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Sheep Can Be Infected with More than One Strain of *Echinococcus granulosus*

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Abstract: *Echinococcus granulosus* is widely distributed throughout Iraq. Characteristics of *E. granulosus* strobila are presented from dogs experimentally infected with protoscoleces from hydatid cyst isolated from the lungs of a domestic sheep in Nineveh Province. They were defined and compared with those of other recognized strains of this parasite. The number, shape and arrangement of rostellar hooks of adult worms and detailed description of male and female genitalea were taken into consideration as key criteria. Adult cestodes are characterized mainly by 32.2-34.8 hooks highly variable in shape and size, 42.8-34.2 testes situated either throughout the proglottis with one rows behind the vitellin gland or they do not exceed the anterior half of the vitelline gland, the ovary variable in shape from compact lung-shaped to elongated with well-demarcated lobules forms. The results revealed the existence of two distinct strains similar to dog/sheep and dog/cattle strains.

Key words: *Echinococcus granulosus*, strain, dog, sheep, cattle

INTRODUCTION

During the past four decades, considerable phenotypic and genetic variability has been observed within the species *E. granulosus* and several strains have been identified (Van Herwerden *et al*., 2000; Thompson and Mcmanus, 2001, 2002; Pearson *et al*., 2002). A common feature for definition of all strain is the utilization of dogs and other canids as definitive hosts, but the strains exhibit several differences in intermediate host spectrum, geographic distribution, adult and metacestode morphology, maturation time in definitive hosts, organ localization of metacestodes and protoscolex production are criteria taken into consideration (Eckert and Thompson, 1997).

The aim of the present research was to substantiate previous observations on *E. granulosus* to determine the possibility of existence of one or more than one strain of *E. granulosus* in sheep in Iraq. Following morphological and structural variations among adult cestodes, obtained from experimentally infected dogs with lung protoscoleces of naturally infected sheep.

MATERIALS AND METHODS

A batch of eight, 4-months-old helminthes free local strain dogs were dosed with approximately, 1×10^4 sediment protoscoleces obtained from fertile hydatid cysts of *E. granulosus*. The intended cysts were isolated from lungs of indigenous sheep slaughtered in Mousl city North of Iraq. Following microscopic examination of the fluid, over 95% of protoscoleces used for infection were viable and had flame cell activity. All the protoscoleces of lung origin were given orally using 20 mL syringes with rubber tubes. The dogs were reared in large kennels located in animal house of the college of veterinary medicine. Each dog was euthanasically sacrificed on day sixty post-infection by IV injection of overdose of 5% ketamin and 2% xylazin. Subsequent autopsy and worm recovery procedures were carried out as previously described by Eckert *et al.* (1989).

Autopsy of puppies: Body cavity of scarified puppies was opened rapidly and the small intestine of each animal was removed, lighted and preserved in 10% formalin. Later, the intestine was opened longitudinally in beaker containing Phosphate Buffer Slain (PBS) pH 7.2. Their contents were carefully examined and washed successively, using graduated brass sieves. Each intestine was left for 30 min in PBS at 37°C. The cestodes collected were allowed to relax further in 10% formalin for 24 (Eckert *et al*., 1989). They were washed carefully with tap water, stained with Acetocarmin, dehydrated in graded ethyl alcohol, cleared in xylol for 5 min and finally mounted in Canada balsam. The obtained cestodes were identified depending upon the criteria mentioned above, using calibrated ocular micrometer.

RESULTS

The characteristics of rostellar hooks of adult worms for the two different groups termed as A and B including

Table 1: Rostellar hooks characteristics of adult *E. granulosus* of Iraqi sheep origin in comparison with data published by other authors

Characters	A	B	Sheep* Australia	Cattle * Switzerland
Total No. of hooks	32.2±1.6 (29.0-360)	34.8±0.3 (30.0-42.0)	33.0±3.4 (19.0-45.0)	38.8±3.0 (28.0-36.0)
Total length of large hooks TL (µm)	27.3±0.8 (20.0-340)	29.8±1.7 (25.0-43.0)	31.8±2.5 (27.3-37.3)	39.1±0.8 (37.0-41.0)
Blade Length BL (µm)	8.9±0.5 (8.10-130)	10.0±0.8 (10.0-15.0)	12.9±1.0 (10.2-14.6)	15.5±0.4 (15.0-16.0)
BL/TL (%)	33.1±2.1 (29.1-42.8)	35.9±1.6 (35.7-41.4)	40.1±2.5 (35.8-43.9)	39.6±1.1 (37.5-42.0)
Total length of small hooks TL (µm)	20.2±1.1 (19.0-220)	25.5±1.7 (21.0-35.5)	24.6±2.9 (21.4-34.7)	32.7±1.1 (31.0-34.0)
Blade Length BL (µm)	7.7±0.8 (7.0-9.30)	9.6±1.0 (08.0-11.0)	9.1±1.2 (7.0-12.00)	11.0±0.7 (10.0-12.0)
BL/TL (%)	36.3±3.8 (33.3-46.0)	36.2±1.2 (31.2-38.7)	37.8±3.9 (30.4-45.4)	33.9±2.3 (30.9-39.0)
Shape of hooks	Rough outline	Somewhat smooth outline	-	-

Table 2: Morphology characteristics of mature *E. granulosus* of Iraqi sheep origin in comparison with data published by other authors

Characters	A	B	Sheep* Australia	Cattle* Switzerland
Total worm length (mm)	3.5±0.2 (2.9-4.1)	3.8±0.2 (2.6-3.7)	2.5±08 (0.7-4.5)	4.2±0.5 (3.4-6.9)
Terminal segment length (mm)	1.3±0.06 (1.1-1.4)	1.4±0.07 (1.4-1.6)	-	-
Position of mature segment	Penultimate	Penultimate	Penultimate or antepenultimate	Always penultimate
Position of genital pore	100% posterior to middle of segment	100% posterior to middle of segment	100% posterior to middle of segment	90% posterior to middle of segment
Terminal segment	100% posterior of middle of segment	100% posterior of middle of segment	100% posterior to middle of segment	100% posterior to middle of segment
Maximum no. of segment	3 in 81%	3 in 90%	4	3
No. of testes	42.8±3.6 (32-50)	34.2±5.4 (24-40)	48±7.0 (30-60)	29.0±3.0 (23-38)
Distribution of testes	Throughout segment with one row behind vitelline gland	Constricted to middle of segment	Throughout segment	Confined to center of segment
Shape of cirrus sac	Piriform	Spherical to piriform	Piriform	Spherical to piriform
Size of cirrus sac (µm)	108.4±11.2×67.2±5.2 (85-50) (55-82)	88.2±7.0×68.2±2.3 (65-100) (60-72)	0.136×0.073±0.047±0.012	0.058×0.052±0.002±0.012
Shape of ovary	Compact lung shape	Elongated lobed butterfly shaped	Compact with less demarcated lobules	Elongated with well demarcated lobules

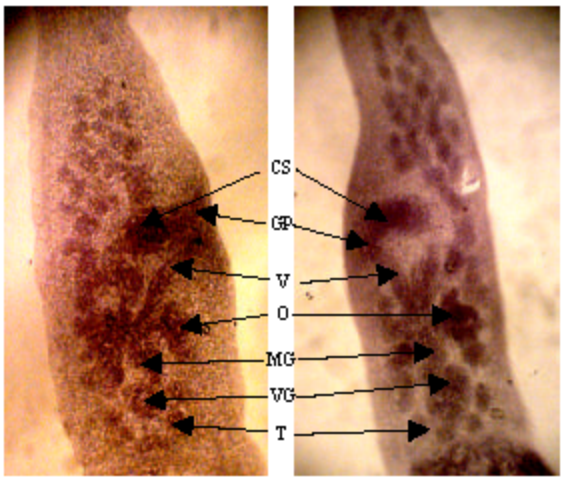

Fig. 1: Morphological characteristics of mature proglottid of *E. granulosus* group A. CS Cirrus Sac; GP: Genital Pore; V: Vagina; O: Ovary; MG: Mehlis Gland; VG: Vitelline Gland and T: Testes

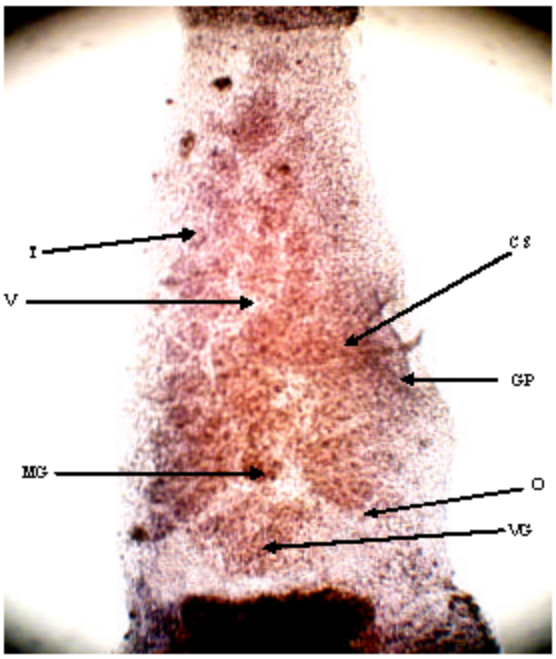

Fig. 2: Morphological characteristics of mature proglottid of *E. granulosus* group B. CS: Cirrus Sac; GP: Genital Pore; V: Vagina; O: Ovary; MG: Mehlis Gland; VG: Vitelline Gland and T: Testes

the number, arrangement of hooks, total length and blade length of large and small hooks are shown in Table 1 and were compared with other (Thompson *et al*., 1984; Eckert *et al*., 1989).

The comparative morphological features of mature proglottids between the two groups (A and B) were taken into consideration, which include, the total length, position of genital pore, number and distribution of testes and anatomy of the female reproductive system are summarized in Table 2 and Fig. 1 and 2. Such criteria were compared with their counterparts (Thompson *et al*., 1984; Eckert *et al*., 1989).

DISCUSSION

An important part of research on echinococci is their taxonomy at the species and interspecies levels. According to Thompson (1995) and Lymbery (1995) and other investigators, 16 species and 13 subspecies have been described in the historical sequence.

Species taxes of these tapeworms have been classified into a series of other interspecies units such as population and geo-graphical form. Those most often differentiated however, are strains (Schantz *et al*., 1995; Thompson *et al*., 1995). The strains are classified into genetically distant clones of the same strain of *Echinococcus* (Lymbery, 1995). Some researchers also, use the term isolate (Bowles *et al*., 1995). Since, opinions on the number of species and on their differential diagnostic characters in the genus *Echinococcus* remain inconsistent, further studies are required on their morphology and other phenotypical and genotypical characters primarily in the localities where these cestodes have not been adequately studied (Dubinsky *et al*., 1998).

This is why research on *E. granulosus* has also, been intensified in different part of world. Emphasis is laid on the differentiation of strains of individual species in general and on the identification of characteristics of dog/sheep strain of *E. granulosus* in particular. It is necessary to ascertain whether there is a single strain of *E. granulosus* adapted to sheeps or whether, >1 strain is capable of being perpetuated in a dog/sheep cycle (Thompson and Mcmanus, 2001).

The present studies on multiple cysts of dog/sheep cycle coming from the Nineveh province, North of Iraq, however, revealed a great morphological and developmental variability of individual cestodes. The variability was documented first of all by scolex of hooks. Specimens of group A support previous suggestion that there is a form of *E. granulosus* that is adapted to sheep and may represent a distinct strain, on the other hand the specimens of group B seem to be identical to those of cattle strain (Eckert and Thompson, 1988) (Table 1).

The characters differentiating *E. granulosus* strain also include the number and arrangement of testes. According to Eckert *et al.* (1993) in the dog/sheep strain the testes are arranged in 1 row posterior to the vitellarium. In the specimens of group A this parameter is typically. While, in specimens of group B, they have similarities with dog/cattle strain in the distribution of testes in that they do not exceed the anterior half of the vitelline gland (Eckert and Thompson, 1988) (Table 2).

The most important features differentiating between the two strains include the shape of the ovary in adult forms (Eckert *et al*., 1989). The specimens of group A refer to dog/sheep strain by having compact lung-shaped ovary Fig. 1. While, the specimens of group B resembling to dog/cattle strain in which the ovary have elongated with well-demarcated lobules forms (Eckert *et al*., 1989). Similarly, specimens of group B differ from those of horse isolate in the shape and position of the cirrus sac (Kumaratilke *et al*., 1986) (Fig. 2).

CONCLUSION

The results obtained imply that increased attention should also, be paid to the variability of echinococci within individual host or prerequisite to confirm the possibility of existence of >1 strain of *E. granulosus*. This will open new clues in the mutter of host specificity of dog/sheep strain. As becomes obvious, the variability of a single host may overlap not only with the morphology of various strains but also with the specificity of dog/sheep strain, as has been documented by previous researchers in different parts of the world (Thompson and Mcmanus, 2001).

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