patients are per-

formed every month by surgeons of the African Medical and Research Foundation (AMREF) and the African Inland Mission (AIM) (African Medical and Research Foundation, 1981).

In the current study, the results of zymogram patterns for two enzymes, glucosephosphate isomerase (GPI), and phosphoglucomutase (PGM), following the isoelectric focusing (IEF) of soluble enzyme extracts of *E. granulosus* from a number of different intermediate hosts, are presented. This technique has previously proved to be successful in helping to differentiate the U.K. horse and sheep strains of the parasite (McManus & Smyth, 1979). The technique has also been used here to examine the possible occurrence of isoenzyme changes between larvae of *E. granulosus* from various hosts and the experimentally produced homologous adults. In addition, the experimental adult isoenzyme patterns are compared with those from some natural dog infections in Turkana, in an attempt to identify the intermediate host source(s) of such infections.

MATERIALS AND METHODS

Hydatid cysts from camels and goats were obtained at Lodwar abattoir (Turkana). Material from Masailand was obtained from cattle, sheep and goats from the Kenya Meat Commission (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 26 naturally infected dogs and a silver-backed jackal examined at Lokichokio (Turkana) and from numerous experimentally infected puppies and a jackal. The experimental carnivores were each fed a gelatine capsule containing 0·2 ml packed protoscoleces and were sacrificed some 36–40 days post-infection. Protoscoleces for IEF were freed from blood capsules using the technique described by McManus & Smyth (1979). Infected carnivore intestines were removed to warm Hanks' Balanced Salt Solution (HBSS), opened longitudinally and incubated at 38°C for 30 min to detach the adult worms. Protoscoleces or adults were rinsed several times in warm HBSS and finally once in distilled water. Parasite material was then transferred to a chilled 1 ml glass homogenizer (Jencons) and hand homogenized on ice with a minimum of either distilled water or 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 ethylenediaminetetracetate (EDTA) made up in distilled water (Kilgour & Godfrey, 1973). All homogenates were centrifuged at 2000 *g* for 5 min in a M.S.E. bench centrifuge operated in a coldroom at 4°C. The final supernatants were then beaded into 15 μl droplets in liquid nitrogen and stored in liquid nitrogen. Some of the supernatants were used freshly with and without enzyme stabilisers whilst others were lyophilised and stored at −20°C for comparison. Protoscoleces 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. All IEF, the staining of ampholine polyacrylamide gels for GPI and PGM and the determination of isoelectric points (pI) w performed as described by McManus & Smyth (1979) with the following minor modifications. Beaded samp [15 μl of supernatant, containing approximately 250 protein (Lowry, Rosebrough, Farr & Randall, 1951)] w thawed on supplied (5 × 10 mm) filter paper applicati pieces and applied to the gel. These applicators w removed 45 min after commencing the isofocusing run order to prevent the tailing of absorbed proteins. The portions were stained in the dark at room temperature 45 min, after which time the zymograms were examin visually and the results were photographed.

RESULTS

During the course of this investigation, mater obtained from 23 human hydatid cysts, five cam cysts, 22 cattle cysts, 40 sheep cysts and 24 goat cy were examined isoenzymatically. The tissue distrib tion of these cysts and the geographical origin of t hosts are presented in Table 1. Additionally, phot graphs of the GPI and PGM zymograms produc by the larvae and also by adult worms from natural and experimentally infected dogs and jackals a shown in Figs. 1–5. The pI values for the maj staining bands of both enzymes for the differe larvae are presented in Tables 2 and 3. The pI valu of minor, weaker staining isoenzymes have not be included in order to facilitate comparison betwe the different larvae from different hosts. There we no qualitative differences in the GPI or PG patterns of any one sample regardless of wheth larvae were treated with or without enzyme stabiliser lyophilised, stored in liquid nitrogen or used freshl

TABLE 1—NUMBER OF CYSTS EXAMINED ISOENZYMATICAL FROM EACH INTERMEDIATE HOST, THE TISSUE SITE OF EAC CYST AND THE GEOGRAPHIC ORIGIN OF THE HOST

Host	Tissue distribution	No. cysts examined	Geographic origin
Human	Hepatic	14	Turkana
	Hepatic	1	Samburu
	Mesenteric	2	Turkana
	Mesenteric	2	Masai
	Splenic	2	Turkana
	Retroperitoneal	1	Turkana
	Retrouterine	1	Turkana
Camel	Splenic	3	Turkana
	Pulmonary	2	Turkana
Cattle	Pulmonary	21	Masai
	Cardiac	1	Masai
Sheep	Hepatic	23	Masai
	Pulmonary	15	Masai
	Splenic	2	Masai
Goat	Pulmonary	10	Masai
	Pulmonary	1	Marsabit
	Pulmonary	1	Turkana
	Hepatic	7	Masai
	Hepatic	1	Turkana
	Splenic	1	Masai
	Splenic	3	Turkana

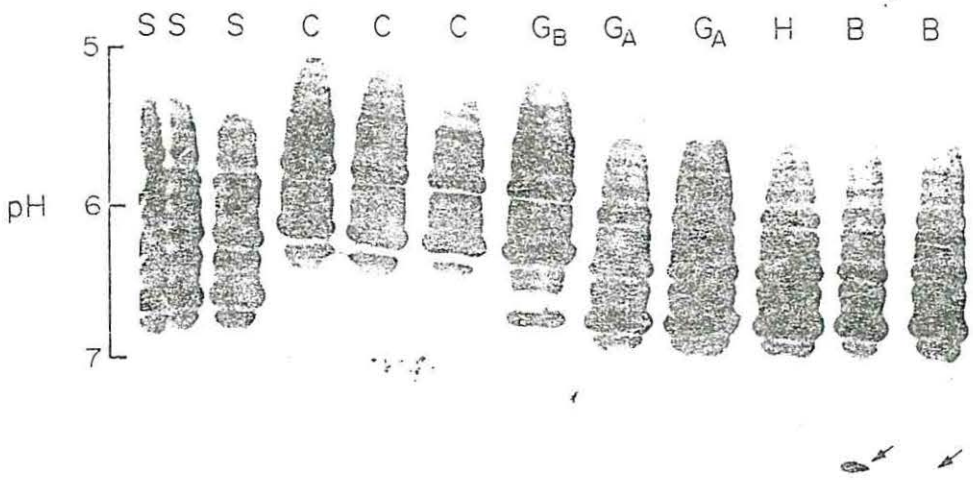

FIG. 1. Electrophoretic patterns obtained with soluble extracts of protoscoleces removed from hydatid cysts from, human (H), camel (C), cattle (B), sheep (S) and goat (G) hosts and stained for the enzyme GPI. The arrows indicate the extra cathodic band seen in some of the cattle samples.

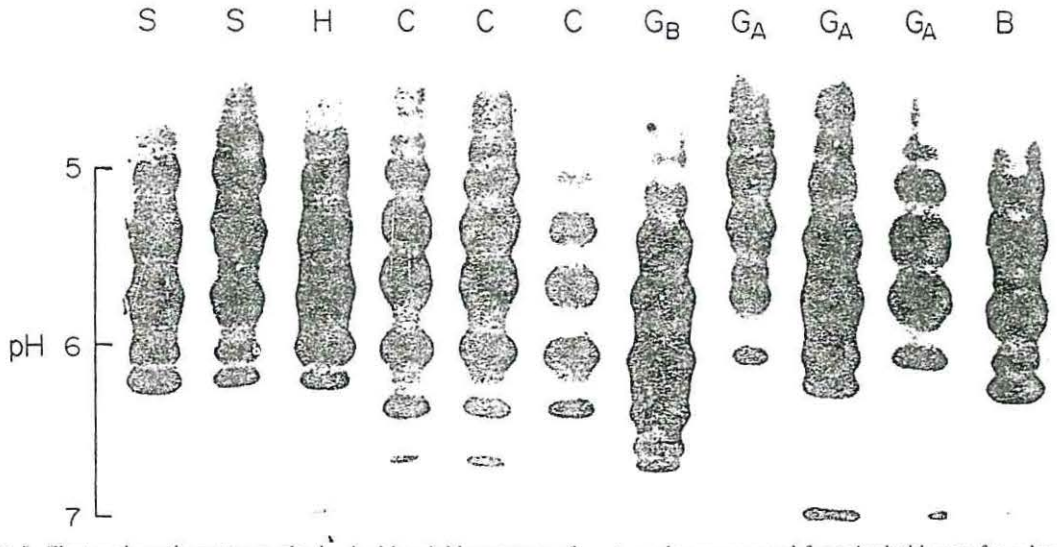

FIG. 2. Electrophoretic patterns obtained with soluble extracts of protoscoleces removed from hydatid cysts from human (H), camel (C), cattle (B), sheep (S) and goat (G) hosts, and stained for the enzyme PGM.

However, additional secondary isoenzymes (Harris & Hopkinson, 1976) were observed in material which had been stored in liquid nitrogen and thawed two or more times.

The isoenzyme patterns produced from each enzyme were not affected by the tissue location of the cyst in the host. Moreover, no polymorphic variations in the isoenzyme profiles of GPI or PGM were observed in *E. granulosus* of human, camel, or sheep origin (Figs. 1 and 2). In cattle material, however, an additional, but weaker staining cathodic band (Fig. 1, arrow, pI = 7·4) was evident in some (seven of 22 samples) of the GPI isoenzyme profiles, although the PGM patterns were always identical. Two distinct banding patterns were apparent in the goat material, for both GPI and PGM (Figs. 1 and 2). One of these patterns (common form, type A) was evident in 20 samples, while the other (rare form, 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 (Figs. 1 and 2). A number of samples of lyophilised protoscoleces from U.K. sheep and a single sample

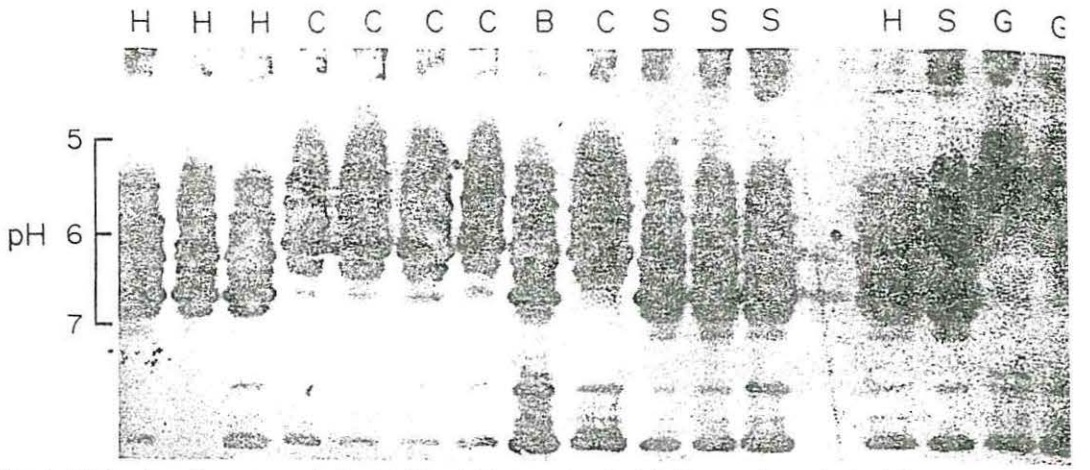

FIG. 3. Electrophoretic patterns obtained with soluble extracts of adult *E. granulosus* obtained from experimental infections and stained for the enzyme GPI. H = dog infected with human protoscoleces, C = dog infected with ca protoscoleces, B = dog infected with cattle protoscoleces, S = dog infected with sheep protoscoleces, G = dog infected v goat (type B) protoscoleces.

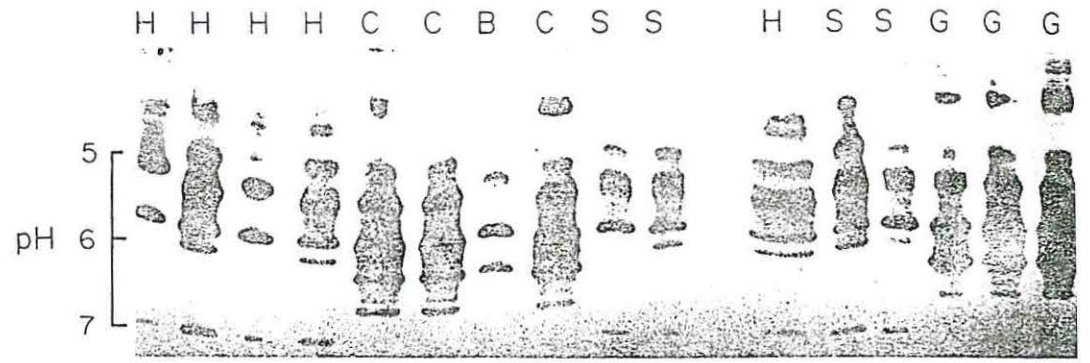

FIG. 4. Electrophoretic patterns obtained with soluble extracts of adult *E. granulosus* obtained from experimental d infections and stained for the enzyme PGM. H = dog infected with human protoscoleces, C = dog infected with can protoscoleces, B = dog infected with cattle protoscoleces, S = dog infected with sheep protoscoleces, G = dog infected wi goat (type B) protoscoleces.

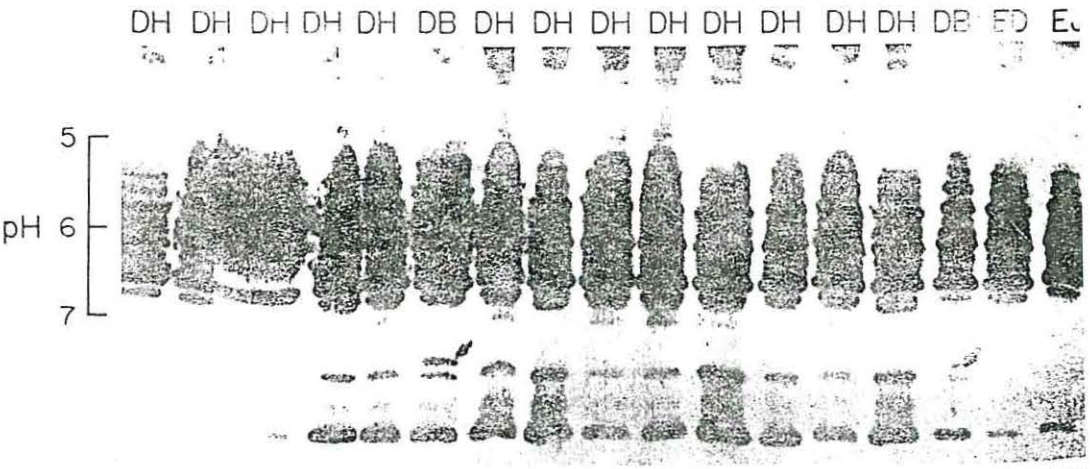

FIG. 5. Electrophoretic patterns obtained with soluble extracts of adult *E. granulosus* obtained from 15 naturally infecte dogs examined around Lokichokio, and from an experimental dog and silver-backed jackal fed human material, an stained for the enzyme GPI. DH = naturally infected dogs with human, sheep or goat (type A) pattern; DB = naturall infected dogs with cattle type pattern, extra cathodic band is arrowed; ED = experimental dog infected with huma material; EJ = experimental jackal infected with human material.

Human	Cattle	Sheep	Goat (type A)	Goat (type B)	Camel
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	6·4	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*				

*Seen in seven out of 22 samples.

TABLE 3—pI VALUES FOR THE MAJOR ISOENZYMES DETECTABLE IN FIG. 2 STAINED FOR THE ENZYME PGM

Human	Cattle	Sheep	Goat (type A)	Goat (type B)	Camel
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			

from an Argentinian sheep produced GPI and PGM patterns which were identical to those obtained from Kenyan sheep.

The banding patterns for both GPI and PGM from camel and goat (type B) material were very similar (Figs. 1 and 2). In contrast, these patterns were substantially different from those obtained from the human, cattle, sheep and goat (type A) material (Figs. 1 and 2). 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 (Figs. 1–4). The only differences between the adult and larval patterns for each particular intermediate host was the extra cathodic bands in the adult GPI patterns. No additional cathodic bands were evident in the PGM isoenzyme profile 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 were also reflected in the corresponding adult worm patterns.

The GPI and PGM isoenzyme patterns of adults produced from an experimentally infected silver-backed jackal, using human hydatid material, were identical to those of adults obtained from similarly infected puppies (Fig. 5).

Interestingly, the GPI isoenzyme patterns for *E. granulosus* adults from 19 out of 26 naturally infected dog samples and one silver-backed jackal sample examined from Lokichokio were very similar to those produced for adults from the experimental human and sheep infections. Three out of the 26 patterns were very similar to the experimental cattle infection and four naturally infected dog patterns had a camel/goat (type B) pattern (Fig. 5).

DISCUSSION

In a recent electrophoretic study, Le Riche & Sewell (1978a) showed that the GPI isoenzyme profiles for *E. granulosus* of U.K. sheep and cattle origin were very similar, but that these patterns were different from 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 their observations, although some of the cattle samples from Kenya differed from the human and sheep material in having one extra cathodic band in the GPI isoenzyme pattern.

One of us (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 largely corroborates that particular study, but indicates that the strain picture in Kenya may be even more complex than was previously suggested. The uniformly consistent and identical GPI and PGM isoenzyme patterns obtained for *E. granulosus* of human and sheep origin suggest that these forms may represent a single, homogeneous (monomorphic) strain in Kenya. In contrast, the camel form appears to be a different monomorphic strain. The situation is not as clear-cut in cattle material due to the inconsistent GPI patterns obtained. Furthermore, the two distinct isoenzyme profiles for both GPI and PGM indicate that *E. granulosus* of goat origin exists in Kenya as two variants, one of which appears to be very similar to the human/sheep and possibly cattle strain, while the other is similar to the camel strain.

There is evidence from biochemical work that the sheep forms of *E. granulosus* from the U.K., Kenya and South America may represent geographically distinct variants which have been termed '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 of *E. granulosus* in any geographical locality and from a particular intermediate (or definitive) host.

An interesting and important aspect of the current study is that the major GPI and PGM isoenzyme patterns exhibited by larvae from all the intermediate hosts investigated are reflected in the homologous adult patterns produced by experimental infection. A similar observation has been reported for several enzymes, including GPI, from larvae and adults of a number of other *Taenia* spp. (Le Riche & Sewell, 1978b). It appears, therefore, that the major enzyme patterns of *E. granulosus* and other taeniids are not influenced by the larval to adult transition or by the change of host environments. In contrast, the extra minor cathodic bands evident in the GPI isoenzyme profiles for the adults of *E. granulosus* may represent new isoenzymes synthesised as an adaption to the differing physiological conditions in the intestine of the definitive host, or, they may be host enzymes absorbed onto the surface of the worms.

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, we have found no differences in the GPI and PGM isoenzyme profiles of *E. granulosus* from horses and *E. granulosus* of horse origin which had been passaged through gerbils (McManus & Macpherson, unpublished observations). Thus it appears likely that the larval GPI and PGM isoenzyme patterns of any particular strain of *E. granulosus* are not influenced by the species of intermediate host invaded. Similarly, the adult isoenzyme patterns appear to be unaffected by the species of definitive host as exemplified by the identical patterns obtained for GPI and PGM from the jackal and dog experimental infections. It would, however, be expedient to examine whether this consistency of isoenzyme patterns occurs with other enzymes and also in cross infectivity studies involving other species of definitive and intermediate hosts.

Of epidemiological significance is the fact that *E. granulosus* adults from 19 out of 26 naturally infected dogs examined in Lokichokio, gave a similar GPI isoenzyme pattern to that of 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 possible that similar GPI and PGM isoenzyme patterns to the experimental human/dog and sheep/dog forms would have been obtained. Therefore, the naturally infected dogs with the human/sheep isoenzyme pattern could also have ingested hydatid material from goats with the type A pattern. Only three of the 26 naturally infected dogs had experimental cattle/dog GPI isoenzyme patterns and four naturally infected dogs had camel or goat (type B) GPI isoenzyme patterns. These results suggest that the majority of dogs naturally infected with *E. granulosus* in Lokichokio obtained their infections by ingesting hydatid material of human, sheep or goat origin.

The present study using the two enzymes GPI and PGM has proved to be useful in helping to identify hydatid material obtained from numerous intermediate and definitive hosts from Kenya, but further strain differentiating techniques are now required to support these observations. The epidemiological implications of this isoenzyme study will be discussed in more detail in subsequent publications concerning the prevalence and infectivity of hydatid disease in humans and their domestic animals from Kenya.

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