



Research Article

Studies on some parameters of echinococcal cysts isolated from ruminants slaughtered at Duhok abattoir, Kurdistan region, Iraq

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ABSTRACT

In this study some parameters like size, shape, pH, fertility and viability of protoscolices of Echinococcal cysts (EC) isolated from slaughtered ruminants (sheep, goats and cattle) at Duhok abattoir were determined. Throughout this study, 226 cysts were collected from 100 sheep, 64 goats and 62 cattle. Out of the examined cysts, 110 were spherical, 100 oval and 16 elongated in sheep, goats and cattle, respectively. Regarding to the pH of the cyst's fluid of different hosts was nearly neutral, ranged between 7.19 ± 0.74 for liver cysts and 7.37 ± 0.73 for lung cysts. Sheep cystic echinococcosis had the highest fertility rate (81%), followed by goats (39.06%), while cattle had the lowest fertility rate (17.74%). Regarding to the calcified, the highest rate (25%) was observed in goats, 6% in sheep and 3.22% in cattle. Protoscolices viability was higher in sheep (87.21%) than in goats (62.5%). This study revealed that most CEs were spherical and oval, the pH range of cystic fluid was nearly neutral. Sheep cysts were characterized by the highest rate of fertility and viability of their protoscolices. This indicate that the sheep are the principle intermediate host in Kurdistan region and are responsible in perpetuating the life cycle of the parasite and in spreading the disease to other hosts.

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Introduction

Cystic echinococcosis (CE) is the larval stage of a helminthic parasite, *Echinococcus granulosus* which causes a zoonotic disease with a worldwide distribution, particularly in developing countries and in regions of the world in which there is a close interaction between both the intermediate and the final hosts, typically sheep and dogs (Alvarez Rojas et al. 2014; Deplazes et al. 2017; Khan et al. 2021). This larval stage develops in various organs of the intermediate host such as sheep, cattle, goats, buffalos, camels, etc., and humans. These hosts acquire infection after accidentally ingesting the parasite eggs with the contaminated pasture, water or food. The eggs develop into a metacestode larva in their organs and tissues mainly the liver and lungs and other internal organs. Additionally, predators, mostly

domestic and wild canids, act as the definitive hosts harbouring the adult tapeworms in their small intestines (Alvaro et al. 2011; Gessese 2020). These definitive hosts acquire the infection through the consumption of the infected organs of the intermediate hosts containing fertile cysts (Deplazes et al. 2017).

Fertile hydatid cysts containing live protoscolices in intermediate hosts are important contributors to the transmission of the diseases including stray dogs that prowl near abattoirs and ingest diseased animal parts, the persistence of the *Echinococcus* life cycle is influenced by several variables, which vary depending on the geographical environment, host, and the infected organs (Vuitton 1997; Fallah et al. 2014).

There are nine species in the genus *Echinococcus*: *E. granulosus*, *E. equinus*, *E. felidis*, *E. canadensis*, *E. ortleppi*, *E. multilocularis*, *E. shiquicus*, *E. vogeli* and *E. oligarthra* (McManus 2013; Nakao et al. 2013). The commonest species that develop in humans and animals is *E. granulosus* causing cystic Echinococcosis (CE), other uncommon species that infect humans are, *E. multilocularis* causing alveolar echinococcosis and the uncommon species *E. vogeli* and *E. oligarthrus* producing polycystic and unicystic echinococcosis, respectively (Eckert et al. 2001; WHO 2013).

Due to the importance of CE as it causes huge health problems in human and losses to animal economy that can be manifested as a decline in the weight gain of animals, a decrease in milk production, a decline in reproduction rates, or a decline in the price of fleeces or other products (Ezatpour et al. 2013).

A considerable work has been performed on echinococcosis in Kurdistan region and rest parts of Iraq, most of it dealt with the prevalence of the disease in different intermediate hosts (sheep, goats and cattle) and its molecular identification (Saida and Nouraddin 2011; Jarjees and Al-Bakri 2012; Mero et al. 2014; Hama et al. 2015; Fadhil and A'aiz 2016; Murtaza et al. 2017; Meerkhan et al. 2018; Alsaady and Al-Quzweeni, 2019; Najim et al. 2020; Mohammed 2021). However, there are limited information on other cysts parameters such as the size, shape, rate of fertility and the viability of the protoscolices which are considered as the most important cysts constituents in disseminating the disease between intermediate host and aiding in the continuation of the parasite life cycle which are the aims of the present study that was performed on cysts isolated from animals slaughtered in Duhok abattoir.

Materials and methods

For this study a total of 226 CEs were isolated from infected animals (100 sheep, 64 goats and 62 cattle) from liver and lungs in Duhok abattoir. These cysts were categorized according to shape, size by measuring the diameter or length and width of isolated cysts in cm, each cyst was opened and the pH of the cyst fluid was measured, then about 3-4 ml of the fluid was centrifuged, and checked for fertility, while the viability of fertile cyst was determined by mixing the cystic fluid with 0.1% eosin (v/v), then examined under microscope and the rate of alive protoscolices was measured according to the following formula:

$$\text{Viability of protoscolices} = \frac{\text{No of viable protoscolices}}{\text{Total No. of protoscolices}} \times 100$$

Result and Discussion

Shape and size of the isolated cysts

The isolated CEs were categorized into various groups according to their shapes, as spherical, oval and elongated. The cyst size was measured in cm and the number of cysts in each infected organ was counted and recorded (Table 1). Out of 100 sheep cysts examined (51 lungs and 49 liver), 43.14% (22/51) lungs cysts were spherical, 49.1% (25/51) were oval and 7.84% (4/51) elongated. While in the liver, 22.44% (11/49) were spherical, 65.30% (32/49) oval and 12.24% (6/49) were elongated. In goats, total of 64 cysts were examined (21 lungs and 43 liver). In the lungs, 42.85% (9/21) were spherical, 47.61% (10/21) were oval and 9.52%

(2/21) were elongated. While in the liver 26 cysts 60.46% (26/43) were spherical, 30.23% (13/43) oval and 9.30% (4/43) were elongated. In cattle, a total of 62 cysts were examined (38 lungs and 24 liver). All of lungs and liver cysts of the cattle were spherical and oval, the rate of lung spherical cysts was 65.78% (25/38) and 34.21% (13/38) oval cysts. In the liver, the rate of spherical cysts was 70.83% (17/24) and 29.16% (7/24) were oval. Statistical analysis of the results using Chi-square analysis showed the presence of a highly significant ($P < 0.001$) difference between spherical, oval and elongated CEs in different intermediate host.

Regarding the number, in sheep the number of the cyst found in infected liver and lungs was equal which ranged between 1-8 cysts, while in goats and cattle the number of the cysts in the liver was slightly higher (1-5 and 1-12, respectively) than in the lungs (1-4 and 1-11, respectively). In Mosul, Jarjees and AL-Bakri (2012) observed that the number of cysts in sheep, goats, and cattle, ranged between 1-16, 1-6, and 1-10 in the infected organs. While Mitrea et al. (2012) found higher number of cysts in the infected organs (liver and lung) per animal, in cattle the number ranged between 1-47 and in sheep ranged from 1-16.

Regarding to the size of the cysts in the infected organs lungs and the liver, the size of spherical cysts of cattle liver was larger (1.8-12.4cm) than in sheep and goats' liver, while the oval shaped cyst of goat's lungs was larger (2.8-10.9cm) than those in sheep and cattle. But in elongated cysts the size in sheep was larger (3.2-9.9cm) than in goats. Statistical analysis using ANOVA test of these results showed the presence of significant differences ($P < 0.05$) between spherical, oval and elongated CEs in different intermediate hosts.

Lung cyst grows in faster rate than that of liver cysts (Larrieu and frideri 2001). It is normal for sheep cysts to develop faster than 1-2 cm in diameter (resulting in cyst volumes between 0.5 and 4.1 cm³ after a year) commonly recorded in sheep (Turner et al. 1937; Yamashita et al. 1957; Gemmell 1966). Thirty months after infection, the sheep cysts reach the size of 51 cm³ (Gemmell 1966). In Argentina and Spain most of cattle cysts ranges from 3-4 cm in diameter (Andresiuk et al. 2009). In Slovakia, Turcekova et al. (2009) stated that the average size of fertile pigs' cysts was 3 times larger than sterile (diameter 4.67 to 1.37cm). Cysts in the human body can range in size from 1 to 15 cm and are completely different in size, but large cysts (more than 20 cm) may occur (Ammann and Eckert 1996; Ahmed 2012). In Ethiopia, Abegaz et al. (2018) and Yideg (2021), found higher number of small sized cysts than the medium and large cysts in the different host. In Egypt, Abo-Aziza et al. (2019) measured the size of lung cysts in camel and cattle and stated that they ranged from 1 to 20 cm in diameter, while liver cysts ranged from 1 to 5 mm in diameter. In a previous study, Abdulla et al. (2020) found that the rate of most CEs measured 5cm in diameter, and that these cysts were more noticeable in the liver than in the lungs.

Types of studied cysts

According to the data in Table 2, the cysts were categorized as fertile, sterile, and calcified. The number of viable cysts was greater in sheep and goats than the sterile and calcified cysts.

However, in cattle, there were more sterile cysts than fertile and calcified cysts. Statistical analysis of the results using chi-square revealed the presence of highly significant differences ($P < 0.001$) between fertile, sterile and calcified cysts.

Most of the sheep cysts in the current investigation were fertile (81%). While the rates of fertility of goats and cattle cysts were lower at rates of 39.06 and 17.7%, respectively. Similar patterns of cyst type have been seen by Mohamad et al. (2008) and Hama (2013) both in Suleimani province.

These results are in line with the findings of Saida and Nouraddin (2011) in Erbil province, they reported the highest rate of fertile cysts in sheep than goats and cattle (94.11, 91.66 and 80, 64%), respectively. Also, in Sulaimani province, Mero et al. (2014) and Hama et al. (2017) reported the highest fertility rate of CE in sheep (86 and 85%) which was higher than that of goats and cattle (60, 60 and 58, 60%), respectively. In Ethiopia Abegaz et al. (2018), worked on the type of CE and found more fertile cyst than sterile and calcified cyst (60.79, 22.97 and 16.94%), respectively. The high fertility rate of CE in sheep indicates the fact that this animal is crucial to the continuation of the life cycle and the spread of the illness for human and other intermediate hosts.

With respect to cattle, Laatamna et al. (2018) in Algeria reported higher rate (96.15%) of sterile cysts than fertile (3.84%). In Ethiopia, Temam et al. (2016) and Yideg (2021) found sterile cyst at higher rate (57.75 and 55.84%) than fertile (20 and 8.61%) and calcified cysts (16 and 35.55%), respectively in slaughtered cattle. In Morocco, El Berbri et al. (2015) stated that the rate of sterile cysts was significantly higher in cattle than sheep (49.7% versus 45.1%) respectively. According to Costin et al. (2015), cattle cysts are mostly sterile and have no role in parasite transmission.

The viability of protoscolices of fertile cyst

The viability of the fertile cyst protoscolices was assessed by using 0.1% eosin (v/v). A total of 68 CEs from sheep and goats were tested as indicated in Table 3. The percentage of viability in sheep was highest (87.21%) than goats (62.5%). Similarly, Hosseini and Eslami (1998) tested the viability rates of cysts, which were 88% in sheep, 75% in goats and 60% in cattle. According to Dalimi et al. (2002), sheep cysts had a higher viability rate as compared to cattle cysts (82% vs. 75%). In Libya, Elmajdoub and Rahman (2015) did not find any variation in the viability rate of protoscolices recovered from all slaughtered animals, as it was 75.6% for all of them. On the other hand, Daryani et al. (2009) and Lahmar et al. (2013) reported lower viability rate in sheep (76.92 and 70.71%) as compared to cattle (82.5 and 78.45%), respectively. Younis (2011) examined the viability of female and male sheep cysts in which the highest (81.1%) was for female cysts. In Ethiopia, Abegaz et al. (2018) reported that the viability of the protoscolices of fertile cysts in slaughtered ruminants (sheep, goats and cattle) were 76.29%, while non-viable protoscolices were 23.71%. Also, Yideg (2021) in Ethiopia collected a total of 31 fertile cyst from liver and lungs of cattle and reported higher viability rate of the protoscolices than non-viable protoscolices (61.29 and 38.71%), respectively.

pH of cystic fluid

As indicated in Table 4, the mean value of lungs cysts pH in sheep, goats and cattle was 7.28 ± 0.06 , 7.25 ± 0.12 and 7.25 ± 0.12 , respectively, which was slightly higher than that of liver cysts (7.04 ± 0.05 , 7.25 ± 0.09 and 7.13 ± 0.15), respectively. However, statistical analysis of the results did not show any significant difference ($P > 0.05$) between pH values of cystic fluid in sheep, goats and cattle. Furthermore, in all studied hosts, the pH range was nearly neutral. Some studies reported the pH range of cystic fluid of *Echinococcus granulosus* cysts at 7.72 ± 0.12 and 7.613 ± 0.21 , respectively (Li et al. 2013; Shanshan et al. 2018).

Conclusion:

The results of this study demonstrated that the fertility and viability rates of CEs in sheep were higher than that of goats and cattle, this means, the sheep play a potential role in the transmission cycle of the parasite in this area. The pH of all CEs was close to neutral (about 7.25). Furthermore, most of liver and lungs cysts isolated from different intermediate hosts were spherical and oval in shape.

Authors' contribution: Both authors contributed equally in this study.

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Table 1: The number, shape and size of in different organs of animals slaughtered at Duhok abattoir

Animal	Infected organ	No. of Exam cyst	Average no. of cysts	Shape of cysts											
				Spherical				Oval				Elongated			
				No.	%	Size (cm)		No.	%	Size (cm)		No.	%	Size (cm)	
						Range	Mean ±SE			Rang	Mean ±SE			Rang	Mean ±SE
Sheep	lung	51	1-8	22	43.13	1.7-5.9	3.03±0.34	25	49.01	L: 2.4-8.4 W: 1.7-4.9	5.32±0.44 3.44±0.30	4	7.84	L: 3-8.9 W: 1.5-3.8	5.57±1.24 2.47±0.60
	liver	49	1-8	11	22.44	1.6-3.9	3.67±0.85	32	65.30	L:2.7-9.4 W: 2-5.8	5.56±0.31 3.63±0.20	6	12.24	L:3.2-9.9 W: 1.4-4.7	5.80±1.06 2.61±0.47
Goats	lung	21	1-4	9	42.85	1.2-6.8	2.78±0.69	10	47.61	L:2.8-10.9 W: 2-6.8	5.38±0.79 3.78±0.55	2	9.52	L: 1.8-3.0 W: 0.7-1.2	3.16±0.84 1.66±0.73
	liver	43	1-5	26	60.46	2.4-5.8	2.33±0.25	13	30.23	L: 1.4-10.1 W: 0.8-7.2	3.70±0.58 2.55±0.48	4	9.30	L:2.1-5.9 W: 0.6-2.4	4.18±0.63 1.92±0.41
Cattle	lung	38	1-11	25	65.78	1.4-5.9	3.19±0.23	13	34.21	L: 1.4-5.9 W: 1.4-3.9	4.17±0.33 2.81±0.20	-	-	-	-
	liver	24	1-12	17	70.83	1.8-12.4	2.81±0.63	7	29.16	L:1.9-10.4 W: 1.2-7.6	4.20±1.00 2.95±0.71	-	-	-	-

Table 2: The types of the studied cysts

Host	No. of cyst exam	Type of cysts					
		Fertile		Sterile		Calcified	
		NO	%	NO	%	NO	%
Sheep	100	81	81	13	13	6	6
Goats	64	25	39.06	23	35.93	16	25
Cattle	62	11	17.74	49	79.03	2	3.22
Total	226	117	51.76	85	37.61	24	10.61

Table 3: The viability rates of protoscolices of cysts isolated from sheep and goats

Host	No. of cysts exam	% of viability
Sheep	44	87.21
Goats	24	62.5
Total	68	

Table 4: The pH range of lung and liver cystic echinococcosis from different intermediate hosts (No=124)

Animal	Organ	No. of cysts exam	Range of pH	Mean ± S.E.
Sheep	Liver	36	6.37 --- 8.13	7.04±0.05
	Lung	35	6.77 --- 8.32	7.28±0.06
Goats	Liver	12	6.84 --- 7.94	7.25±0.09
	Lung	7	6.79 --- 7.78	7.25±0.12
Cattle	Liver	6	6.71 --- 7.80	7.13±0.15
	Lung	28	6.61 ---7.96	7.34±0.07
Mean value	Liver	54	6.37---8.13	7.19±0.74
Mean value	Lungs	70	6.61--- 8.32	7.37±0.73

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