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Original Article

Morphometric Analysis of Larval Rostellar Hooks in *Taenia multiceps* of Sheep in Iran and Its Association with Mitochondrial Gene Variability

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Abstract

Background: The purposes of the present study were morphometric characterization of rostellar hooks of *Taenia multiceps* and to investigate the association of hook length variation and the variability within two mitochondrial genes of sheep isolates of the parasite.

Methods: Up to 4500 sheep brains were examined for the presence of *C. cerebralis*. Biometric characters based on the larval rostellar hook size were measured for each individual isolate. Representative mitochondrial CO1 and 12S rRNA gene sequences for each of the isolates were obtained from NCBI GenBank. Morphometric and genetic data were analyzed using cluster analysis, Interclass Correlation Coefficient (ICC) and random effects model.

Results: One hundred and fourteen sheep (2.5%) were found infected with the coenuri. The minimum and maximum number of scoleces per cyst was 40 and 550 respectively. Each scolex contained 22–27 hooks arranged in two rows of large and small hooks. The average total length of the large and small hooks was 158.9 and 112.1 µm, respectively. Using ICC, statistically significant clusters of different hook sizes were identified within the isolates. The length of the large and small hooks was significantly associated with the variability in mitochondrial 12S rRNA gene.

Conclusion: *Taenia multiceps,* is a relatively important zoonotic infection in Iranian sheep with the prevalence rate of 2.5%. Hook length analysis revealed statistically significant difference among individual isolates. Associations between the rostellar hook length and variability in the mitochondrial 12S rRNA was documented.

Introduction

Coenurosis, also known as gid or sturdy, is a larval helminth infection of herbivorous animals. It is caused by the metacestode stage of *Taenia multiceps*, *Coenurus cerebralis*. Adult tapeworm of *T. multiceps* inhabits small intestine of some domestic and wild carnivores e.g. dogs, Jackals, foxes and coyotes. Eggs excreted in the environment by the definitive hosts are ingested by herbivorous intermediate hosts including sheep, goat, horse, cattle, camel, deer and pig. As a result the oncosphere passes through the intestinal wall and via bloodstream primarily localizes in the CNS. This causes neurological symptoms and even death in young animals (1-3).Furthermore coenurosis is a zoonotic disease in which human may be accidentally infected and subsequently be suffered fromserious neurological problems. A few human cases has been reported from different countries including Italy, Egypt and the United States (4, 5).

Iran is an endemic region for *T. multiceps*. Several studies indicated different prevalence rates in different regions. The infection rate of *C. cerebralis*, varied from 0.32-18.7% in sheep, goat, and wild sheep. Ovine cenurosis has been reported in 18.7% (6), 9.8% (7), 3.8% (8) and0.3% (9) of animals. Investigations on definitive hosts in different endemic regionsof Iran indicated rather high rates between 3 and 40% (10-13) in dogs, 7.5% in jackals,18.2% in foxes and 40% in wolves (10, 13). In other parts of the world similar prevalence rates of ovine cenuriasis have been recorded e.g. 3% in Jordan (1), 1.3–36.8% in Turkey (14, 15), and 2.5% in Bangladesh (16). Achenef et al. recorded prevalence rate ranging from 2.3 to 4.5% of sheep in Kenya (2). Significant economic losses due to livestock morbidity and mortality caused by *T. multiceps* have been documented in several investigati-onsi-n endemic countries (17, 18). In Iran the financial damage resulting from the condemnation of meat and viscera of sheep due to coenurosis is estimated about US$ 15,700 (7).

Rostellar hook characters have been used to differentiate species and strains of cyclophyllidean cestodes. One accepted method for distinguishing taeniid tapeworms at intra- and inter-specific levels has been the use of larval rostellar hook dimensions particularly total length of large and small hooks (19-24). Due to their keratin-like contents, rostellar hooks are hard and are not affected by different physical and chemical factors. Hook measurement is not a complexprocedure and this makes hook morphometry a suitable tool for identification of different species of tapeworms.

Hook size is believed to be influenced by a combination of genetic and host factors. Using enzyme electrophoresis on six loci in the taeniid tapeworm, *Echinococcus granulosus*, Lymbery (1998) showed that the total larval hook lengths particularly the total length of small hooks was the most affected hook character in the isolatesfrom different intermediate hosts (25). Similar studies have been conducted on other organisms. For example in bacteria a sound genetic basis is documented for the flagellar morphology in *Salmonella* and *Shigella* (26). In passerine birds' microsatellite and mtDNA variations have been significantly associated with phenotypic traits like bill length (27). However no study has been undertaken to investigate possible association of variability within different genes and the larval rostellar hook length in taeniid cestodes.

This study was conducted to investigate the rostellar hook morphometry and the influence of mitochondrial gene variations on the hook length in sheep isolates of *T. multiceps*.

Materials and Methods

Parasite collection

A total of 4500 sheep heads were examined for the presence of *T. multiceps* metacestodes in the period of October 2010 to May 2011 in

three major food processing companies in Tehran, Alborz and Qom provinces of Iran.After opening the skulls, coenuri were detached from the brain and were transferred to the Helminthology lab in the School of Medicine, Kerman University of Medical Sciences. The metacestodes were then rinsed three times in normal saline and the specimens were stored at -20°C until used.

Morphometric study

The fluid-filled coenuri were contained several scoleces surrounded by a thin, transparent membrane. All the scoleces within each coenure were counted and biometric characters based on the larval rostellar hook size were measured. For each metacestode five scoleces were randomly selected. Each individual scolex was dissected under a stereomicroscope. The rostellum was carefully cut and transferred on a slide. Total lengths of each of three large and three small hooks per scolex were measured by a calibrated eyepiece micrometer under medium power magnification (Fig. 1). All measurements were taken by a single person (S.R).Representative mitoch-ondrial CO1 and 12S rRNA gene sequences of all the isolates were obtained from NCBI GenBank to find out possible association between mitochondrial gene variability and hook morphometry.

Statistical analysis

The large and small hook length data as well as the corresponding CO1 and 12S rRNA haplotypes were managed in the Statistical Package for the Social Sciences (SPSS, v. 21). Cluster analysis was applied to classify the subjects into homogeneous subgroups using Interclass Correlation Coefficient (ICC). Random effects model was applied to estimate how much of variation in thehook length was attributable to the genetic differences between the subjects. Dendrogram and scatter plot were generated based on the large and small hook length using hierarchical cluster analysis.

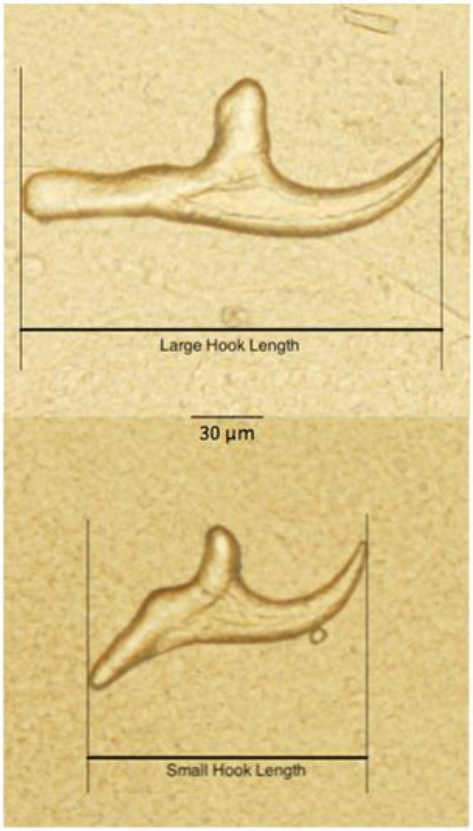

Fig. 1: Measurement of the large and small rostellar hooks of *Taenia multiceps* as used in the present study

Results

Inspection of 4500 sheep brains revealed that 114 (2.5%) heads were infected by *T. Multiceps* metacestodes. The minimum and maximum number of scoleces per cyst was 40 and 550 respectively. Each scolex contained 22–27hooks arranged in two rows ofthelarge and small hooks. The average total length of thelarge and small hooks was 158.9 µm (range: 110-195) and 112.1 µm (range: 63-132), respectively.

Significant ICC's were obtained from random effects models showing that the large and small hook length*s are* significantly different among *T. multiceps* isolates (P<0.001, Table 1). The results indicated that respectively 57.0% and 22.6% of variation in large and small hook

lengths are attributable to different individuals in *T. multiceps* isolates. This means that based on large and small hook length, statistically significant clusters are distinguishable within the isolates.The results of hierarchical analysis are presented as a dendrogram in Fig. 2.

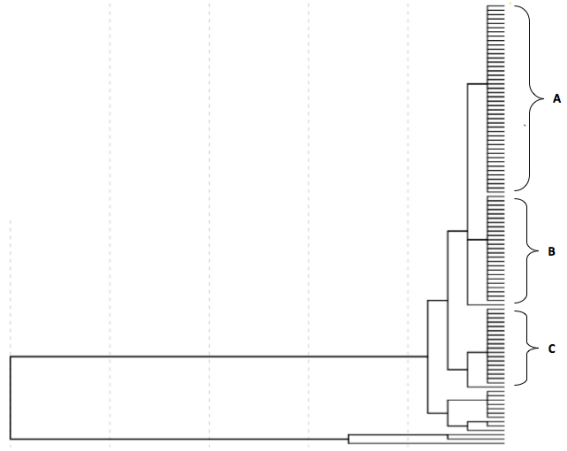

Fig. 2: Dendrogram generated by hierarchical cluster analysis using large and small hook length of 102 Iranian sheep isolates of *Taenia multiceps*, showing the majority (85.3%) of the isolates clustered in three main sub clades: A (43.1%), B (24.5%) and C (17.6%)

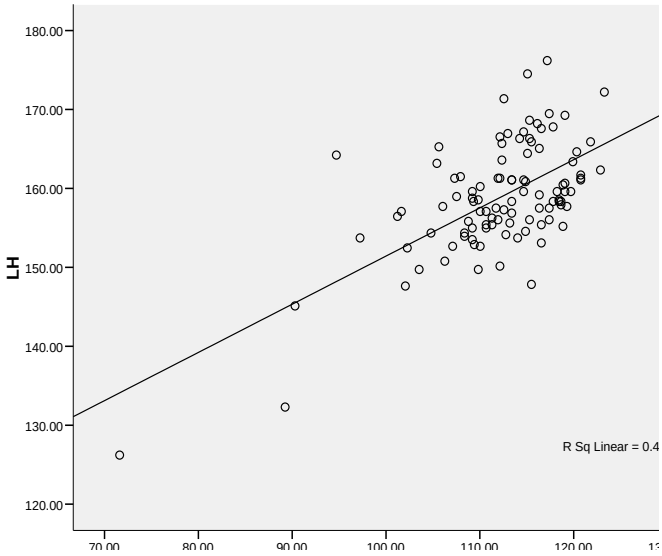

Fig. 3: Scatter plot of the length of large (LH) and small (SH) hooks for 102 sheep isolates of *Taenia multiceps* from Iran

The dendrogram contained two main clades one of which comprised 97.1% of the isolates.Subclades A, B and C contained the majority of the isolates i.e.44 isolates (43.1%) in the subclade A, 25 isolates (24.5%) in the subclade B and 18 isolates (17.6%) in the subclade C.No associations were found between hook length and CO1 gene variability, however 12S rRNA variability was significantly associated with theboth large and small hook length (Table 1).

Table 1: Inter-class Correlation Coefficient (ICC) of large and small hook length differences within sheep isolates of *Taenia multiceps* and between hook length values and two mitochondrial genes variability

Hook length	ICC	*P* value
LHL[1]	0.57	0.000
SHL[2]	0.23	0.000
Gene-Hook length		
CO1[3]-LHL	0.11	0.22
CO1-SHL	0.04	0.17
12S rRNA[4]-LHL	0.38	0.002
12S rRNA-SHL	0.08	0.003

1. Large Hook Length. 2. Small Hook Length. 3. Cytochrome c oxidase subunit I. 4. 12S ribosomal RNA

Discussion

Coenurus cerebralis is a serious disease of herbivores with a worldwide distribution caused by the larval form of the cestode *T. multiceps*. Different prevalence rates for coenurosis have been reported depending on various geographical, climatic and socio-economic conditions as well as environmental factors and livestock husbandry systems(28). Coenurosis is more prevalent in developing countries of Africa and Asia(2). Apparently, estimating precise prevalence of coenurosis is difficult because animal brains are not usually inspected during routine veterinary examinations.

According to the present study the prevalence of ovine coenurosis was 2.5%.Previous studies in Iran indicated a range of relative frequency between 0.3 and 18.7%. The pre-

sent prevalence is higher than those obtained by Yuossefi (0.3%) in Iran, Abedl-Maogood (2005) in Egypt (1.5%) and Scala et al. (2007) in Italy (0.35%)(9, 29, 30).Other studies indicated higher prevalences in Urmia (18.7%), Tabriz(3.8%) and Shiraz(9.8%) (6-8).In the neighboring Turkey similar prevalence rates were recorded as 1.3-36.8%(14, 15). In Bangaladesh and Ethiopia the prevalence of *T. multiceps* metacestode was obtained as 2.5% and 2.7% respectively (2, 16). Regarding the relatively high prevalence of ovine cenuriasis in Iran and the resulting economic losses due to the disease (7), implementation of control and prevention programs in the endemic regions are recommended.

The present study revealed that the average number of scoleces in the metacestode is 85 with a range of 40-550 scoleces per coenure. Our finding is almost similar to the findings of other studies in which the highest number of scoleces per cyst reached 550 ranging from 10 to 370 scoleces per cyst (2, 31-33). Presence of different numbers of scoleces may be related to the differences in the age of the coenuri.

Table 2 compares the rostellar hook morphometric characters of *T. multiceps* derived from existing data in the literature.The results of morphometric study showed that the mean± SD total length of the large and small hooks were 158.9± 9.3 µm and 112.1± 9.4 µm respectively. The range of large hook length was 110.3-195.3 µm, and 63.0-132.3 µm for the small hooks. As it is illustrated in the scatter plot (Fig.3) hook lengths of the majority of the isolates were found to be 150-165 µm and 105-120 µm for the large and small hooks respectively. The classical work of Verster indicates the average large and small hook lengths as 166.7 and 125.0 respectively (23). Our results are in agreement with those of Verster as well as the other published morphometric studies on *T. multiceps* hooks (Table 2).

Table 2: Comparison of *Taenia multiceps* rostellar hook number and size obtained from different studies

Author	No. of hooks	Total Hook Length Min-Max in µm		Reference
		Large hook	Small hook	
Verster	22-30	157-177	98-136	(23)
Hall	22-32	150-170	90-130	(37)
Clapham	24-32	120-170	76–130	(38)
Elowni et al.	ND	120-190	73-150	(39)
Abedl-Maogood	ND	130-156	78 - 104	(29)
Edwards and Herbert	20-32	132-171	81–126	(22)
Rostami et al.	22-27	110.2-195.3	63.0-132.3	Present study

Based on the small and large hook values for each individual isolate, two main clusters were identified in the dendrogram (Fig.2). Most of the isolates were located in the three main sub clades A, B and C. However, morphologically defined variants have not been described in *T. multiceps* so far. Varcasia et al. described genetic diversity within Sardinian populations of *T. multiceps*, however morphometric analysis was not carried out on that population (34). Obviously more comprehensive morphological studies in other regions are required to clarify possible morphometric diversity within *T. multiceps* populations from different intermediate hosts.

Mixed model analysis in the present study established a significant association between 12S rRNA variability and larval rostellar hook lengths. According to the results of the present study 38% and 8% of the large and small hook

length variations are significantly attributable to sequence variations in 12S rRNA gene respectively ($P<0.05$). However the corresponding values for CO1 were not statistically significant (Table 1). Taeniid rostellar hook size is known to be affected by genetic and environmental factors. Our knowledge on genetic basis and determinants of hook characters is very limited. Rostellar hooks are known to be made of keratin-like proteins (35, 36), however further genomic studies on keratin-related genes are required to improve our understanding on the genetic basis of larval hook development.

Conclusion

Prevalence rate of ovine cenuriasis was 2.5%. Hook length analysis revealed statistically significant difference among individual isolates, indicating intraspecific variation within *T. multiceps* in Iran. Morphometric analyses in the present study showed an association between the rostellar hook length and sequence variability in the mitochondrial 12S rRNA.

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