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Fertility of Hydatid Cysts and Viability of Protoscoleces in Slaughtered Animals in Qazvin, Iran

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Abstract

This study was conducted to estimate the fertility of hydatid cysts and viability of protoscoleces based on the location, size and type of cysts in slaughtered animals in Qazvin, Iran. Cysts were collected from the livers and lungs of 247 sheep and 275 cattle infected with hydatid cysts. Fertility of the cysts was assessed by examining the cysts' fluid for the presence of protoscoleces and the viability of the protoscoleces was determined using staining with an aqueous solution of 0.1% eosin. The highest and lowest rate of fertility was observed in hepatic cysts of sheep (81.05%), and cattle (1.27%) respectively. Most fertile cysts had medium size (45.03%), while the lowest fertility rate belonged to the small cysts (22.8%). Relationship between fertility of the cysts and type of infected organs, type of animals and size of cysts were significant ($p<0.05$). The highest rate of viability was found in cattle's lungs (88.06%) and the lowest rate was seen in sheep's liver cysts (46.49%). There was significant difference in viability of fertile cysts between cattle and sheep organs ($p<0.05$). Most of sterile, suppurative and calcified cysts were found in cattle's lungs (84.7%), cattle's liver (89.87%) and sheep's liver (6.58%) respectively.

In conclusion it can be said that fertility rates of liver and lung hydatid cysts of sheep and viability of their protoscoleces is considerable. In addition, although the fertility of cysts in cattle was low, but they had high viability rate.

Keyword: *Echinococcus granulosus*, hydatid cyst, fertility, protoscoleces, viability

1. Introduction

Cystic Echinococcosis (CE)/Hydatidosis is one of the most important zoonotic diseases caused by the larval stage of *Echinococcus granulosus*. It is a worldwide distributed disease and is prevalent mostly in countries that animal husbandry is common. CE is considered endemic in some regions of the world such as India, South America, Australia, and Middle East including Iran (Dueger & Gilman, 2001; Qaqish et al., 2003; Small & Pinch, 2003; Ahmed et al., 2006; Dopchiz et al., 2009; Pednekar, Gatne, Thompson, & Traub, 2009; Saeed, Kapel, Saida, Willingham, & Nansen, 2000; Jenkins, Allen, & Goullet, 2008; Ibrahim, 2010). In Iran the rate of the infection in carnivores and herbivores such as sheep, cattle, and goat is significant. Also human infections are frequently reported from most parts of this country (Daryani et al., 2007; Ahmadi & Hamidi, 2008; Sadjjadi, Sedaghat, Hosseini, & Sarkari, 2009; Ahmadi & Meshkehkar, 2010; Shahnazi, Hejazi, Salehi, & Andalib, 2011; Dalimi et al., 2002; Mamishi, Sagheb, & Pourakbari, 2007). Hereby, hydatidosis is a major health-economic problem, that has become one of the WHO's active plans for controling the disease (Vuitton, 1997).

Fertile hydatid cysts with viable protoscoleces in intermediate hosts are important factors in transferring of infection, and stray dogs that wandering around abattoirs, spread the disease due to feeding on infected organs. These factors which differ according to the geographical situation, host and type of infected organs, affect on *Echinococcus* cycle persistence (Vuitton, 1997). Therefore the aim of this survey was to evaluate the fertility of hydatid cysts and viability of their protoscoleces based on the site, size and type of cysts in slaughtered animals in Qazvin, central region of Iran.

2. Materials and Methods

The study was conducted in Qazvin City from Qazvin province, at central district of Iran between 45 - 48 degrees of Eastern longitude from Greenwich Meridian and 35 - 36 degrees of Northern latitude from the Equator. Qazvin province situated on southern hillside of Alborz Mountain is considered of temperate areas of Iran due to its various highlands and average rainfalls. At the present time, this province is one of the important places of Iran involved in raising livestock and production of farm animal products.

The current descriptive, analytic and cross-sectional research was periodically conducted from June 2008 to June 2009 on infected organs (lung and liver) of sheep and cattle with hydatid cysts. Our sample had a volume of 522 slaughtered animals' cysts (247 sheep and 275 cattle). Removed hydatid cysts were counted and classified according to the type, macroscopic characteristics (calcified, suppurative and active), and size [small (<2cm), medium (2-4cm) and large (>4cm)]. The fluid of active cysts was aseptically aspirated and transferred separately into tubes.

After being washed with normal saline, the cyst fluid was centrifuged at 500 rpm for 5 min. The precipitate of each sample was observed under a light microscope. Cysts with no protoscoleces were considered as sterile. Using staining with an aqueous solution of 0.1% eosin together with considering the motility of flame cells, viability of protoscoleces was assessed in fertile cysts et al., 2002). Viable protoscoleces did not take the stain up whereas the dead ones did.

3. Results

The study included a total number of 522 slaughtered animals' cysts, 247 (47.32%) from sheep and 275 (52.68%) from cattle. Ninety five sheep cysts (38.46%) were found in liver and 152 of them (61.54%) in lung. Among all cattle cysts, 79 liver cysts (28.73%) and 196 lung cysts (71.27%) were observed.

Sterile cysts were the most common type of cysts (33.52%) and the calcified cysts were the least (5.18%). After sterile cysts, fertile and suppurative cysts allocated the highest percentage of infection. The highest rate of fertility was revealed in sheep's livers (81.05%) and the lowest in cattle's livers (1.27%). Most of sterile, suppurative and calcified cysts were found in cattle's lungs (84.7%), cattle's livers (89.87%) and sheep's livers (6.58%), and the least of them were in sheep's livers (0%), cattle's lungs (6.16%) and cattle's livers (3.8%) respectively (table 1). It was revealed that there was a significant relation between type of cysts and kind of animals and infected organs ($p<0.05$).

Table1. Hydatid cysts in different organs of slaughtered animals based on type

Animals	Infected organs	Type of cysts				
		Sterile cysts n (%)	Suppurative cysts n (%)	Calcified cysts n (%)	Fertile cysts n (%)	Total n (%)
Sheep	Liver	0 (0.00)	12 (12.63)	6 (6.32)	77 (81.05)	95 (100)
	Lung	5 (3.29)	50 (32.89)	10 (6.58)	87 (57.24)	152 (100)
Cattle	Liver	4 (5.06)	71 (89.87)	3 (3.80)	1 (1.27)	79 (100)
	Lung	166 (84.7)	16 (8.16)	8 (4.08)	6 (3.06)	196 (100)
Total		175 (33.52)	149 (28.54)	27 (5.18)	171 (32.76)	522 (100)

From 175 sterile cysts, most of them were found in cattle's lungs having less than 2 cm length, while there were not any sterile cysts in sheep's livers. In addition, we didn't observe any sterile cysts in sheep's lungs with medium and large sizes and cattle's livers with large size (Table 2). Highest rate of suppurative cysts was possessed by cattle livers with small size, however cattle lungs did not have any large suppurtaive cysts (Table 3). Most of calcified cysts were found in sheep's lungs with small size, while there were not any calcified cysts having either large or medium sizes in sheep's livers or in large size among cattle's livers (Table 4).

Table 2. Sterile hydatid cysts in different organs of slaughtered animals based on size

Animals	Infected organs	Size of cysts (cm)			
		<2 n (%)	2-4 n (%)	>4 n (%)	Total n (%)
Sheep	Liver	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
	Lung	5 (100)	0 (0.00)	0 (0.00)	5 (100)
Cattle	Liver	3 (75)	1 (25)	0 (0. 00)	4 (100)
	Lung	75 (45.18)	63 (37.95)	28 (16.87)	166 (100)
Total		83 (47.43)	64 (36.57)	28 (16)	175 (100)

Table 3. Suppurative hydatid cysts in different organs of slaughtered animals based on size

Animals	Infected organs	Size of cysts (cm)			
		<2 n (%)	2-4 n (%)	>4 n (%)	Total n (%)
Sheep	Liver	4 (33.33)	5 (41.67)	3 (25.00)	12 (100)
	Lung	28 (56.00)	15 (30.00)	7 (14.00)	50 (100)
Cattle	Liver	36 (50.70)	31 (43.66)	4 (5.64)	71 (100)
	Lung	11 (68.75)	5 (31.25)	0 (0.00)	16 (100)
Total		79 (53.02)	56 (37.59)	14 (9.39)	149 (100)

Table 4. Calcified hydatid cysts in different organs of slaughtered animals based on size

Animals	Infected organs	Size of cysts (cm)			
		<2 n (%)	2-4 n (%)	>4 n (%)	Total n (%)
Sheep	Liver	6 (100)	0 (0.00)	0 (0.00)	6 (100)
	Lung	8 (80.00)	2 (20.00)	0 (0.00)	10 (100)
Cattle	Liver	1 (33.33)	2 (66.67)	0 (0.00)	3 (100)
	Lung	4 (50.00)	3 (37.50)	1 (12.50)	8 (100)
Total		19 (70.37)	7 (25.93)	1 (3.70)	27 (100)

Most of 171 fertile cysts had medium size (45.03%), while the lowest rate belonged to the small cysts (22.8%). There were significant relationship between fertility of the cysts, type of infected organs, type of animals and size of cysts ($p<0.05$) (Table 5). Also significant difference was observed between viability of cattle and sheep's cyst protoscoleces (86.22±8.47% and 51.14±27.43% respectively, $P<0.05$), and viability of cattle's lung fertile cysts and that of cattle livers (88.60±70.60%, 75.20±7.60% respectively, $P<0.05$). However there was not significant difference in relation to viability of fertile cysts of sheep's lungs and livers (55.30±28.64% and 46.49±25.39% respectively $P>0.05$). The highest and lowest viability rate of protoscoleces were seen in small size cysts of cattle lungs (81.48%) and sheep's livers (41.09%). No significant difference was observed between these cysts in aspects of viability and size ($p>0.05$).

Table 5. Fertile hydatid cysts in different organs of slaughtered animals based on size

Animals	Infected organs	Size of cysts (cm)			
		<2 n (%)	2-4 n (%)	>4 n (%)	Total n (%)
Sheep	Liver	24 (31.17)	34 (44.16)	19 (24.67)	77 (100)
	Lung	13 (14.94)	41 (47.13)	33 (37.93)	87 (100)
Cattle	Liver	1 (100)	0 (0.00)	0 (0.00)	1 (100)
	Lung	1 (16.67)	2 (33.33)	3 (50.00)	6 (100)
Total		39 (22.80)	77 (45.03)	55 (32.17)	171 (100)

4. Discussion

For all examined hydatid cysts, there were significant relations between the type of animal and infected organ with type of cysts. In addition, the highest percentage of infection belonged to sterile cysts, while calcified cysts

possessed the least (Table 1). In agreement with our findings, Kebede et al., in Northwestern Ethiopia, reported the sterile cysts as the most prevalent type of removed hydatid cysts (Mohebali & Sammak, 1996; Kebede, Abebe, & Tilahun, 2009). Additionally, Mohebali et al. in Arak province of Iran, found the calcified cysts as the least type among all hydatid cysts (Kebede, Abebe, & Tilahun, 2009), however opposite to our finding, in Sardinia (Italy) calcified cysts were observed as the most prevalent (Scala, Garippa, Varcasia, Tranquillo, & Genchi, 2006).

In the present study, the observed number of sterile cysts in cattle (n=170) was higher than that of sheep (n=5), that is in agreement with findings of studies carried out in Mazandaran province (Iran), western of Iran, northwest Iran and Northwestern Ethiopia (Daryani et al., 2007; Dalimi et al., 2002; Kebede, Abebe, & Tilahun, 2009).

In our study it was demonstrated that, the most of sterile cysts were in cattle's lungs, whereas there was not any sterile cyst in sheep liver. Similar to our finding, in the other parts of Iran, the highst rate of sterile cysts were 69.7% and 80.7% in cattle lungs, while the lowest rate of that cysts were 25.7% and 31.5% in sheep's liver in North and Northwest respectively (Daryani et al., 2007).

The current research revealed that, the most calcified hydatid cysts were found in sheep's lungs and the least in cattle's liver. In Mazandaran, cattle's liver infected with the highest percentage of both calcified and suppurative cysts (44.4%), while sheep's liver had the lowest (27.2%) percentage of infection (Daryani et al., 2007). Contrary to our findings, in Northwestern Ethiopia, the highest and the lowest number of calcified cysts were found in cattle's lungs and sheep's liver respectively (Kebede, Abebe, & Tilahun, 2009).

The most and the least of suppurative cysts were observed in cattle's and sheep's liver respectively (Table1). In other studies carried out in several parts of Iran, calcified and suppurative cysts have been categorized together (Scala, Garippa, Varcasia, Tranquillo, & Genchi, 2006), therefore there was no isolated information. In relation to sheep's organs, most of suppurative cysts were found in lungs. This finding was the same as those obtained in Sardinia of Italy (Scala, Garippa, Varcasia, Tranquillo, & Genchi, 2006).

Considering the significant relation between cyst fertility and type of infected host and organs in this study, the highest and the lowest fertility were obtained in sheep's liver (81.05%) and cattle's liver (1.27%), respectively. Furthermore, here the highest fertility of cysts found in sheep's liver was similare to findings reported from studies in Mazandaran (47.1%), northwest Iran (68.5%), western Iran (36.9%) and Saudi Arabia (56.33%) (Mohebali & Sammak, 1996; Daryani et al., 2007; Dalimi et al., 2002; Ibrahim, 2010). However, other surveys demonstrated that sheep's lungs in Sardinia and Peruvian Andes as well as cattle's lungs in Northwestern Ethiopia had the maximum cyst fertility (Dueger & Gilman, 2001; Scala, Garippa, Varcasia, Tranquillo, & Genchi, 2006; Kebede, Abebe, & Tilahun, 2009).

Our statistical analysis showed a significant relation of cysts' fertility and their size. Most of the fertile hydatid cysts had medium size in cattle livers (Table 6). In western Iran, most of the fertile cysts in sheep and cattle were found in large size (Dalimi Malaki et al., 2002). In Northwestern Ethiopia, most of the considered fertile cysts were small (Kebede, Abebe, & Tilahun, 2009). In current study, in relation between type and size of the cysts, most of sterile cysts were small (Table 2), which the results were similar to finding of Dalimi et al. in western of Iran (Dalimi et al., 2002). Most of calcified and suppurative cysts had small size (Tables 3 and 4). In western Iran, these cysts often had small size in sheep but in cattle were medium size (Dalimi et al., 2002).

Table 6. Viability of protoscoleces of fertile hydatid cysts in different organs of slaughtered animals based on size

Animals (%)	Infected organs	Size of cysts (cm)			
		<2 average viability %	2-4 average viability %	>4 average viability %	mean±S.D.
Sheep	Liver	41.09	49.88	47.04	46.49±5.39
	Lung	57.58	51.31	58.57	55.30±28.64
Cattle	Liver	75.20	-	-	75.20±7.60
	Lung	81.48	89.55	89.28	88.06±7.60

In this study, the highest and lowest viability of cysts were seen in cattle's lungs and sheep liver respectively (Table 6). In similar study in Mazandaran, the maximum and minimum viabilities of cysts were observed in

cattle's lungs and sheep's liver,respectively (Daryani, Sharif, Amouei, & Nasrolahei, 2009), but in western Iran and Saudi Arabia, lung and liver cysts in sheep had the highest viability (Dalimi et al., 2002; Ibrahim, 2010). Among the fertile cysts, maximum viability in the cysts of sheep's lungs and also cattle's liver belonged to the small-sized and in sheep's liver and cattle's lungs, to the medium-sized ones. Dalimi et al. reported the highest viability of small-sized fertile cysts in sheep but of large-sized ones in cattle (Dalimi et al., 2002). We didn't find any significant relation between viability and cyst size. The difference in viability of protoscoleces can be due to the various factors, including the impact of different situations, such as temperature and humidity, and difference of research methods, for example time of sampling, viability assessment method and genetic variations of *Echinococcus granulosus* (Manterola et al., 2006; Dicker, Tinar, & Senlik, 2007).

This research didn't show any hydatid cyst of goats. Since goats feed mainly by browsing, rather than grazing, they usually show lower levels of infection (Dalimi et al., 2002). Furthermore, in the other regions, the prevalence of hydatidosis in goats has been less reported than in cattle and sheep (Mohebali & Sammak, 1996; Daryani et al., 2007; Dalimi et al., 2002; Arbabi Hooshyar, 2006; Ibrahim, 2010).

According to the results, fertility rates of liver and lungs hydatid cysts of sheep and viability of their protoscoleces is considerable in Qazvin, Iran. Thus this region is of great importance from epidemiological aspect and also disease distribution. In addition, although the fertility of cysts in cattle organs is low, but they showed high viability rate. Therefore it is necessary that a preventive operations such as: perishing the infected organs of animals, controlling stray dogs and keeping them away from abattoirs, need to be monitored.

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