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Fertility and viability rates of hydatid cysts in camels slaughtered in Kerman region, southeast of Iran

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Abstract. The aim of this study was to determine the fertility and viability of hydatid cysts in slaughtered camels. Cysts were collected from the liver and lungs of 217 camels infected with Echinococcus granulosus when slaughtered in Municipal abattoir in Kerman, Iran, during October 2009 to October 2010. Out of 217 camels slaughtered in Kerman Municipal abattoir 45 (20.73%) animals were found harboring hydatid cysts. A significantly higher infection was detected in older camels ($P<0.05$) than younger ones. Of the total of 45 infected, 21 (46.66%) had hydatid cysts only in the lung, nine (20%) in the liver, while the rest of 15 (33.33%) had multiple organ infections. Thorough meat inspection in the abattoir revealed that 62 visceral organ were found harboring one or more hydatid cysts. Of the 62 viscera harboring hydatid cysts, the highest (58.06%) was lung followed by liver (38.75%), spleen (3.22%). In addition, out of the total of 361 cysts collected, 58.17% were fertile, 25.20% sterile, and 16.62% calcified or purulent cysts. The rate of cyst calcification was higher in the liver than in the lung. There was a significant difference in fertility of cyst from different organs ($P<0.05$), those of lung origin being highly fertile. Likewise, Hydatid cyst viability rate of 57.14% was observed. The present study provides baseline data on the current status of the disease in kerman area. Based on the finding in the present study, effort should be made to control transmission of cystic

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Introduction

Hydatid disease, due to infection in animals or human with larval stages of the tape worm Echinococcus granulosus, poses significant economic and public health problems in many temperate and tropical areas of the world (Nahmias et al., 1991). Livestock infection leads to protein and economic loss, and the feeding of stray dogs with offal discarded from various animals slaughtered for human consumption helps to maintain the life-cycle of E. granulosus, the causative agent (Khan et al., 2001). The suitable climatic and ecological

features, traditional situations such as large numbers of small, ill-equipped, and unsupervised abattoirs, home slaughtering, and large populations of stray dogs, are only the main factors influencing the persistence of *E. granulosus* in the Mediterranean area (Seimenis et al., 2006). The ability of *E. granulosus* to adapt to a wide variety of host species contributes to the broad distribution of this parasite; in addition, probably due to this wide spectrum of hosts, there is a great genetic variability among *E. granulosus* strains (Thompson and McManus, 2002). Various surveys throughout the country have indicated that hydatid cyst is commonly found in sheep, camels, cattle and goats (Mobedi et al., 1970; Moghaddar et al., 1992; Oryan et al., 1994; Dalimi et al., 2002; Ahmadi, 2005). Furthermore, human cases are regularly observed in the country and widespread recovery of adult worms has been reported from dogs, jackals and wolves (Mobedi et al., 1973; Mehrabani et al., 1999; Maleky and Moradkhan, 2000; Dalimi et al., 2002; Meshgi et al., 2009). So far three distinct cycles of *E. granulosus* have been suggested in Iran: A domestic cycle between dogs and livestock, a desert cycle between dogs and camels and a sylvatic cycle between wild carnivores and wild ruminants. In domestic cycle, the mean prevalence of *E. granulosus* in domestic dogs is 23.45%, which vary widely from 3.3 to 63.3% depending on the local condition (Eslami and Hosseini, 1998). Sheep and camel (with 88% and 70% of fertile cysts, respectively) are the most important intermediate hosts, and cattle (with 19% fertile cysts), have been considered as the weakest intermediate host of *E. granulosus*. In Iran (Hosseini and Eslami, 1998; Rokni, 2009). The prevalence of infection and cyst fertility rates in sheep are high (Oryan et al., 1994; Hosseini and Eslami, 1998; Mehrabani et al., 1999; Dalimi et al., 2002). Camels are found in the majority of arid regions and commonly infected with *E. granulosus*, possessing a high cyst fertility rate (Mobedi et al., 1970; Moghaddar et al., 1992; Hosseini and Eslami, 1998; Dalimi et al., 2002).

On epidemiological grounds, camels appear to be an important reservoir for human infection (Eckert et al., 1989; Rokni, 2009), also few reports showing infection of camels of the southern and central provinces of Iran with this parasite. Data on the prevalence and fertility of hydatid cysts in different domestic herbivores are very necessary to be determined in surveys of hydatidosis, because they provide reliable indicators of the importance of each type of animal as a potential source of infection to dog. Therefore, the aim of this study was to determine the frequency of infection with hydatid cysts in camel, and to study the localization and fertility/sterility rates of hydatid cysts and the viability of their protoscoleces will also be investigated in Kerman Abattoir, southeast Iran.

Materials and methods

Study area

The study was conducted in Kerman city of Kerman province. Kerman is located at 30°17’13"N and 57°04’09"E southeast of Iran. The city's many districts are surrounded by mountains which bring variety to Kerman's year round weather pattern, thus the northern part of the city is located in an arid desert area, while the highland of the southern part of the city enjoys a more moderate climate. The mean elevation of the city is about 1755 m above sea level. Kerman city has a hot and arid climate and the average annual rainfall is 135 mm. Because it is located close to the Kavir-e lut (Lut Desert), Kerman has hot summers and in the spring it often has violent sand storms. Otherwise, its climate is relatively cool.

Study animals and sampling method

This crosssectional study was carried out on 217 camels in the Kerman Municipal abattoir, southeastern Iran, from October 2009 to October 2010. For this purpose, the industrial slaughterhouse was visited periodically to examine the liver, lungs and other organs of slaughtered animals for the presence of cystic echinococcosis. All camels presented on each visit day were examined. During antemortem examination, each study animal was given an identification number and the age, sex, breed and origin of animals were recorded. The age was determined based on dentition and owner’s information. A total of 217 camels (83

females and 134 males) in three age groups (<5, 5–10 and >10 years old) were inspected for infection with cystic echinococcosis. In the abattoirs, thorough meat inspection was carried out on different organs of each of the slaughtered camels, particularly lung, liver, spleen, kidney, heart and the muscles. Each organ was assessed macroscopically by visual inspection and palpation, and where necessary one or more incisions were made to detect small hydatid cysts (Soulsby, 1982). The infected organs from each positive animal were collected; the total number of hydatid cysts were counted per infected organ and recorded.

Examination of cyst fertility and viability of protoscoleces

The pressure of the cyst fluid was reduced by using a sterile hypodermic needle. Then cyst wall was incised with a sterile scalpel blade, and the content was transferred into a sterile container and examined microscopically (40x) for the presence of protoscoleces. Similarly, the germinal layer was put in glycerin between two microscopic glass slides and examined for the presence of protoscoleces. The presence of protoscoleces either attached to the germinal epithelium in the form of brood capsule or theirs presence in the cyst fluid was considered as indicative of fertility (Macpherson et al., 1985). Cysts which contained no protoscoleces as well as heavily supurative or calcified were considered infertile. Fertile cysts were subjected to viability test. A drop of the sediment containing the protoscoleces was placed on the microscope glass slide and covered with a cover slip and observed for amoeboid like peristaltic movements with 40x objective.

The viability of protoscoleces was assessed by the motility of flame cells together with staining with a 0.1% aqueous eosin solution (Smyth and Barrett, 1980). The viability of protoscoleces was tested for each fertile cyst per animal species and organ. For clear vision, a drop of 0.1% aqueous eosin solution was added to equal volume of protoscoleces in hydatid fluid on the microscope slide, with the principle that viable protoscoleces should completely or partially exclude the dye, while the dead ones take it up (Smyth and Barrett, 1980; Macpherson et al., 1985). Furthermore, infertile cysts were further classified as sterile or calcified. Sterile hydatid cysts were characterized by their smooth inner lining, usually with slightly turbid fluid in its content. Typical calcified cysts produce a gritty sound feeling up on incision (Soulsby, 1982; Parija, 2004).

Statistical analysis

Data collected from antemortem, postmortem, and laboratory finding were entered in to MS Excel and statistical packages such as SPSS Version 9.0 for Windows (SPSS Inc., Chicago, IL, USA) were employed to analyze the results and Chi-square (χ^2) test was applied for comparison of rate of infections with regard to the hypothesized risk factors like age and cyst characteristics.

Results

Prevalence

The prevalence mean number and viability and of hydatidosis in organs of 217 camels slaughtered at Kerman slaughterhouse, Iran, in different age groups is summarized in Table 1. Forty five out of 217 (20.73%) camels slaughtered and examined were infected with hydatid cysts, harboring one or more cysts involving different visceral organs (lung, liver, spleen,). As many as 29 out of 135 males (21.48%) and 16 out of 82 females (19.51%) were found to be positive, but no significant difference was observed between males and females ($p>0.05$).

Rate of infection in different age groups (<5, 5–10 and >10 years old) was assessed and described (Table 1). Age prevalence has shown a statistically significant variation ($P<0.05$) with older group having higher infections, but no significant difference was observed between males and females ($P > 0.05$). There was a direct relationship between the rate and intensity of infection and host age.

Cyst distribution and cyst characterization

Single and multiple hydatid cyst distribution was recorded in different organs (Table 2).

Table 1. Frequencies and percentages of positive camels by age class, mean number, and viability of hydatid cysts at Kerman Municipal abattoir

Age groups (years)	No. of infected camels (%)	Data on hydatid cysts (mean number and viability)						
		No.	Mean no.	Fertile	Sterile	Calcified/ Caseous	Viable	Nonviable (%)
<5	4 (9.75)	29	7.25	21 (72.41)	5 (17.24)	3 (10.34)	16 (76.19)	5 (23.80)
5-10	10 (13.88)	68	6.80	52 (76.47)	10 (14.70)	6 (8.82)	33(63.74)	19 (36.53)
>10	31 (29.80)	264	8.51	137 (51.89)	76 (28.78)	51 (19.31)	71 (51.82)	66 (48.17)
Total	**45 (20.73)**	**361**	**8.02**	**210 (58.17)**	**91 (25.20)**	**60 (16.62)**	**120 (57.14)**	**90 (42.85)**

Most of the hydatid cysts were found concentrated in great number in the lungs. Out of a total of 45 camels harboring hydatid cysts 30 (66.66%) were found involving only a single organ and the remaining 15 (33.33%) had a multiple organ involvement. About 96.77% (60 of 62) of all infected viscera is attributed to overall involvement of lung and liver. The chi-square test for differences of location was significant (P< 0.05). The total number, relative prevalence and mean number of cysts harboured by each affected organ are shown in Table 3. Among the different organs affected, lung and liver constituted almost 98.06% of the overall infection of the organs. The distributions of hydatid cyst between organs of infected animals were significantly different in camel (p<0.05).

Cyst fertility, viability and sterility

Out of 361 cysts tested for fertility, 150 (63.55%) cysts of lung, 57(48.30%) cysts of liver, and three (42.85%) cysts of spleen origin had detected protoscoleces and hence, fertile. The rest were either sterile or calcified (Table 4). Fertility status of cysts from different organs have shown a significant difference (P<0.05), with cysts of lung origin being highly fertile. A total of 210 fertile cysts originating from lung, liver, and spleen were tested for viability and described (Table 4). The viability rate of protoscoleces of liver fertile cysts (71.92%) was significantly higher than that of lung cysts (51.33%) and spleen (66.66%), (P<0.05). The numbers of protoscoleces and viable protoscoleces is summarized in Table 5.

Table 2. Distribution of hydatid cysts in different organs of positive camel at Kerman Municipal abattoir

Relative frequency (%)	Percentage	Number infected	Infected organ
46.66	9.67	21	Lung only
20	4.14	9	Liver only
28.88	5.99	13	Lung and liver
4.44	0.92	2	Lung, liver, and spleen
100	**20.73**	**45**	**Total**

Table 3. Distribution and number of organs with hydatid cysts in infected camel slaughtered in Kerman Municipal Abattoir

Cyst count				Relative Prevalence	No. organs affected	Organ
%	Total	Range	Mean/organ			
65.37	236	1-18	6.55	58.06	36	Lung
32.68	118	1-9	4.91	38.70	24	Liver
1.93	7	1-4	3.5	3.22	2	Spleen
100	**361**	**1-18**	**6.01**	**100**	**62**	**Total**

Table 4. Fertility/sterility and viability statuses of cysts collected from different organs of camel slaughtered at Kerman Municipal abattoir

Nonviable cysts (%)	Viable cyst (%)	Calcified (%)	Sterile cyst (%)	Fertile cyst (%)	Organ
73 (48.66)	77 (51.33)	23 (9.74)	63 (26.69)	150 (63.55)	Lung
16 (28.07)	41 (71.92)	35 (29.66)	26 (22.03	57 (48.30)	Liver
1 (33.33)	2 (66.66)	2 (28.75)	2 (28.57)	3 (42.85)	Spleen
90 (42.85)	**120 (57.14)**	**60 (16.62)**	**91 (25.20)**	**210 (58.17)**	**Total**

Table 5. Viability statuses of protoscoleces

Infected organ	Average volume of cysts/ml	Average number of protosco-leces/ml	Average number of live protoscoleces/ml (%)
lung	19.86	285	(186) 65.26
liver	8.56	114	60 (52.63)
Spleen	3.14	32	13 (40.62)

Discussion

Hydatid disease is an important medical and veterinary problem in Iran. Domestic intermediate hosts are major reservoirs for the disease in humans. The widespread distribution and nature of the life cycle of E. granulosus suggest that there will always be a risk of re-introducing the cestode as long as live animals are imported. Most prevalence studies have relied on slaughter data. as these are an economical way of collecting and analyzing information on livestock disease, particularly subclinical conditions. Also lesions of cystic echinococcosis usually remain for the life of the animals, and so, at post-mortem it is possible to tell whether or not an animal is infected (Njoroge et al., 2000). The prevalence of hydatid cysts has been reported in Iran from 11.4% to 70% in camels (Ahmadi, 2005; Rokni, 2009). So, that reports showed that camel hydatidosis is widespread in Iran. The findings of this study showed the existence of high prevalence (20.73%) in camels slaughtered at municipal abattoirs. However, the extent to which results were documented from different locations tends to show variable scales. This variation could be attributed to differences in culture, social activity, attitude to dog in different regions, strain differences, host age factors, abundance of final infected hosts, stocking rate of livestock and the management of studied animals (Macpherson et al., 1985; Mc Manus, 2006; Ibrahim, 2010).

Livers and lungs were the most frequently infected visceral organs in camels examined. This is explained by the fact that livers and lungs possess the first great capillaries sites encountered by the migrating echinococcus oncosphere (hexacanth embryo) which adopt the portal vein route and primarily negotiate hepatic and pulmonary filtering system sequentially before any other peripheral organ is involved (Kebede et al., 2009).

The finding that the lungs of camels, were found to be more commonly infected with hydatid cysts than the livers is in agreement with the previous findings of Ahmadi (2005) and Ibrahem and Craig (1998). This might be due to the fact that camel are slaughtered at older age, during which period the liver capillaries are dilated and most oncospheres pass directly to the lungs; additionally, it is possible for the *Echinococcus* oncosphere (hexacanth embryo) to enter the lymphatic circulation and be carried via the thoracic duct to the heart and lungs in such a way that the lung may be infected before or instead of liver (Regassa et al., 2010).

The overall percentage of fertile cysts in the present study was 58.17. Various surveys throughout the country have indicated that high cyst fertility rate is commonly found in camel (Mobedi et al., 1970; Moghaddar et al., 1992; Hosseini and Eslami, 1998; Dalimi et al., 2002) hence they are potential sources of infection to dogs. The difference in fertility and the proportion of viable protoscoleces from fertile cysts may be related to the difference in immunological response per host, Moreover, the fertility of hydated cysts in the intermediate hosts may be also genotype dependent (McManus, 2006).

In comparison of the fertility rate among the organs, it was higher in lungs than liver and spleen. It has been stated that the relatively softer consistency of lung tissue allows the easier development of the Cyst (Himonas 1987), whilst the viability rate of protoscoleces of liver fertile cysts was significantly higher than that of lung cysts and spleen ones, respectively. This is in agreement to the finding of Ahmadi, (2005) in camels slaughtered at different abattoirs of five province regions in Iran. Ahmadi (2005) reported that the fertility of cysts in the lung (69.7%) of camels in Iran was higher than that in the liver (58.7%) and spleen (50.0%), whilst the viability rate of liver fertile cysts (80.3%) was higher than that of lung (55.8%) and other organs (57.1%) cysts. Furthermore, the greater prevalence and higher fertility rate of pulmonary cysts over

hepatic cysts of camel indicate the importance of each internal organ as a potential source of infection to dogs. In *E. granulosus* endemic areas of Iran it is evident that, the majority of *E. granulosus* infected livestock animals can potentially act as reservoirs of human infection (Daryani et al., 2009).

This has important implication for cystic echinococcosis control and public health. Hydatidosis is one of the major parasitic diseases in the study area. In light of the result obtained and the current situation in Kerman Municipal abattoir and it's surrounding, warranting serious attention for its prevention and control. Moreover, promoting the construction of abattoirs with their appropriate disposal pits, particularly in rural areas, and conducting obligatory meat inspection services and further detailed investigation into the basic local epidemiological factors governing the spread of hydatidosis in the area to establish regional control strategy are recommended.

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