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STUDIES ON MORPHOLOGY OF *ECHINOCOCCUS GRANULOSUS* FROM DIFFERENT ANIMAL-DOG ORIGIN

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Abstract: Morphological studies on larval and adult Echinococcus granutosus of buffalo, cattle, sheep, goats and camel origin revealed non-significant difference as regards the total number of hooks as well as shape and arrangement of the hooks. The mean total length of large and small hooks did not vary significantly in protoscoleces from different animal origin but mean total length of large and small hooks of adult worm differed significantly. No significant differences were observed in segmentation of adult worms, number and distribution of testes, shape of cirrus sac, position of genital pore and in histological examination hydatid cysts.

Key words: *Echinococcus granulosus*, protoscoleces, morphology, dog origin.

INTRODUCTION

chinococcus granulosus is the most important tapeworm of dog and other carnivora (Dent and Relly, 1976). Its larval forms causes hydatidosis in domestic animals and man (Baldock *et al.,* 1985). It is a significant economic and public health problem in Pakistan (Tang *et al.,* 2004). Various strains of this parasite are now recognized in Australia, England and Switzerland (Thompson and Kumaratilake, 1982; Kumaratilake and Thompson, 1983, 1984). In Pakistan incidence of the disease in some localities and chemical composition of hydatid cyst fluid has been reported (Munir, 1980). In adult animals, hydatid cysts were present in 34.88, 30.35, 6.35, and 4.33 percent in buffaloes, cows, sheep and goats, respectively (Hussain *et al.,* 1992). He also reported that rate of infection was higher in females and aged animals. Studies on morphology of *E. granulosus* from different geographical areas and intermediate hosts have not so far been conducted in Pakistan. The present investigation was, thus, planned to study the morphology of protoscoleces from different intermediate hosts to find the intraspecific variants of the parasite. This study would help in understanding the identification and evaluation of local strains and would make basis from a rational approach to develop a central strategy.

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MATERIALS AND METHODS

Protoscoleces of *E. granulosus* were collected from fertile viable hydatid cysts in livers, lungs or spleens of naturally infected buffaloes, cows, sheep, goats and camels slaughtered at Faisalabad Abattoir. The viability of the protoscoleces was determined by observing flame cell activity (Calero *et al.,* 1978). Viable protoscoleces used for experimental infection were washed five times in fresh sterile Hank's Balanced Salt solution containing antibiotic at 4°C (Smyth and Davies, 1974). Samples of viable protoscoleces were fixed in 10 per cent Buffered formalin for morphological studies. Five mongrel dogs 12-16 weeks of age from internal and external parasitism were dosed with approximately 0.5 ml (60,000) of sedimented protoscoleces in a solution obtained from hydatid cysts of buffaloes, cattle, sheep, goats and camels. All the dogs were kept under similar feeding and managemental conditions throughout the course of study.

Each dog was euthanized 30 days after the experimental infection by intravenous injection of Pentothal sodium (Abbott Labs. Ltd.). The adult worms were collected by incising the small intestine of dogs and fixed in 10% buffered formalin in separate containers for morphological studies (Thompson, 1977, Turcekova *et al*., 2003). The protoscoleces fixed in 10% buffered formalin were mounted in Lactophenol, the number, shape and arrangement of rostellar hooks was studied. Total lengths of large and small hooks were also measured using a calibrated eyepiece micrometer under oil immersion objective (Sweatmen and Williams, 1963). Rostella were dissected free from the adult worms and mounted lactophenol and same measurements were made as described above for protoscoleces.

Total worm length measurements we made on unstained, fixed specimens by ordinary measuring ruler under dissecting microscope. Strobillar morphology was studied by staining the adult was with Modified Masson's Trichome (Heren, 1957, Fitzgerald *et al.,* 2001).

Laminated memberane of hydatid cysts along with brood capsules preserved and fixed in 10% buffered formalin were dehydrated in ascending grade of alcohol, cleaved with xylol and infiltrated in paraffin sections of 5-8μm, thick we made and stained with haematoxylin and eosin (Humason, 1972). These were then seen under microscope for histological differences.

The data was analyzed by using the ANOV A (Steel and Torrie, 1980) to detect any significant differences among the measurable characteristics of parasitic material of different host origin. Student's t-test were employed to detect the variation between different hosts.

RESULTS AND DISCUSSION

Protoscoleces -Rostellar Hook morphology

The total number of hooks and length of large and small hooks in different batches of protoscoleces obtained from hydatid cysts of liver and lungs of cattle, buffaloes, sheep, goats and camels are presented in Table-1. The results indicated non-significant differences in these parameters. The arrangement of large and small hooks was alternate. The shape of the hooks was smooth in their outline. These findings of protoscoleces from cattle and sheep origin are in line with the findings of Thompson *et al.* (1984) and Kumaratilake and Thompson (1984), Turcekova *et al.* (2003), Tang *et al.* (2004) and Ahamdi (2004). In this study morphological characters of protoscoleces from buffalo, goat and camel origin were also similar to those of cattle and sheep origin, although report on such study could not be traced from the available literature.

Table 1: Rostellar hook characteristics of protoscoleces of *Echinococcus granulosus* from domestic animals in Pakistan

Characteristics	Parasite Material [Mean ±S.D. (Range)]					
	A Buffalo Origin	B Cattle Origin	C Sheep Origin	D Goat Origin	E Camel Origin	Statistics
Total No. of hooks	36.0 ± 5.6 (26.0 - 46.0)	36.0 ± 5.1 (30.0 - 46.0)	35.0 ± 4.2 (26.0 - 46.0)	33.0 ± 4.2 (22.0 - 38.0)	35.0 ± 5.2 (26.0 - 44.0)	A=B=C=D=E
Large hooks Total length (µm)	27.5 ± 3.5 (22.5 - 33.2)	28.0 ± 2.2 (24.6- 31.0)	25.8 ± 1.8 (23.5 - 28.9)	27.1 ± 2.5 (23.5 - 31.0)	25.0 ± 2.90 (21.4 - 29.9)	A=B=C=D=E
Small hooks Total length (L/m)	17.8 ± 2.6 (15.0 - 2.5)	17.5 ± 2.2 (13.09 - 0.3)	18.3 ± 2.6 (12.8 - 21.4)	17.8 ± 2.4 (13.9 - 21.4)	17.3 ± 1.4 (14.5 - 19.3)	A=B=C=D=E

Adult worm -Rostellar Hook morphology

The results indicated non-significant differences in the total number of hooks of adult worms obtained from different animal-dog origin but total length of large and small hooks of adult worms of buffalo and dog origin differed significantly ($P< 0.01$) from others (Table 2). Shape and arrangement of hooks was also quite similar. Kumaratilake *et al.* (1984) also reported that the total number of Rostellar hooks are same in adult 35 day-old *E. granulosus* from cattle, sheep and pigs. They observed significant difference in the number of Rostellar hooks in adult *E. granulosus* from macropodes and other animal species. However, they did not include *E. granulosus* of buffalo, goat and camel origin. The difference in total length of large and small hooks of 30-day-old *E. granulosus* of

buffalo-dog origin from those of adult Echinococcosis of cattle-dog-sheep-dog, sheep-dog and camel-dog origin might indicate the existence of some new strain of *E. granulosus* in the area. These studies are in partial agreement with those of Gill and Rao (1986), Tang *et al.* (2004) who reported the occurrence of gravid worms with only two segments.

Table 2: Rostellar hook characteristics of 30 day old adult *Echinococcus granulosus* from domestic animals in Pakistan

Characteristics	Parasite Material [Mean ±S.D. (Range)]					
	A Buffalo Origin	B Cattle Origin	C Sheep Origin	D Goat Origin	E Camel Origin	Statistics
Total No. of hooks	38.0 ± 7.0 (27.0 - 50.0)	36.0 ± 3.3 (30.0 - 44.0)	37.0 ± 3.3 (32.0 - 42.0)	35.0 ± 4.1 (28.0 - 42.0)	34.0 ± 4.0 (28.0 - 40.0)	A=B=C=D=E
Large hooks	37.9 ± 1.9	32.2 ± 2.5	29.2 ± 1.9	30.2 ± 2.6	31.4 ± 1.8	A=B=C=D=E
Total length (L/m)	(36.4 - 41.7)	(28.9 - 37.5)	(25.7 - 31.0)	(25.7 - 33.2)	(28.9 - 34.3)	(P<0.001)
Small hooks	32.2 ± 1.7	22.8 ± 1.8	20.1 ± 2.5	20.1 ± 2.8	23.1 ± 2.5	A=B=C=D=E
Total length (L/m)	(30.0 - 34.3)	(20.3 - 26.8)	(15.0 - 26.6)	(14.0 - 24.6)	(19.6 - 27.8)	(P<0.001)

Strobillar Morphology

The mean total worm length (mm) of 30 day-old buffalo-dog, cattle-dog-sheep-dog, goat-dog and camel-dog origin worm are presented in Table-3. No significant differences in total worm length were observed. Similarly no significant difference was observed in the segmentation of the worms. Majority of isolates of 30 day-old worms were possessing three segments and less than 20% of the population possessing four segments. There was no statistically significant difference in the total number of testes and in their distribution anterior or posterior to the genital pore between worms of different animal-dog origin. The position of the genital pore was observed in relation to the mid-point of the segment. The genital pore was present posterior to the mid-point of the segment in the majority of the adult worms of different isolates. However, in some worms, the genital pore was present at the mid-point of the segment. No significant difference was observed in position of the genital pore with relation to the adult worms. The cirrus sac was characteristically piriform in shape (pear shaped) in isolates of 30 day-old *E. granulosus* of buffalo-dog, cattle-dog, sheep dog, goat-dog and camel-dog origin. Differences were not apparent among these isolates. Kumaratilake and Thompson (1984) and Thompson *et al.*, (1984) also reported similar strobillar morphology in worms of cattle-dog and sheep dog origin in Australia. However, literature about the strobillar morphology of *E. granulosus* of buffalo-dog, camel-dog and goat-dog origin could not be

traced. The morphological characteristics of all the isolates examined were similar to that of morphological description of Kumaratilake and Thompson (1982), Turcekova *et al.* (2003). The present study confirmed the existence of a single species of *E. granulosus* in Pakistan. However, there is also evidence that more than one strain of *E. granulosus* exist in this country like *E. granulosus* (sheep, bovines, dogs and white mice), *E. granulosus canadensis* (dogs, sheep, pigs and mice), *E. granulosus borealis* (wolves, mice and deers), and *E. granulosus equines* (dogs, horses and rarely in sheep).

Table 3: Rostellar hook characteristics of protoscoleces of *Echinococcus granulosus* from domestic animals in Pakistan

Characteristics	Parasite Material [Mean ±S.D. (Range)]					
	A Buffalo Origin	B Cattle Origin	C Sheep Origin	D Goat Origin	E Camel Origin	Statistics
Total worm length	2.1 ± 0.57 (1.7 - 3.5)	2.23 ± 0.55 (1.4 - 3.2)	2.01 ± 0.5 (1.3 - 3.0)	2.0 ± 0.48 (1.1 - 2.9)	1.9 ± 0.48 (1.0 - 2.7)	A=B=C=D=E
Maximum No. of segments (% of worms)	4 (18%)	4 (20%)	4 (20%)	4 (17%)	4 (15%)	
No. of testes	40.0 ± 8.86 (28.0 - 7.0)	37.0 ± 7.85 (24.0 - 52.0)	37.0 ± 3.94 (30.0 - 40.0)	34.0 ± 4.06 (26.0 - 40.0)	35.0 ± 9.96 (24.0 - 48.0)	A=B=C=D=E

Histological studies

Histological examination showed the cysts to be typical unilocular hydatid cysts characteristics of *E. granulosus*. The germinal layers from different hydatid cysts were found in the active, proliferative phase and brood capsules and protoscoleces formation were still occurring. Many protoscoleces had hooks which were not fully developed. Even though the age of different hydatid cysts was not confirmed, no significant difference was observed in thickness of laminated membranes as well as in germinal layers of different hydatid cysts from different animal origin. Details about the histological studies of hydatid cysts were not found from the literature available. However, further well-planned investigations are required to confirm to histological details of the hydatid cysts from different animal species.

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