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Research Article

Availability, Cyst Characteristics and Hook Morphology of *Echinococcus granulosus* Isolates from Livestock (Cattle, Sheep and Goats) in Central Punjab, Pakistan

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Abstract

Cystic Echinococcosis (CE) is a zoonotic infection caused by larval (metacestode) stages of cestodes belonging to the genus *Echinococcus* and the family *Taeniidae*. The aim of current study was to determine the availability and organ placement of hydatid cysts in some ruminants of Pakistan and to study of rostellar hooks morphology of protoscoleces of *E. granulosus*. A total of 2803 animals comprising 925 sheep, 939 goats and 939 cattle (n=) from both sexes were examined to find out the prevalence of hydatid cysts in different regions of Central Punjab from January to December 2013. The overall prevalence of hydatidosis was determined as 3.24%, 2.44% and 2.44% in examined sheep, goats and cattle, respectively. The localization of hydatid cysts in the livers of infected sheep, goats and cattle was found as 1.4%, 1.17% and 1.17%, respectively while lung localizations were determined as 1.83%, 1.27% and 1.27% in the same order. Among the 33, 25 and 30 examined hydatid cysts in sheep, goats and cattle, 8 (24.2%), 11 (33.3%), 8 (24.2%), 6 (18.1%); 9 (36%), 9 (36%), 4 (16%), 3 (12%) and 10 (33.3%), 11 (36.6%), 4 (13.3%), 5 (16.6%) were characterized as fertile, sterile, calcified and under-developed, respectively. The total number of hooks on protoscoleces was 28.68±3.80 (sheep origin), 26.0±2.59 (goat origin) and 27.70±1.11 (cattle origin). In conclusion our investigation revealed that availability of hydatid cysts is still significantly higher among all examined livestocks.

Keywords: *Hydatid cyst, Protoscoleces, Availability, Cattle, Sheep, Goat, Pakistan*

Pakistan'ın Pencap Eyaletindeki Çiftlik Hayvanlarında (Sığır, Koyun ve Keçi) *Echinococcus granulosus* Izolatlarının Mevcudiyeti, Kist Karakteristiği ve Çengel Morfolojisi

Özet

Kistik Ekinokokkozis (KE) *Taeniidae* ailesinde *Echinococcus* soyuna bağlı sestodların larval (metasestod) dönemlerinin sebep olduğu zoonotic bir enfeksiyondur. Bu çalışmanın amacı, Pakistan'daki bazı ruminantlarda hidatik kistlerin mevcudiyeti ve organ yerleşimini tespit etmek ve *E. granulosus* protoskolekslerinin çengel morfolojisini belirlemekti. Ocak - Aralık 2013 tarihleri arasında Pencap eyaletinin farklı bölgelerindeki 2803 ruminant (925 koyun, 939 keçi ve 939 sığır) hidatik kist yaygınlığını belirlemek için muayene edilmiştir. Hidatidozisin ortalama yaygınlığı koyunlarda %3.24, keçilerde %2.44 ve sığırlarda %2.44 olarak belirlenmiştir. Organlara göre yaygınlık koyun karaciğerinde %1.4, akciğerlerinde %1.83; keçi karaciğerinde %1.17, akciğerlerinde %1.27 iken sığır karaciğerlerinde %1.17 akciğerlerinde %1.27 olarak belirlenmiştir. Koyunlarda muayene edilen 33 hidatik kistin keçilerde ise muayene edilen 25 kistin fertile, steril, kalsifiye ve yeni gelişmekte olma durumları koyunlarda sırasıyla 8 (%24.2), 11 (%33.3), 8 (%24.2), 6 (%18.1) ve keçilerde sırasıyla 9 (%36), 9 (%36), 4 (%16) ve 3 (%12) olarak belirlenmiştir. Sığırlarda muayene edilen 30 hidatik kistin 10'u (%33.3) fertil, 11'i (%36.6) steril, 4'ü (%13.3) kalsifiye ve 5'i (%16.6) de yeni gelişmekte olan kist olarak belirlenmiştir. Protoskolekslerdeki toplam çengel sayısı koyun kistlerinde 28.40±1.72, keçi kistlerinde 21.0±1.06 ve sığır kistlerinde 27.70±1.11 adet olarak belirlenmiştir. Bu çalışma ile çiftlik hayvanlarındaki hidatik kist mevcudiyetinin halen yüksek olduğu belirlenmiştir.

Anahtar sözcükler: *Hidatid kist, Protoskoleks, Yaygınlık, Sığır, Koyun, Keçi, Pakistan*

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INTRODUCTION

Livestock plays a vital role in economic development and play a major role in the life of farmers in developing Asian countries. In the Asian region, livestock contributes a major role to agriculture through draft power, manure, fuel, and as a fertilizer. Parasitism is a significant problem affecting livestock in many parts of the world. The low productivity in livestock sectors is due to the parasitism. The parasitic infestation is very common in Pakistan and responsible for about 26.5 million (Pakistani Rupees) costs annually to livestock sector [1]. Hydatidosis is an important zoonosis and the disease is widespread worldwide [2,3]. In the developed countries such as in North American continent this disease is about 1/100.000 but in developing countries the prevalence was 10% [4]. In the central Asia, echinococcosis results serious health problems such as about 466 million people mostly inhabitants and farmers in Kyrgyzstan, Turkmenistan, Kazakhstan, Tajikistan, Uzbekistan, Afghanistan, Mangolia, Iran, western China and Pakistan are under high risk of *E. granulosus* and *E. multilocularis* as well [5-9]. The strains of *E. granulosus* larvae can also be identified by studying an appropriate principle of protoscolex hook morphology and have been considered by [10-12] the rostellar hooks of protoscolex are positioned in two rows of larger and smaller [13,14].

The objectives of the current study was to determine the availability and organ localizations of hydatid cysts in ruminants of Central Punjab (Sargodha district), Pakistan and to identify the types of cysts on the basis of fertile, sterile, calcified and under-developed configurations as well. Besides, we aimed to study rostellar hooks morphology of protoscoleces from some livestock *E. granulosus* isolates.

MATERIALS and METHODS

Location

Sargodha district has a latitude and longitude of 32° 10′ north and 72° 40′ east respectively, with an average temperature in summer is ranging from 25-49°C and in winter 5-23°C and yearly rainfall of 526 millimeter.

Study Design

The present study was conducted to find out the prevalence of hydatidosis in goats, sheep and cattle. The clinical data on sampled animals were collected from different abattoirs and butcher shops of Central Punjab (Sargodha), Pakistan. A cross-sectional study was conducted to find out the occurrence of hydatidosis. The cysts were collected from infected organs in different abattoirs and butcher shops. These abattoirs were visited twice a week in 2013 (January to December) for the collection of hydatid cysts from lungs and liver of slaughtered sheep, cattle and goats.

Sample Collection and Laboratory Investigations

Following parameters was carried out in order to investigation of hydatid cysts: Prevalence of hydatidosis, organ specificity (lungs and liver) and types of cysts (fertile, sterile, calcified, under-developed).

Postmortem Examination

During postmortem examinations, detected hydatid cysts from liver and lungs were collected and distribution of cysts in different organs was recorded. Hydatid cysts were carefully detached and individually collected (in organ basis) into the clean containers for further cyst description.

Cyst Characteristics and Viability

Cysts were incised with a sterile scalpel blade and the substance (fluid) was poured into the glass Petri dishes and examined. The existence of protoscoleces either closes to the germinal layer in the form of brood capsule or presence in the cyst fluid was considered as indicative of fertility. Fertile cysts were more subjected to viability test. A drop of fluid from cyst containing the protoscoleces were placed on the microscope glass slide and covered with cover slip and observed for amoeboid like peristaltic movements with ×40 objective. For clear vision, a drop of 0.1% aqueous eosin solution was added to equal volume of protoscoleces. Sterile hydatid cysts are characterized by their smooth inner lining, generally with a slight turbidity of the enclosed fluid and usually calcified cyst that produced a rough sound feeling upon opening [15]. Calcified cysts were hard and nodular with at least one internal chamber or had calcified, chalky deposits in the cyst wall. Under-developed cysts were firm in texture and quite small in calibrate (1-2 mm), had a little fluid in the cyst wall and defined germinal layer but no protoscoleces [16].

Morphology of Protoscoleces

Protoscoleces were mounted in polyvinyl-lactophenol and sufficient pressure was applied to the cover slip to flatten but not to damage the hooks. All measurements were made by single person using a calibrated eye-piece micrometer under oil immersion. Three large and small hooks were individually measured and all the hooks were counted from each of 6 protoscoleces from each isolate. Morphometric analysis was done as described by Hobbs *et al.*[13].

Statistical Analysis

The statistical analyses were carried out by using SPSS (Version 18).

RESULTS

Prevalence of Hydatid Cysts

Among the examined 2803 livestock, the overall prevalence of hydatid cysts was determined as 3.24%,

2.44% and 2.44% in sheep, goats and cattle, respectively *(Table 1)*. The statistical analysis (chi-square) showed that there were no differences between the prevalence of hydatid cysts in sheep and the others.

Organ Specificity of Hydatid Cysts

The prevalence of hydatid cysts in sheep liver and lungs was 1.40% and 1.83% while 1.17% and 1.27% of the liver and lungs from goats were found to be infected with hydatid cysts. The distribution of hydatid cysts in the liver and lungs of the examined cattle and goats were designated as 1.17% and 1.27% *(Table 1)*. The analysis indicated that there were no statistical differences in the prevalence of different infected organs from goats, sheep and cattle.

Types of Cysts

Prevalence of cysts was recorded on the basis of fertile, sterile, calcified and under-developed type. In sheep 8 (24.2%), 11 (33.3%), 8 (24.2%) and 6 (18.1%) of the examined hydatid cysts (n=33) were classified as fertile, sterile, calcified and under-developed, respectively. While in goats 9 (36%), 9 (36%), 4 (16%) and 3 (12%) out of 25 and in cattle 10 (33.3%), 11 (36.6%), 4 (13.3%) and 5 (16.6%) out of 30 hydatid cysts were characterized in the same order as in sheep *(Table 2)*. Among all animal species various numbers of cysts selected for comparisons. Cattle showed comparatively maximum number of fertile cysts (n=10) than in sheep and goats. Maximum number of sterile cysts (n=11) were found in sheep and cattle and maximum numbers of calcified cysts (n=8) were observed in sheep. Sheep showed relatively maximum number of under-developed cysts (n=6) while goats showed minimum under-developed cyst. The statistical analysis (chi-square) showed that there were no statistical differences between fertile and sterile cysts also between calcified and under developed cysts ($P<0.05$). While there were statistical differences among the groups ($P>0.05$) *(Table 2)*.

Rostellar Hooks Morphology

In present study the measuring factors were consist of the total number of hooks (NH), their total length (TL) (µm) and blade length (BL) (µm), (in case of small hook blade length was measured in L/m) *(Table 3)*.

Average Number of Hooks (NH)

The average number of hooks on protoscoleces from hydatid cysts in sheep (28.68±3.80), goat (26.0±2.59)

Table 1. *Showing the infection rate of hydatid cysts in examined livestock*
Tablo 1. *İncelenen çiftlik hayvanlarında hidatik kist enfeksiyon oranları*

Animals	Examined (n)	Infected Liver		Infected Lung		Total	
		Inf (n)	Prev (%)	Inf (n)	Prev (%)	Inf (n)	Prev (%)
Sheep	925	13	1.40	17	1.83	30	3.24
		X^2=1.067 P=0.302					
Goat	939	11	1.17	12	1.27	23	2.44
		X^2= 0.087 P=0.768					
Cattle	939	11	1.17	12	1.27	23	2.44
		X^2=0.087 P=0.768					
Total	2803					76	2.71

Table 2. *Cyst types of hydatid cyst in sheep, goats and cattle*
Tablo 2. *Koyun, keçi ve sığırlardaki hidatik kistlerin kist tipleri*

Animals	Total No. of Cysts	Fertile		Sterile		Calcified		Under-developed	
		Cysts (n)	Infected (%)	Cysts (n)	Infected (%)	Cysts (n)	Infected (%)	Cysts (n)	Infected (%)
Sheep	33	8	24.2	11	33.3	8	24.2	6	18.1
		X^2=2.06 P=0.560							
Goat	25	9	36	9	36	4	16	3	12
		X^2=6.56 P=0.087							
Cattle	30	10	33.3	11	36.6	4	13.3	5	16.6
		X^2=6.57 P=0.087							
Total	89	27	30.3[a]	31	34.8[a]	16	17.9[b]	14	15.7[b]
		X^2=12.43 P=0.006							

[a,b] *Different letters represent statistically significant (P>0.05) difference between groups*

Table 3. *Rostellar hooks morphology of protoscoleces of Echinococcus granulosus from various hydatid cyst isolates. Values are given for (Mean±S.E) 10 samples of three reciprocal groups*

Tablo 3. *Çeşitli hidatik kist izolatlarından elde edilen Echinococcus granulosus protoskolekslerinin çengel morfolojisi. Değerler üç karşılıklı gruptan 10 örnek için verilmiştir (Ortalama±S.H)*

Parameters	Mean + S.E			P
	Sheep	Goat	Cattle	
Total Number of Hooks (NH)	28.68±3.80 [a]	26±2.59[b]	27.70±1.11[ab]	**
Large Hook Length (LHL) (µm)	28.15±1.77[a]	27.14± 1.84[a]	25.87±1.92[b]	***
Large Hook Blade Length (LBL) (µm)	9.55±0.89[b]	9.39±0.9[b]	15.22±0.96[a]	***
Small Hook Length (STL) (L/m)	18.62±2.28	17.22±2.08	18.59±2.79	NS
Small Hook Blade Length (SBL) (L/m)	7.31±0.45[b]	7.20±0.84[b]	8.53±0.88[a]	***

NS: *Not significant; ** P<0.01; *** P<0.001*

and cattle origin (27.70±1.11) are shown in *Table 3*. The results showed that the maximum number of hooks were present on protoscoleces from sheep origin and minimum on those from goat origin. The values of NH significantly varied among all examined species. Average number of hooks in goat isolates found significantly varied from other species but showed least variance from NH of sheep isolates. The one way ANOVA applied to the data of total number of hooks on protoscoleces indicated a significant difference among the isolates from all animal species.

Total Large Hook Length (LHL) (µm)

The mean length of large hooks was measured as 28.15±1.77 µm, 27.14±1.84 µm and 25.87±1.92 µm for the protoscoleces from sheep, goats and cattle origin, respectively *(Table 3)*. It is evident from these values that the maximum length of large hooks was recorded in protoscoleces of sheep origin and minimum in those of cattle origin. Total length of large hook (LHL) was significant among all these species (*P*<0.001).

Large Hook Blade Length (LBL) (µm)

The blade lengths of large hooks on protoscoleces from sheep (9.55±0.89), goats (9.39±0.9) and cattle origin (15.22±0.96) were shown in *Table 3*. It is evident from these values that the LBL was found maximum in cattle origin and minimum in goat origin. Blade length showed significant variation in large hooks (LBL) (*P*<0.001).

Small Hook Length (SHL) (L/m)

The total length of small hooks measured on protoscoleces from sheep (18.62±2.28), goats (17.22±2.08) and cattle origin (18.59±2.79) was presented in *Table 3*. It is evident from these values that the maximum and minimum values of SHL were observed in sheep and goats origin, respectively. Small hook length (SHL) was not-significant among all species (*P*>0.05).

Small Hook Blade Length (SBL) (L/m)

The blade length of small hooks on protoscoleces was measured as 7.31±0.45 L/m, 7.20±0.84 L/m and 8.53±0.88 L/m from sheep, goat and cattle origin, respectively *(Table 3)*. It is evident from these values that the SBL was measured maximum in cattle, while minimum in goat origin. There was significant difference among small hook blade lengths (SBL) from all these species (*P*<0.001).

DISCUSSION

The results of the present study showed the actual prevalence of hydatidosis in sheep (3.24%), goats (2.44%) and cattle (2.44%) in Pakistan. The prevalence of hydatidosis was also reported by various investigators as 49% in buffaloes, 33% in cattle, 14.8% in sheep, and 5.9% in goats in Pakistan. Pal and Jamil [17] reported the hydatidosis prevalence of 31.5%, 1.79% and 5.3% in cattle, goats and sheep, respectively at Rawalpindi abattoir. Our results are different from previously reported those results because they studied before 20 to 25 years and they collected information only from one abattoir from each selected city. Only the prevalence in goats determined in this study was similar with the findings of Iqbal *et al.*[18]. Our results also showed that the location of hydatid cysts in various organs (lungs and liver) differed significantly among sheep, goats and cattle as well as between different organs of the same species of animal. Ahmed *et al.*[19] also reported the prevalence of hydatidosis in liver of sheep (46.74%) and goats (23.28%). The liver was the predominant site of infection in sheep and goats [20]. Tavakoli *et al.*[21] also reported the prevalence of hydatidosis in liver and lung in cows as 4.84% and 4.41%, in sheep as 5.05% and 6.84% respectively. In the present study lung was found to be more infected organ as compared to livers among all animals. The present study revealed that significantly higher occurrence of hydatid cysts (*P*<0.05) in lungs and liver of sheep than goats and cattle. Similar observations were reported by Getachew *et al.*[22]. They reported that the prevalence of hydatid cyst in sheep was 60% in lung and 36% in liver. In goats, hydatid cysts were recovered from 70% of the lung, 18.5% of the liver. In both sheep and goats the infection was more in lungs, followed by liver.

The results showed that total number of hooks (NH), large hook length (LHL), large hook blade length (LBL) and small hook blade length (SBL) of protoscoleces were varied significantly among all species. Blade length showed non-significant variation in small hook length (STL). The results showed that large hook length (28.15±1.77) and blade length (9.55±0.89) of sheep isolate was similar to sheep strain reported by some other researchers [23,24]. Small hook length (18.62±2.28) and blade length (7.31±0.45) of protoscoleces from sheep isolates in the present study was similar to sheep isolates identified by Gordo and Bandera [10]. Our investigations presented the morphometric analysis of protoscoleces, total number of hooks, large hook length, large hook blade length and small hook and blade length in sheep, goat and cattle origin. Morphological study and statistical analyses showed that hooks morphology is not sufficient for strains identification of *E. granulosus* in Central Punjab, Pakistan. Molecular study is required for describing better criteria for strain identification among different species.

There is a trend of declining prevalence of cystic echinococcosis among livestock likely due to the presence of over 180 local council or municipal owned abattoirs in peri-urban regions. However, in stating this, it is likely that in the present study, the government-run abattoirs that were sampled are more likely to attract livestock from large-scale livestock production facilities that are intensively managed rather than the poorly resourced rural farmer. This study is therefore unlikely to represent the prevalence of hydatid disease of food producing animals in poorly resourced rural communities, which is expected to be significantly higher.

In conclusion, our investigation revealed a mild prevalence of fertile, sterile and under-developed cysts among all animal species. These findings showed variations from previous reports which might be due to geographical distributions. Keeping in view, it is concluded that hydatidosis is still out of control due to stray dogs and their easy approach to the abattoirs, improper disposal of hydatid organs and unhygienic conditions of abattoirs in Pakistan. Thus it is suggested that more effort should be done for the prevention of hydatidosis.

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REFERENCES

1. Anwar AH, Buriro SN, Phulan A: A hydatidosis veterinary perspective in Pakistan. *The Veterinarian*, 4, 11-14, 1995.

2. Pednekar RP, Gatne ML: Molecular and morphological characterization of *Echinococcus* from food producing animals in India. *Vet Parasitol*, 165, 58-65, 2009. DOI: 10.1016/J.VETPAR.2009.06.021

3. Ibrahim MM: Study of cystic echinococcosis in slaughtered animals in Al Baha region, Saudi Arabia: Interaction between some biotic and abiotic factors. *Acta Tropica*, 113, 26-33, 2010. DOI: 10.1016/J.ACTATROPICA.2009.08.029

4. Hussain A, Maqbool A, Hussain S, Athar M, Shakoor A, Amin MK: Studies on prevalence and organ specificity of hydatidosis in ruminants slaughtered at Karachi and Faisalabad abattoir, Pakistan. *Ind J Dairy Sci*, 45, 454-456, 1992.

5. Bai Y, Cheng N, Wang Q, Cao D: An epidemiological survey of cystic echinococcosis among Tibetan school pupils in western China. *Ann Trop Paed*, 21, 235-238, 2001. DOI: 10.1080/027249301200777817

6. Craig PS: Epidemiology of human alveolar echinococcosis in China. *Parasitol Int,* 55, 221-225, 2006. DOI: 10.1016/j.parint.2005.11.034

7. Wang Q, Qiu J, Yang W, Schantz PM, Raoul F, Craig PS, Giraudoux P, Vuitton DA: Socioeconomic and behavior risk factors of human alveolar echinococcosis in Tibetan communities in Sichuan, People's Republic of China. *Am J Trop Med Hyg,* 74, 856-862. 2006.

8. Yang YR, Sun T, Li Z, Zhang J, Teng J, Liu X, Liu R, Zhao R, Jones MK, Wang Y, Wen H, Feng X, Zhao Q, Zhao Y, Shi D, Bartholomot B, Vuitton DA, Pleydell D, Giraudoux P, Ito A, Danson MF, Boufana B, Craig PS, Williams GM, McManus DP: Community surveys and risk factor analysis of human alveolar and cystic echinococcosis in Ningxia Hui autonomous region, China. *Bull World Health Organ,* 84, 714-721, 2006.

9. Yu SH, Wang H, Wu XH, Ma X, Liu PY, Liu YF, Zhao YM, Morishima Y, Kawanaka M: Cystic and alveolar echinococcosis: An epidemiological survey in a tibetan population in southeast Qinghai, China. *Jpn J Inf Dis,* 61, 242-246, 2008.

10. Gordo FP, Bandera CC: Differentiation of Spanish strains of *Echinococcus granulosus* using larval rostellar hook morphometry. *Int J Parasitol,* 27, 41-49, 1997. DOI: 10.1016/S0020-7519(96)00173-7

11. Thompson RCA, Boxell AC, Ralston BJ, Constantine CC, Hobbs R, Shury T, Olson ME: Molecular and morphological characterization of *Echinococcus* in cervids from North America. *Parasitology,* 132, 439-447, 2006. DOI: 10.1017/S0031182005009170

12. Latif AA, Tanveer A, Riaz-Ud-Din S, Maqbool A, Qureshi AW: Morphometry of protoscoleces rostellar hooks of *Echinococcus granulosus* isolates from Punjab, Pakistan. *Pak J Sci*, 61, 223-228, 2009.

13. Hobbs RP, Lymbery AJ, Thompson RCA: Rostellar host morphology of *Echinococcus granulosus* (Batsch, 1786) from natural and experimental Australian hosts, and its implication for strain recognition. *Parasitology*, 101, 273-281, 1990.

14. Almeida FB, Rodrigues Silva R, Neves RH, Romani ELS, Machado-Silva JR: Intraspecific variation of *Echinococcus granulosus* in livestock from Peru. *Vet Parasitol*, 143, 50-58, 2007. DOI: 10.1016/J.VETPAR.2009.06.021

15. Parija SC: Medical Parasitology, Protozoology and Helminthology Text and Atlas. 2nd ed., 221-229, Chennai Medical Book Publisher, India, 2004.

16. Simsek S, Koroglu E: Evaluation of enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunoelectrotransfer blot (EITB) for immunodiagnosis of hydatid diseases in sheep. *Acta Tropica*, 92, 17-24, 2004. DOI: 10.1016/J.ACTATROPICA.2009.08.029

17. Pal RA, Jamil K: Incidence of hydatidosis in goats, sheep and cattle. *Pak Vet J*, 6, 65-69. 1986.

18. Iqbal Z, Hayat CS, Hayat B, Khan MN: Prevalence of organ distribution and economics of hydatidosis in meat animals at Faisalabad abattoir. *Pak Vet J*, 9, 70-74, 1989.

19. Ahmed S, Nawaz M, Gul R, Zakir M, Razzaq A: Some epidemiological aspects of hydatidosis of lungs and livers of sheep and goats in Quetta, Pakistan. *Pak J Zool*, 38, 1-6. 2006.

20. Baswaid SH: Prevalence of hydatid cysts in slaughtered sheep and goats in hadramout (Yemen). *Ass Univ Bul Environ Res*, 10, 67-71, 2007.

21. Tavakoli H, Junaedi JN, Izadi M, Parsa M: Epidemiological investigation of hydatidosis infection in Iran. *18th European Congress of Clinical Microbiology and Infectious Diseases,* 19-22 April, Barcelona, 2008.

22. Getachew D, Almaw G, Terefe G: Occurrence and fertility rates of

hydatid cysts in sheep and goats slaughtered at Modjo Luna Export Slaughter House, Ethiopia. *Eth Vet J*, 16, 83-91, 2012. DOI: 10.4314/evj.v16i1.7

23. Ahmadi N, Dalimi A: Characterization of *Echinococcus granulosus* isolates from human, sheep and camel in Iran. *Inf Gen Evol*, 6, 85-90, 2006. DOI: 10.1016/J.ACTATROPICA.2009.08.029

24. Karimi A, Dianatpour R: Genotypic and phenotypic characterization of *Echinococcus granulosus* of Iran. *Biotechnology*, 7, 757-762, 2008.