Türkiye Parazitoloji Dergisi, 33 (2): 199 - 202, 2009
© Türkiye Parazitoloji Derneği

Türkiye Parazitol Derg.
© Turkish Society for Parasitology

The Detection of *Echinococcus granulosus S*trains Using Larval Rostellar Hook Morphometry

Kader YILDIZ[1], I. Safa GURCAN[2]

[1]Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Kırıkkale,
[2]Ankara University, Faculty of Veterinary Medicine, Department of Biostatistic, Ankara, Türkiye

SUMMARY: The purpose of this study was to determine the morphometrical characteristics of the larval hooks of *Echinococcus granulosus* in Turkey. The number of rostellar hooks (NH) and the total length of long blades, length of the blade of the long hooks, the total length of small blades and the length of the blade of the small hooks were measured in sheep and cattle isolates. The principal component and discriminant function analyses were used to analyze the data. Rostellar structure of protoscoleces was very similar in the sheep and cattle samples. According to the correlation matrix, the hook number and the hook length was negatively correlated. However, the correlation between the hook lengths was positive. It was found that the morphometric characteristics of the samples from the sheep and cattle closely resembled each other.

Key Words: *Echinococcus granulosus*, protoscoleces, rostellar morphometry

Protoskoleks Çengel Morfometrisi Kullanılarak *Echinococcus granulosus* Suşunun Tespiti

ÖZET: Bu çalışmada *Echinococcus granulosus* protoskoleks çengellerinin morfometrik karakterini belirlemek amaçlanmıştır. Koyun ve sığır *E.granulosus* izolatlarındaki protoskolekslerin çengel sayısı, uzun çengelin toplam uzunluğu, uzun çengeldeki kılıç uzunluğu, kısa çengelin toplam uzunluğu ve kısa çengeldeki kılıç uzunluğu mikrometrik okuler kullanılarak ölçülmüştür. Elde edilen veriler principal component analizi ve discriminant fonksiyon analizi kullanılarak değerlendirilmiştir. Koyun ve sığır izolatlarındaki protoskolekslerin çengel yapısının oldukça benzer olduğu gözlenmiştir. Korrelasyon matriksine göre çengel sayısı ve çengel uzunluğu arasında negatif, buna karşılık çengel uzunlukları arasında ise pozitif ilişki olduğu belirlenmiştir. Koyun ve sığıra ait örneklerde morfometrik karakterlerin oldukça benzer olduğu izlenmiştir.

Anahtar Sözcükler: *Echinococcus granulosus*, protoskoleks, morfometri

INTRODUCTION

Echinococcus granulosus is a zoonotic parasite belonging to the genus *Echinococcus*. Larval infection, cystic echinococcosis, is characterised by long term development of hydatid cysts in the intermediate host (5, 6). This parasite seems to be the major cause of human cystic echinococcosis in Turkey like in the other parts of the world. The approximate surgical case rate of cystic echinococcoccosis is 0.87-6.6/100 000 people in Turkey (2).

Several reports have been found on *E.granulosus* infection in livestock animals in Turkey (8, 14 16, 25, 26). The prevalence of infection has varied from 5.9 to 50.9% in sheep (14, 25), from 4.5 to 31.25% in cattle (8, 26) and from 1.6 in goats (14). Stray dogs with high rates of echinococcosis (28-40.5%) play an important role in epidemiology of echinococcosis in Turkey (3, 22).

Makale türü/Article type: **Araştırma / Original Research**
Geliş tarihi/Submission date: 10 Şubat/10 February 2009
Düzeltme tarihi/Revision date: 26 Mayıs/26 May 2009
Kabul tarihi/Accepted date: 30 Haziran/30 June 2009
Yazışma /Correspoding Author: Kader Yıldız
Tel: (90) (318) 357 33 01 Fax: (90) (318) 357 33 04
E-mail: kaderyildiz@hotmail.com
The study was presented in the 3rd National Congress of Hydatidology (06-09 September 2006, Samsun, Turkey).

The intraspecific variation in *E.granulosus* may be related with host specifity, development rate, life-cycle patterns, sensitivity to chemotherapeutic agents and pathology (6, 21). *E.granulosus* has different subspecific variations (G1-10) (6, 17). Sheep strain (G1) is the most common strain which is widespread especially in Mediterranean countries (17). The life cycle of this strain is nearly domestic, dogs as definitive and sheep as intermediate hosts in general (6). A recent molecular genetic study revealed that G1 to G3 strain are now designated as *E.granulosus* sensu stricto (13).

Different methods, based on morphology, physiology, biochemistry or molecular genetics, have been used for strain differentiation of *E.granulosus* (6, 17). In morphological analysis, the strains of *Echinococcus* can be identified by the shape, size and number of the rostellar hooks of protoscoleces (1, 7, 9, 11, 15, 18, 19).

The larval hook characters remain mostly unchanged by passage through different definitive hosts (4). The measurements of hook characters in adult worms can provide a useful tool for determining the transmission route of *E.granulosus* (4).

The data on the morphology of the protoscoleces isolated from Turkish cystic echinococcosis is lacking. The aim of the present study was to determine the morphometrical characteristic of the larval hooks of *E.granulosus* in Turkey.

MATERIAL AND METHODS

In the present study, hydatid cysts were collected in liver and lung of naturally infected sheep (n: 50) and cattle (n: 10). Protoscoleces were collected in hydatid cysts aseptically in laboratory and were fixed in glycerine alcohol (1:1). Each individual cyst was considered as a sample or isolate. These samples were placed in polyvinyl lactophenol on slide. A slight pressure was applied on cover slide to flatten rostellar hook. The invaginated and live protoscoleces were only analysed, and all the rostellar hook measurements were made by one investigator. The protoscoleces were viewed on light microscope (BX50 light microscopy, Olympus Optical Co., Ltd., Tokyo, Japan) with immersion oil using objectives x100. The rostellar hooks were measured using micrometric ocular.

The rostellar hook number (NH) and arrangement of hooks were observed. Long blade long (LBL), long total hook long (LTL), small blade long (SBL) and small total hook long (STL) were measured on hooks of protoscoleces selected randomly. The rostellar hook number was taken on 30 different protocoleces. The LBL, LTL, SBL, STL measurement were measured on 10 different protoscoleces of each cyst samples (3+3) (9).

Five variables (NH, LBL, LTL, SBL and STL) were evaluated statistically. The principal component analysis (PCA) and the discriminant function analysis (DFA) were used to analyse the data. All procedures were carried out using version 15.0 of the SPSS software package program.

RESULTS

In the present study, the rostellar structure of protoscoleces investigated was very similar (Figure 1). Table 1 shows the morphologic structure of rostellar hook. The relationship between variations in samples analyzed with PCA was seen Table 2. According to correlation matrix, the relationship between NH and the hook longevity variation was weak and negative. However, the relationship between the hook longevity was positive and strong. The positive relationship among longevity measurements was seen in Figure 2.

The principal component analysis was used to detect the relationship between groups and characters. The first and the second components consisted of the hook longevity and the hook number, respectively. These two components represented 79.38 % of the total variation. When samples plotted with two components, only one group was observed (Figure 3). According to Figure 2, the cattle samples were located in the sheep samples.

Table 1. Rostellar hook characteristics of the protoscoleces from animal samples

	Sheep origin (n: 50)	Cattle origin (n: 10)
No.of hooks analysed (NH)	24-42	28-42
Large hooks		
Blade lenght (LBL)*	20-28	22-27
Total lenght (LTL)*	9-15	11-14
Small hooks		
Blade lenght (SBL)*	12-25	17-23
Total lenght (STL)*	6-12	7-9

*Lenghts in µm

Table 2. Correlation matrices for the five variables considered (NH, LTL, LBL, STL and SBL)[a]

Samples	NH	LTL	LBL	STL	SBL
Animal (n.60)					
NH	1				
LTL	-,040	1			
LBL	,078	,743*	1		
STL	-,005	,874*	,658*	1	
SBL	,178	,386*	,618*	,429*	1

[a] **NH**: The rostellar hook number, **LTL**: Long total hook long, **LBL**: Long blade long, **STL**: Small total hook long, **SBL**: Small blade long
*Correlation is significant at the 0.05 level (2 tailed)

Table 3. Results from discriminant function analysis were supplied animal samples.

Group		Predicted group membership		Total
		Sheep	Cattle	
Original	sheep	38 (76 %)	12 (24%)	50
	cattle	1 (10 %)	9 (90%)	10

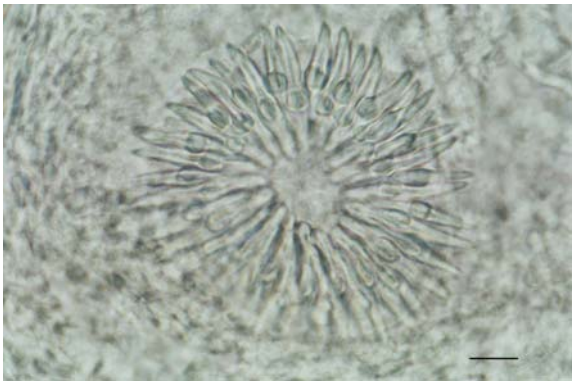

Figure 1. Rostellar hooks raw of protoscolex obtained from hydatic cyst (with immersion oil). Bar: 10 micron.

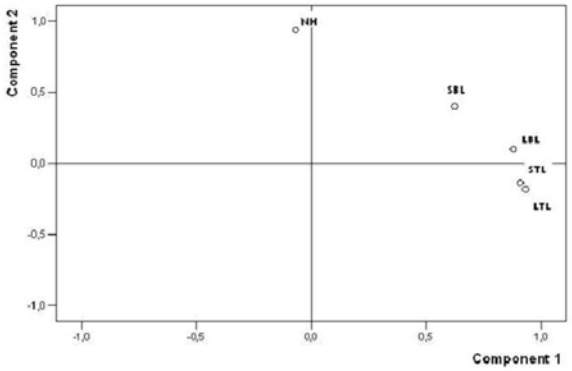

Figure 2. The positive relationship among longevity measurements with Principal Component Analysis in animal samples.

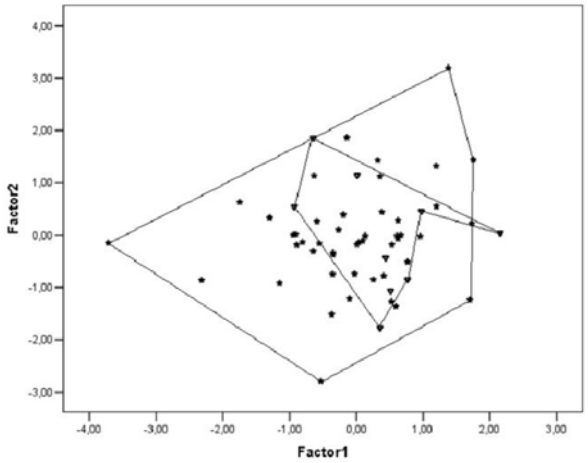

Figure 3. Plot of 60 animal samples of *E.granulosus* for the 2 components extracted by Principal Component Analysis.
Key to symbols: (*) sheep, (Δ) cattle samples.

Variables which affect the differentiation were selected using Wilk's lambda. The hook number, LTL and LBL characters were detected in differentiation analysis as a functionary characters. The rate of animal species using DFA was seen in Table 3. The hook number, LTL and LBL measurements used were detected in hydatid cysts as 76% in sheep and 90% in cattle with DFA analysis. According to the analysis, the sheep and cattle samples were in close relationship due to analysed characters.

DISCUSSION

Turkey is an endemic area for *E.granulosus* according to the results of different studies in the country (5, 8, 14, 16, 25, 26).

For differentiated strains of Echinococcus, several rostellar characters have been used in various studies (1, 7, 9, 11, 15, 18, 19). In the present study, 30 protoscoleces per sample were used to determine the mean number of rostellar hooks per protoscolex. Three large and three small hooks of each rostellum have been measured. Ten protoscolex per sample were used for the hook measurements.

Among the rostellar hook characters analysed, two functional characters (length of hooks and number of hooks) could be grouped in the present study. This result agrees with that of other studies (1, 7, 15).

Analysing of morphological, biochemical and genetic data of *Echinococcus* have been done using different statistical techniques (4, 9, 15). The results of such analyses have been used as the basis for a major taxonomic revision of the genus (21). In the present study, the results of statistical analysis conducted on the morphology data was similar with the results obtained from genetic analysis (23, 24, 27). The result of DNA analysis study indicated that there is one distinct strain of *E.granulosus* (G1, the sheep strain) in Turkey (23, 24, 27). This strain nominated as *E.granulosus* sensu stricto with G2 and G3 strain recently (13).

Sheep strain of *E.granulosus* consisted of sterile cysts in cattle and pig in general, whereas most cysts were found fertile in sheep tissue (10). The fertility rate was detected in hydatid cysts of sheep liver and lung as 81.53% and 76.47%, respectively while the fertility rate was reported in cattle as 6.6% in previous studies (25, 26). This situation supports the results obtained from the samples examined with morphometric analysis in present study as sheep strain.

Morphology is considered the basis of the both identification and taxonomy in *Echinococcus* genus (12). Also, the hook characteristic remained unchanged even after passage during the definitive hosts (9) and can be used to determine the origin of infections in the carnivorous definitive hosts (4). It is suggested that the morphology is rapid and economical strain identification method for *E.granulosus* in epidemiological research (9, 20). The practical values of using morphology for differentiating between strains of *Echinococcus* were reported and claimed that both sheep and camel strains can be readily differentiated only using of hook morphology (7). However, host-induced morphological variation is problem (9, 18). For this reason, the results from morphological analysis are in agreement with data from other techniques such as genetic analysis.

The results of the present study support those of previously genetic studies based on DNA analysis (23, 24, 27). According to this, *E.granulosus* sensu stricto is distinguishable by rostellar morphology in Turkey. Also, morphometry has potential use in epidemiological studies of cystic echinococcosis in this country.

ACKNOWLEDGEMENT

This work was supported by a grant from the Kırıkkale University Research Fund (Project no: 03/09.02.02)

REFERENCES

1. **Ahmadi NA,** 2004. Using morphometry of the larval rostellar hooks to distinguish Iranian strains of *Echinococcus granulosus*. *Ann Trop Med Parasitol,* 98: 211-220.
2. **Altintas N,** 2003. Past to present: echinococcosis in Turkey. *Acta Trop,* 85: 105-112.
3. **Ataş AD, Özçelik S, Saygı, G,** 1997. Sivas sokak köpeklerinde helmint türleri ve bunların halk sağlığı bakımından önemi. *Turkiye Parazitol Derg,* 21: 305-309.
4. **Constantine CC, Thompson RCA, Jenkins DJ, Hobbs RP, Lymbery AJ,** 1993. Morphological characterization of adult *Echinococcus granulosus* as a means of determining transmission patterns. *J Parasitol,* 79: 57-61.
5. **Eckert J, Deplazes P,** 2004. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev,* 17: 107-135.
6. **Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS,** 2001. WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern. World Organisation of Animal Health, Paris, France.
7. **Fasihi Harandi M, Hobbs RP, Adams PJ, Mobedi I, Morgan-Ryan UM, Thompson RCA,** 2002. Molecular and morphological characterization of *Echinococcus granulosus* of human and animal origin in Iran. *Parasitology,* 125: 367-373.
8. **Gıcık Y, Arslan MO, Kara M, Köse M,** 2004. Kars ilinde kesilen sığır ve koyunlarda kistik ekinokokkozisin yaygınlığı. *Türkiye Parazitol Derg,* 28: 136-139.
9. **Hobbs RP, Lymbery AJ, Thompson RCA,** 1990. Rostellar hook morphology of *Echinococcus granulosus* (Batsch, 1786) from natural and experimental Australian hosts, and its implications for strain recognition. *Parasitology,* 101: 273-281.
10. **Kamenetzky L, Canova SG, Guarnera EA, Rosenzvit MC,** 2000. *Echinococcus granulosus:* DNA extraction from germinal layers allows strain determination in fertile and nonfertile hydatid cysts. *Exp Parasitol,* 95:122-127.
11. **Kumaratilake LM, Thompson RCA,** 1984. Morphological characterization of Australian strains of *Echinococcus granulosus*. *Int J Parasitol,* 14: 467-477.
12. **McManus DP, Bowles J,** 1996. Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. *Int J Parasitol,* 26: 687-704.
13. **Nakao M, McManus DP, Schantz M, Craig PS, Ito A,** 2007. A molecular pylogeny of the genus *Echinococcus* inferred from complete mitochondrial genomes. *Parasitology,* 134: 713-722.
14. **Oge H, Kalınbacak F, Gıcık, Y, Yıldız K,** 1998. Ankara yöresinde kesilen koyun, keci ve sigirlarda bazı metasestodların (*Hidatid kist, Cysticercus tenuicollis, Cysticercus bovis*) yayılışı. *Ankara Univ Vet Fak Derg,* 45: 123-130.
15. **Ponce Gordo F, Cuesta Bandera C,** 1997. Differentiation of Spanish strains of *Echinococcus granulosus* using larval rostellar hook morphometry. *Int J Parasitol,* 27: 41-49.
16. **Poyraz O, Özçelik S, Saygı G, Genç S,** 1990. Sivas Et ve Balık Kurumu Kombinasında kesilen koyun ve sıgırlarda kist hidatiğin görülme oranları. *Türkiye Parazitol Derg,* 14: 41-44.
17. **Romig T, Dinkel A, Mackenstedt U,** 2006. The present situation of echinococcosis in Europe. *Parasitol Int,* 55: 187-191.
18. **Sweatman GK, Williams RJ,** 1963. Comparative studies on the biology and morphology of *Echinococcus granulosus* from domestic livestock, moose and reindeer. *Parasitology,* 53: 339-390.
19. **Tashani OA, Zhang LH, Boufana B, Jegi A, McManus DP,** 2002. Epidemiology and strain characteristics of *Echinococcus granulosus* in the Benghazi area of eastern Libya. *Ann Trop Med Parasitol,* 96: 369-381.
20. **Thompson RCA, Lymbery AJ,** 1988. The nature, extent and significance of variation within the genus *Echinococcus*. *Adv Parasitol,* 27: 209-258.
21. **Thompson RCA, McManus DP,** 2003. Towards a taxonomic revision of the genus *Echinococcus*. *Trends Parasitol*, 18: 452-457.
22. **Umur S, Aslan MO,** 1998. Kars yöresi sokak köpeklerinde görülen helmint türlerinin yayılışı. *Turkiye Parazitol Derg*, 22: 188-193.
23. **Utuk AE, Simsek S, Koroglu E, McManus DP,** 2008. Molecular genetic characterization of different isolates of *Echinococcus granulosus* in east and southeast regions of Turkey. *Acta Trop,* 107: 192-194.
24. **Vural G, Baca AU, Gauci CG, Bagci O, Gicik Y, Lightowlers MW**, 2008. Variability in the *Echinococcus granulosus* cytochrome C oxidase 1 mitochondrial gene sequence from livestock in Turkey and a re-appraisal of the G1-3 genotype cluster. *Vet Parasitol,* 154: 347-350.
25. **Yıldız K, Gurcan S,** 2003. Prevalence of hydatidosis and fertility of hydatid cysts in sheep in Kirikkale, Turkey, *Acta Vet Hung,* 51: 181-187.
26. **Yıldız K, Tuncer C,** 2005. Kırıkkale'de sığırlarda kist hidatik'in yayılışı. *Türkiye Parazitol Derg,* 29: 247-250.
27. **Yolasigmaz, A, Turcekova L, Turk M, Reyhan E, Snabel P, Dubinsky P, Gunes K, Altıntas N,** 2004. Genetic variation in *Echinococcus granulosus* from Turkey and Slovakia demostrated by sequence and SSCP analysis. *Inter Arch Hydatid,* 35: 183.