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Morphometric differentiation between camel and sheep strains of *Echinococcus granulosus* using computer image analysis system (CIAS)

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ABSTRACT

Objective: To find importance of morphometric criterion of larval rostellar hook of *Echinococcus granulosus (E. granulosus)* and the easy and reliable method for distinguish sheep and camel strains in epidemiologic studies. **Methods:** Larval rostellar hooks (n=1860) of 31 camel and sheep isolates in Iran, which already had been characterized by PCR, were carefully processed by computerized imagime analysis system (CIAS) and acquired data about rostellar hooks were analyzed using software SPSS. **Results:** Measurement analysis of rostellar hooks [mean length (24.23±3.12) μm] indicated that length of the large hook was a remarkable parameter for strain differentiation. Data analysis demonstrated that CIAS could be used as a reliable tool to distinguish camel from sheep strains with high sensitivity (95.2%) and specificity (91.5%). **Conclusions:** CIAS as a specific, sensitive, economic, fast, and reliable means might be used for differentiation of *E. granulosus* strains. Although perimeter and area were measured by digital technology, they were not shown as discriminative criterion as total hook length did.

1. Introduction

Hydatid cyst due to *Echinococcus granulosus (E. granulosus)* is an important zoonotic helminthic disease throughout the world[1]. Despite of dramatically reduction of many other helminthic infections in Iran, human hydatidosis is still of great public health importance[2,3]. Regarding to different genetically distinct strains of *E. granulosus* that may affect the patterns of transmission among wide variety of intermediate hosts including human beings, a raising interest on characterization of its variants has been arisen. Different methods consisting of old fashion morphology, biochemistry, immunology, physiology and molecular genetics have been employed to determine *E. granulosus* bio-diversity[4-6]. Literatures witness the fact that there are some strains of *E. granulosus*, which could cause human hydatidosis poorly. Concerning the host specificity in Iran, sheep strain which has been isolated from cattle, goat, human and sheep itself seems to be the most common variants. Accordingly, the role of sheep strain in public health is remarkable[7].

Until recently by the time of flourishing modern techniques such as polymerase chain reaction (PCR), morphometric morphology had been widely used as the merely criterion in helminthes taxonomy. Measurement of morphological characteristics for both adult and protoscoleces of this tiny cestod has always been a feasible means of differentiation among the samples obtained from different definitive and intermediate hosts. Although the number of hooks in protoscoleces has not been stated as a reliable tool for strain classification itself, the length of hooks, however, was always distinguishing. To date based on morphometric analysis carried out on hooks from isolated hydatid cysts, two distinct strains of *E. granulosus*, including "sheep" and "cattle" are distributed among herbivores in Iran. Understanding the origin of infection in final host is another attractive viewpoint in Echinococcosis/hydatidosis when the circulation of parasite in feral and domestic lifecycle

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becomes controversial. This can have implications for surveillance and control. Therefore, according to previous publications as the larval hook characteristics will remain unchanged even during the passage from intermediate to definitive hosts, thus the careful measurements could be helpful to determine the origin of infection among carnivorous final hosts[8,9]. Within similar findings in Iran, measurements analyzed on rostellar hooks from human isolates have indicated their relevancy to sheep strain[10].

Subsequent to speedy growth of digital technology and its extension to manufacturing precise cameras beside the arty software, which are perpetually advancing, a new means of imaging and measurements have provided in the field of sciences with a broad range of utility. Computer imaging analysis system (CIAS), as a practical means of digital taxonomy, has recently been appeared as a new term in parasitology and has been employed in helminthic taxonomy. Taking advantage of this reliable method is reflected in papers describing characteristic parameters of *Fasciola hepatica* (*F. hepatica*) and related species[11,12]. Biometrical analysis on *E. granulosus* rostellar hooks; using CIAS for describing its intraspecific variation has also been investigated by Peruvian parasitologists[13].

The present study aimed to differentiate morphometricaly between Iranian camel and sheep strains of *E. granulosus* using CIAS to promote the knowledge of distinguishing different variants of the parasite strains.

2. Materials and methods

Overall, 1 860 protoscoleces of 31 isolates (15 of sheep and 16 of camel) which had already been characterized by PCR in a previous study[14] were selected to be measured and analyzed using CIAS. For each isolate, 10 protoscoleces were picked up and of every individual, three large, and three small hooks prepared for measurement[7]. Sample size and pattern of measurement for rostellar hooks were performed after reviewing of similar studies[14,15]. In this study, protoscoleces with sufficient pressure were gently crashed under cover slip in a drop of lactophenol solution. Delicate manipulation of cover slip to cause the hook to lie flat was always helpful. Parameters for measurement in CIAS were defined for each large and small rostellar hook as total hook length (THL), largest width length (LWL), handle length (HL), blade length (BL), and area as well as perimeter. In Figure 1, four selected parameters, measured in this study are illustrated.

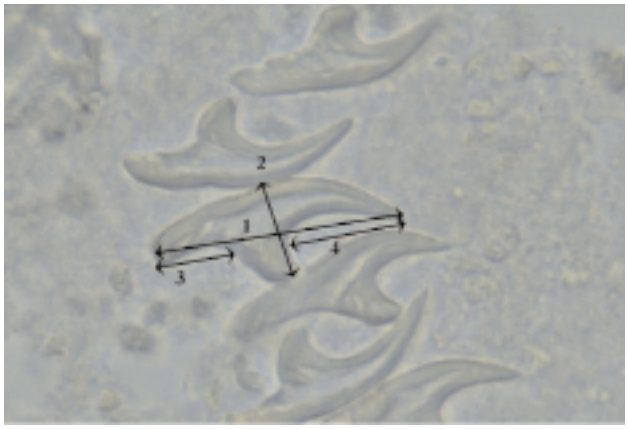

Figure 1. Different parameters selected for morphometric measurements; 1–THL, 2–LWL, 3–HL, 4–BL.

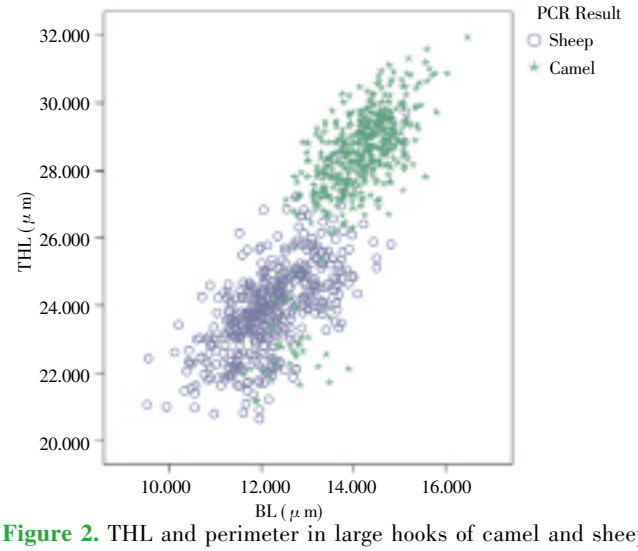

Figure 2. THL and perimeter in large hooks of camel and sheep strains.

Olysia Software (Olysia zoom 3.2 Soft Imaging Systems 2003) compatible with Olympus microscope (SZX12) and digital camera (DP12) installed on a personal computer (Pentium 4 CPU 3.0 GHz) was employed to measure the hooks and data management. According to the protocol planned in the software, calibration was adjusted for all specimens and was rechecked regularly in each session on the system for every magnification.

Collected data were transferred to SPSS ver. 13 and statistical analysis was calculated for all defined criteria. Multivariable logistic regression was used as statistical method to identify the most important factors in distinguishing between sheep and camel strains. Odds ratio of each factor is reported and P-value less than 0.05 were considered as statistically significant.

3. Results

3.1. Descriptive results

Statistical description for 900 hooks (450 large, 450 small) of 15 protoscoleces of sheep strain, and 960 hooks (480 large, 480 small) of 16 protoscoleces of camel strains measured by CIAS system, is demonstrated in Table 1. According to these findings the mean length of hooks for Iranian *E. granulosus* hydatid cyst protoscoleces, was (24.23±3.12) μm. Camel and sheep strains could be significantly distinguished by hook parameters.

3.2. Analytic results

Binary logistic regression showed that correct distinguished percentage of hooks was 82.5%. When small and large hooks were analyzed separately, this percentage increased to 93.3% for the entered parameters of large hooks and 84.0% for entered parameter (BL) of small hooks, respectively. It seems that overall considering large and small hooks made a bias in analysis. These data indicates valuable importance of large hooks in identification of strains. Table 2 shows the

details of these analyses. Table 2 shows the results of fitting multivariable logistic regression between type of strains (sheep/camel) and different measured parameters for all hooks too. According to these findings, percentage correctly identified for sheep is 83.7% and for camel is 81.4%, respectively. Excluding small hooks and usage of large hooks leads to better results. Fitting multivariable logistic regression analysis between type of strains (sheep/camel) and different measured parameters for small hooks showed no notable percentage regarding correct identification of sheep and camel strains. However, results of this analyze considering large hooks demonstrated promising output for this goal, *i.e.* 93.3% and 91.5 for sheep and camel, respectively (Table 2).

3.3. Applicable result

According to above-mentioned calculations, we propose a formula for distinguish camel/sheep strains in ordinary laboratories. If K=211.38 and Index = (THL×BL) − K, then if the Index is less than 0 (Negative value) the case is sheep strain and when the Index is more than 0 (Positive value) the case is camel strain. Our proposed formula, have 86.4% sensitivity and 85% specificity for diagnosis the sheep strains and vice versa for camel strain. Table 5 shows comparing the results of PCR and CIAS techniques. In other words, if THL×BL is more than 211.38, 85% the hook belongs to a camel strain hosteller hook in Iran, and less than that with 86.4% sensitivity, is sheep strain.

4. Discussion

Variability among strains of *E. granulosus* on the scene of parasitology, which is elucidated by showing differences in nucleic acid sequences, has dragged the mind of researches to discuss on other epidemiological aspects of Echinococcosis/hydatidosis rather than taxonomy itself. Those genetic traits illustrated in phenotypes of individuals might be also effective in many other aspects including controlling measures towards cystic echinococcosis[16].

To date, investigations supported by DNA analysis, have introduced 10 genotypes of *E. granulosus* among different animals in the nature[17]. According to documented findings horse and cattle strains, illustrate relatively high intermediary host specificity, while this peculiarity which shows a broader range of intermediate hosts for camel and swine host, is capable to acquire more genotypes[18]. The

Table 1
Mean of different variables of hooks of *E. granulosus* in Iran (Mean ± SE).

Variable	Strain	Large	Small	Mean
THL (μ m)	Sheep	23.94±0.06	21.03±0.07	22.48±0.07
	Camel	28.24±0.08	23.50±0.10	25.87±0.10
BL (μ m)	Sheep	12.23±0.05	8.69±0.04	10.46±0.07
	Camel	14.09±0.03	10.06±0.03	12.08±0.07
LWL (μ m)	Sheep	8.76±0.04	7.55±0.04	8.15±0.03
	Camel	9.99±0.04	8.10±0.04	9.05±0.04
DL (μ m)	Sheep	7.32±0.04	8.60±0.05	7.96±0.04
	Camel	9.35±0.06	9.41±0.06	9.38±0.04
Area (μ m^2)	Sheep	91.76±0.58	73.20±0.50	82.48±0.49
	Camel	123.69±0.72	84.92±0.61	104.31±0.48
Perimeter (μ m)	Sheep	62.01±0.17	54.24±0.19	58.13±0.18
	Camel	71.96±0.21	60.31±0.25	66.14±0.25

Table 2
Results of fitting multivariable logistic regression between type of strains (sheep/camel) and different measured parameters*.

Variable	Hook type	B±SE	*OR*	95% *CI* for *OR*		*P*-value	Correctly identification for/in		
							Sheep	Camel	Overall
THL	All	0.785	2.192	1.840	2.610	0.000	–	–	–
	Large	0.714	2.043	1.291	3.232	0.002	–	–	–
BL	All	−0.195	0.823	0.690	0.979	0.030	–	–	–
	Small	2.058	7.834	6.104	10.060	0.000	–	–	–
	Large	1.377	3.961	2.572	6.099	0.000	–	–	–
LWL	All	−0.679	0.507	0.420	0.611	0.000	–	–	–
	Large	−0.687	0.503	0.324	0.782	0.002	–	–	–
HDL	All	0.405	1.499	1.280	1.760	0.000	–	–	–
Area	Large	0.103	1.109	1.061	1.158	0.000	–	–	–
Perimeter	Large	−0.245	0.783	0.654	0.938	0.008	–	–	–
Constant	All	−14.350	0.000	–	–	0.000	83.7%	81.4%	82.5%
	Small	−19.283	0.000	–	–	0.000	80.9%	86.9%	84.0%
	Large	−24.916	0.000	–	–	0.000	93.3%	91.5%	92.4%

*Forward stepwise (likelihood ratio) method logistic regression.

prevalence rate for sheep strain of *E. granulosus* in Iran is higher and from the epidemiological points of view, the fertility rate of its cystic stage is higher as well[19].

A criterion that has been constantly on debate among new researchers and classical taxonomists was the power of accuracy in drawings and measurements. This argues was regarded seriously until the recent appearance of computer based digital technology. One of the remarkable instances in helminthology in terms of CIAS was comparison of allopatric populations of *F. hepatica* and *F. gigantica*[11]. Accuracy and repeatable results provided in CIAS have exhorted a number of parasitologists to revise their knowledge concerning helminthes classification, as well as intraspecific variation of *E. granulosus*[13]. The aim of this study was to determine whether CIAS could be employed as an alternative means of strain specification among *E. granulosus* isolates with confidence or should not be considered as a reliable tool in comparison with other modern techniques such as DNA analysis. Prior to study a review of literature was performing to decide sampling for rostellar hooks and number of parameters to measure. Some researchers have preferred to select 10 protoscoleces and of each four or five large and equal number for small hooks to perform measurement[5]. Several rostellar characters have been use to differentiate *E. granulosus* strains indifferent parts of the world. Total length of the large and small hooks, besides the blade length for the both has been consider as main morphometric criterions by various researchers[7]. In almost all surveys, total length of the large hook was mention as a landmark character.

In conclusion, based on this analysis we intend to offer a new pattern for differentiation among sheep and camel strains. Overall, statistical analyses based on hook parameters in this study indicate that small hooks of two strains are not as valuable as large hooks in strain discrimination. Our precise observation throughout the parameters measured here beside statistical evaluations revealed that THL and perimeter of large hooks are of two best-proposed parameters to distinguish strains from each other. The above-mentioned formula seems to be reliable, easy, economic, and fast method to distinguish between Iranian sheep and camel strains and may use in small laboratories for recording data for epidemiological applications.

Conflict of interest statement

The authors declare that there is no conflict of interests.

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