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Research Article

Epidemiology and the types of isolated echinococcal cysts from sheep and cattle slaughtered at Zakho abattoirs, Zakho District. Kurdistan Region, Iraq

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ABSTRACT

Echinococcus granulosus sensu lato species complex is the parasite responsible for cystic echinococcosis (CE), which has a worldwide distribution. This study involved the prevalence of cystic echinococcosis in sheep and cattle slaughtered at Zakho abattoirs from February 2020 to January 2021, in which 21565 animals were slaughtered. They included 14547 sheep and 7018 cattle. The total rate of infection was 1.18% (255/21565), with the highest rate (1.34%) in sheep and the lowest (0.85%) in cattle. Among infected sheep, 45.64% of the cysts were seen in both the liver and lungs, 32.82% in the liver and 21.53% in the lungs. While among infected cattle, 76.66% of the cysts were found in both the liver and the lungs, 16.66% in the liver and 6.66% in lungs. Concerning animal gender, females of sheep and cattle showed the highest infection rate as compared to males (37.01 and 83.33% versus 0.49 and 0.71%), respectively. Regarding the yearly prevalence of CE, sheep showed the highest rate of infection throughout the year with a peak during April which was 4.43%, while the highest rate of infection in cattle was during August (2.63%). According to cyst types, sheep CE showed the highest rate (63.04%) of fertility, followed by cattle (14.29%). While the highest rate (77.77%) of sterile cysts were in cattle. Furthermore, 16.67% of sheep cysts and 7.95% of cattle cysts were calcified. Sheep cysts are characterized by the highest rate of protoscolices viability than cattle cysts (58.36 vs 27.36%).
These results showed that the prevalence of CE infection is lower than that previously reported in the same regions of Iraq, but the disease is still present and there is the possibility of its transmission to sheep and cattle which play an important role in the life cycle and transmission of this zoonotic disease to definitive host and from them to humans and other susceptible intermediate hosts.

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Introduction

Cystic echinococcosis (CE) is a silent helminthic zoonotic disease affecting human and animals, inflicting not only severe sickness and perhaps death in intermediate hosts but also causing economic losses due to treatment expenses, missed income, and livestock-related productivity losses (Day et al. 2012; Benyan et al. 2013). This disease has a global distribution and is mainly endemic in rural sheep-raising areas where both the definitive and intermediate hosts are in close contact to each other (Lett 2013; Deplazes et al. 2017). The parasite life cycle is completed in two hosts, the definitive host, the dogs and other members of family Canidae in which the adult stage inhabit their intestine, and the intermediate hosts including many ruminants such as sheep, goats, cattle, camel etc., in these hosts the larval stage lives in visceral organs (Romig and Deplazes 2017; Karamian et al. 2017; Mulinge et al. 2018). Intermediate hosts are infected by ingesting the infective eggs released with dog feces that contaminate the pasture (Adanir and Tasci 2013; Romig et al. 2017). The dogs and other carnivores acquire infection by eating infected organs from slaughtered animals such as the liver and lungs containing fertile cysts (Otero-Abad and Torgerson 2013; Craig et al. 2017). Many studies have been performed on *E. granulosus* in Iraq and Kurdistan Region, which are considered an endemic area of animal CE, causing a great economic loss in animals due to damage in the infected organs (AL-Nakeeb 2004; Al-Jawady 2009; Al-Bosely 2013; Mero et al. 2014; Meerkhan et al. 2018; Abdulhameed et al. 2018; Mohammed 2021). The objectives of this study were to determine the prevalence of the disease in slaughtered animals in Zakho abattoir, Zakho district, Kurdistan region, Iraq, and their correlation with gender, cyst location, and the monthly rate of infection, in addition, the cyst fertility and the viability of fertile cysts protoscolices were also determined.

Materials and Methods

Experimental design

Samples collection

The cysts were collected during the period from February 2020 to January 2021 from Zakho abattoir (Fig. 1), which was visited twice a week for sample and data collections included: the number of animals slaughtered, those infected, the host type, gender, the month of slaughtering and the infected organs. During this period, 21565 slaughtered animals, 14547 sheep, and 7018 cattle were examined for Echinococcal cysts. The detected cysts were isolated from the infected visceral organs of the inspected infected hosts. Each cyst was placed in a sterile, fully labeled container, then all collected cysts at each visit were placed in a cool box and transferred to the laboratory at the Biology Department, Faculty of Science, University of Zakho, for examination and further processing.

Determination of the type of cysts

In the laboratory, a total number of 201 CEs were isolated from the infected organs of sheep and cattle, these included: 138 sheep cysts and 63 cattle cysts. Individual cysts were grossly examined to determine their type (fertile, sterile or calcified) by examining a piece of the germinal layer of each cyst under the microscope or by withdrawing 5ml of cyst fluid with a sterile syringe, then was transferred to a clean test tube. This liquid was centrifuged for 5 minutes at 3000 rpm or allowed to settle, and after that, one drop of the precipitate was placed on a microscopic slide, covered with a cover slip, and inspected for the presence of protoscolices under a light microscope at 10 x followed by 40 x. The cysts that lacked protoscolices were regarded as sterile (Daryani et al. 2006).

The viability of cysts protoscolices

Fifty-three echinococcal cysts including 41 sheep and 12 cattle cysts were examined to determine the viability of their protoscolices using eosin dye for the invaginated and evaginated protoscolices by direct microscopical examination under 40X magnification (Daryani et al. 2006). The procedure was carried out by adding a drop of 0.1% aqueous eosin solution (v/v) and a drop of each centrifuged cyst sample to a slide, covering it with a cover slip, and examining it at 40X. The stain was not absorbed by the living protoscolices, but it penetrates the dead protoscolices making them red (Esfahani and Youssefi 2010). The viability was measured by counting the number of live protoscolices using the following formula:

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

Data analysis

The obtained epidemiological data were analyzed using the SPSS statistical analysis program (version 25), to evaluate the probability value, the Chi-square (X2) test was performed. (P<0.05) regarded as significant.

Results

The prevalence of cystic echinococcosis in slaughtered sheep and cattle:

Table 1 shows the prevalence of echinococcal cysts in sheep and cattle. It is clear from the results that the overall infection rate was 1.18% (255/21565) in animals, with sheep having the highest rate, which was 1.34% (195/14547). On the other hand, the infection rate in cattle was 0.85% (60/7018), which was lower than in sheep. Statistical analysis revealed the presence of highly significant differences (P<0.01) between infected animal species.

Table 1: The prevalence of cystic echinococcosis from sheep, and cattle at Zakho abattoir

Hosts	No. of slaughtered animals	No. of infected	% of infection
Sheep	14547	195	1.34
Cattle	7018	60	0.85
Total	21565	255	1.18
X^2:9.551	P=0.002		

The prevalence of CE in animals according to the gender:

In this study, the rate of CEs among slaughtered sheep and cattle and their gender are presented in Table 2. In general, females of infected animals showed a very high rate of infection as compared with males (38.62% versus 0.57%). When comparing each host separately, we found that female sheep and cattle have greater CE rates than males (37.01 and 83.33% versus 0.50 and 0.71%), respectively. Statistically highly significant differences between these two groups (*P*<0.01) were observed.

Fig.1: Zakho abattoir, Zakho city, Kurdistan Region, Iraq

Table 2: The prevalence of cystic echinococcosis in both genders of slaughtered animals

Hosts	Gender	No. of examined animals	No. of infected	% of infection
Sheep	Male	14212	71	0.50
	Female	335	124	37.01
Cattle	Male	7006	50	0.71
	Female	12	10	83.33
Total	Male	21218	121	0.57
	Female	347	134	38.62
X^2: 4444.036		*P*<0.001		

The cyst location in slaughtered animals

Table 3 shows the distribution of CE in several organs of infected slaughtered animals. It is obvious from the results that the highest infection rates among sheep and cattle were in the liver and the lungs (45.64 and 76.66%, respectively). These rates were followed by the liver alone, accounting for 29.10%, and the lowest rate (18.03%) was in the lungs. Different organs showed statistically significant variations (*P*<0.05) in rates of infection.

Table 3: The rates of cystic echinococcosis in various organs of slaughtered sheep and goats

Hosts	No. Infected	Liver		Lung		Both (liver and lung)	
		No.	%	No.	%	No.	%
Sheep	195	64	32.82	42	21.53	89	45.64
Cattle	60	10	16.66	4	6.66	46	76.66
Total	255	74	29.01	46	18.03	135	52.94
X^2: 18.094	*P<0.05*						

The monthly rate of cystic echinococcosis in slaughtered animals

Table 4 presents the monthly infection rates of CE in the slaughtered animals. As indicated from the table, the highest rates of infection occurred throughout the year in the slaughtered sheep, with a peak in April which was 4.43%, the lower rate of infection (0.24%) was in November, while infection was not detected during October and January. These differences were statistically significant (*P*<0.05). On the other hand, the monthly prevalence of CE in cattle, during the period of the study, showed the highest rate of infection during August (2.63%) and the lowest rate (0.19%) was in November (0.19%). Also, infection was not reported during October. Statistical analysis revealed significant differences (*P*<0.05) between the rates of infection in different months.

Table 4: The monthly rates of cystic echinococcosis in slaughtered sheep and cattle.

Date in months	Sheep			Cattle		
	No. of slaughtered	No. of infected	% of infection	No. of slaughtered	No. of infected	% of infection
Feb. 20	1124	30	2.66	640	2	0.31
Mar. 20	1580	44	2.78	666	2	0.30
Apr. 20	1264	56	4.43	699	2	0.28
May. 20	1482	28	1.88	754	9	1.19
Jun. 20	1053	6	0.56	519	6	1.15
Jul. 20	1599	9	0.56	655	10	1.52
Aug. 20	1213	9	0.74	456	12	2.63
Sep. 20	1119	6	0.53	495	6	1.21
Oct. 20	900	0	0.00	456	0	0.00
Nov. 20	806	2	0.24	518	1	0.19
Dec. 20	1297	5	0.38	616	5	0.81
Jan. 21	1110	0	0.00	544	5	0.91
Total	14547	195	1.34	7018	60	0.85
X^2: 198.875 *P*<0.05				X^2: 36.745 *P*<0.05		

The types of cysts isolated from slaughtered animals

The isolated cysts were classified based on their types as fertile, calcified, and sterile, as shown in Table 5. A total of 138 sheep and 63 cattle cysts were examined. The highest proportion (63.04%) of fertile cysts was recorded in sheep, while the highest percentage (77.77%) of sterile CEs was found in cattle. Furthermore, 16.67% of sheep cysts were calcified. Statistical analysis using chi-square (X2) indicated that the species of the intermediate host and the types of the cysts were dependent, meaning that the fertile, sterile, and calcified cysts were significantly highly affected by the species of inspected animal (*P*<0.01).

Table 5: The types of cysts isolated from infected slaughtered sheep and cattle in Zakho abattoir.

Hosts	No. of cyst exam	Types of cysts					
		Fertile	%	Sterile	%	Calcified	%
Sheep	138	87	63.04	28	20.29	23	16.67
Cattle	63	9	14.29	49	77.77	5	7.94
Total	201	96	47.76	77	38.31	28	13.93
X^2: 61.211 *P*<0.01							

The viability rate of cyst protoscolices isolated from sheep and cattle

The vitality of fertile cyst protoscolices was assessed by the activity of flame cells, peristaltic motility, and the capacity to stain with 0.1% aqueous eosin solution by testing a total of 53 CEs isolated from 41 sheep and 12 cattle, as shown in Table 6. Protoscolices of sheep cysts had a higher rate of viability (58.36%) than those of cattle (27.36%).

Table 6: Viability rates of protoscolices of cysts isolated from slaughtered sheep and cattle.

Hosts	No. of cysts examined	% of viability
Sheep	41	58.36
Cattle	12	27.36
Total	53	

Discussion

Cystic echinococcosis is a zoonotic infection that is prevalent all over the world, including the Kurdistan region and other parts of Iraq. Usually, ruminants are more susceptible to infection due to the close association between them and the dogs, which contaminate the pasture with viable echinococcal eggs. The primary source of CEs in domestic animals is stray dogs, which acquire the infection by consuming infected offal from animals slaughtered outside abattoirs. However, in most nations, the government has authority over this procedure at urban abattoirs where veterinarians oversee the slaughtering of animals and burn their infected organs (Assefa et al. 2015; Elmajdoub and Rahman 2015). In this study, the highest infection rate with CE in Zakho abattoir was observed in sheep (1.34%), followed by cattle (0.85%). Similarly, in other studies in Iraq and Kurdistan region, there have been reports of a high infection rate in sheep, such as Saida and Nouraddin (2011) in Erbil; Jarjees and Al-Bakri (2012) in Mosul; Sargali and Mero (2013) in Duhok; Al Bosely (2013) in Zakho; Mero et al. (2014) in Slemani; Hassan (2017) in Erbil; Meerkhan et al. (2018) in Duhok and Mohammed (2021) in Baghdad, these studies recorded the highest rates in sheep, as they had the highest risk of infection, even though these rates were variable (11.1, 2, 12.3, 24.63, 12.7, 9.07, 4.25 and 2.0%), respectively. These results indicate the high susceptibility of sheep to this parasite. The high infection in sheep from other animals, might be due to the host specificity and strain availability since most of the performed studies in this area indicate the presence of sheep strain, in addition, sheep are more desirable for rearing due to their consumption preference to fulfill religious and social requirements and they are more adapted with dog than goats or cattle, also the feeding habits of goats and cattle differ from that of sheep (Mero et al. 2014).

The decreased frequency of CE among cattle in the current study might be attributed to better care in the cowshed, where they have less interaction with infection sources (Thompson and McManus 2002), or it may be due to difference in strains of *E. granulosus* in the area as strains exhibit a different degree of infectivity for certain intermediate hosts and the most prevalent strain in this area is the sheep strain (Ahmed 2012; Hama 2013; Hassan 2017).

On the other hand, some studies found higher infection in cattle than in sheep. For example, Bajalan (2006) in Kalar reported a rate of 0.57% in cattle versus 0.27% in sheep. Furthermore, Meerkhan and Abdullah (2012) in Duhok, also, reported a higher CE rate in cattle versus sheep (10.57 and 9.92%), respectively. They attributed it to the fact that adult and old cattle are slaughtered thus long duration of life increases the risk of exposure to eggs of *E. granulosus* with a higher possibility of acquiring the infection while sheep and goats are slaughtered at early ages, so the chances of acquiring are reduced, or cysts are small and undetectable (Kebede et al. 2009).

The results of CE prevalence in slaughtered sheep and cattle at Zakho abattoir of both genders showed the presence of significant differences between infection rates in females which showed the highest rates vs males in both sheep and cattle (37.01 vs 0.50%) and (83.33 vs 0.71%) respectively. Similar findings were reported in other studies in Kurdistan regain, Iraq including Mero et al. (2014) in Slemani (Iraq); Murtaza et al. (2017) and Abdulhameed et al. (2018) in Basrah (Iraq), all these studies reported higher infection rate in females. The lower rate of infection in males is due to their slaughtering at younger ages (6 months to less than 18 months), since this time is not enough for the development and detection of echinococcal cysts, so they are too small and can be easily missed during meat inspection. On the other hand, female livestock were usually maintained for longer periods to give offspring several times before slaughtering (Ibrahim 2010; Muqbil et al. 2012).

The present study revealed that the co-infection of the liver and lungs was the predominant situation, as the liver is the primary site of infection followed by the lungs. Similar findings were reported by many researchers from Iraq and different parts of the world (Saida and Nuraddin 2011; Muqbil et al. 2012; Jarjees and Bakri 2012; Hama 2013; Mero et al. 2014; El Berbri et al. 2015; Toulah et al. 2017; Murtaza et al. 2017; Mohammed 2021). According to their claims, the liver serves as a first barrier for the oncosphere after it penetrates the intestinal mucosa and enters the portal circulation before being carried by the bloodstream to all parts of the body, they also claimed that the liver is the first filter, and the lungs are the second filter. Furthermore, the large size of the oncosphere lodges it in the liver (Kebede et al. 2009). On the other hand, the results of this study did not coincide with those of some other workers in which they found that the lungs were the most predominant sites for cyst development (Ghaffar 2008; Abdullah 2010; Latif et al. 2010; Tappe et al. 2010; Sargali and Mero 2013; Temam et al. 2016; Roostaei et al. 2017; Meerkhan et al. 2018). The common route for larva to reach the lungs is through the alimentary canal of the intermediate host, where the hatched oncospheres pierce the intestinal wall to access blood vessels, and then go to the liver and lungs through blood. The oncosphere may also be released from the eggs during rumination in the second method, providing direct access to the lungs through the trachea (Qingling et al. 2014; Temam et al. 2016).

Throughout the year, sheep exhibited a high prevalence of disease with a peak during April which was 4.43%, while infection was not reported during October and January. On the other hand, in cattle the high rate of infection occurred during August, which was 2.63% and no infection was found in October. The present results partially agree with those reported by Al-Berwari (2012) in Duhok province, as he reported the highest rate of infection in sheep (25.6%) during April and the lowest during September (13.7%). This might be due to the source of slaughtered sheep. The current findings, however, did not agree with those of Meerkhan and Abdullah (2012), who observed the highest (13.3%) infection of CE in sheep in July and the lowest (7.88%) in June, while in cattle the highest (13.24%) infection rate of CE was in June and the lowest (7.46%) in February. Sargali and Mero (2013) also found the highest rate (22.8%) in sheep in July, while the lowest (8.1%) was in April.

The cysts were classified as fertile, sterile, and calcified. Cysts isolated from sheep were characterized by higher rate of fertility than those isolated from infected cattle (63.04 vs 14.29%), and cysts isolated from the liver and the lungs of both sheep and cattle showed higher rates of fertility than those isolated from other organs. It is important to determine the cyst fertility rate, since it plays a significant role in the epidemiology, because fertile cysts have a significant role in disseminating the disease. Similar observations have been documented by Saeed et al. (2000); Azlaf and Dakkak (2006); Daryani et al. (2007); Hama (2013) and Tesfaye et al. (2016), they stated that out of all organs; the liver had the most prevalence of viable cysts than other organ, followed by the lungs and then other body organs. In Erbil, Saida and Nouraddin (2011) reported the highest rate of fertile cysts in sheep than goats and cattle (94.11, 91.66 and 80.64%), respectively. In Duhok province, Al-Bosely (2013) found the higher rates of fertile cysts in sheep (81%) followed by goats (39.06%) and the least in cattle (17.74%). 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. The higher CE fertility rate in sheep suggests that this species is essential to the maintenance of the life cycle and the transmission of the disease to humans.

Concerning cattle, Al-Bosely (2013) reported a higher rate (79.03%) of sterile cysts as compared to fertile (17.74%) and calcified cysts (3.22%). Laatamna et al. (2018) in Algeria reported higher rate (96.15%) of sterile cysts than fertile (3.84%) cysts. In Ethiopia, Temam et al. (2016) found sterile cyst at higher rate (57.75%) than fertile and calcified cysts (20 and 16%), 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 variation in protoscolices viability rates from fertile cysts might be explained by the host's immune response. Furthermore, the genotype of the parasite may influence the fertility of the cysts in intermediate hosts (McManus 2006; Assefa et al. 2015).

The viability of the protoscolices of viable cysts was evaluated by using 0.1% eosin. Sheep had a greater viability rate (58.36%) than cattle (27.36%). Similarly, Hosseini and Eslami (1998); Dalimi et al. (2002); Kouidri et al. (2012) and Hassan (2017) reported higher rates of protoscolices viability (88, 82, 61.22 and 86.36%), respectively in sheep than that in cattle (60, 75, 4.22 and 57.13%). On the other hand, Daryani et al. (2009) and Lahmar et al. (2013) reported lower viability rates in sheep (76.92 and 70.71%) as compared to cattle (82.5 and 78.45%), respectively. The variations in viability rate using a 1% eosin stain may be related to the time duration in which the protoscolices absorb this stain. It is important to estimate the time taken by the live protoscolices to absorb the stain, since living protoscolices did not absorb the stain until they died, and the dye normally enters the protoscolex within 5-8 minutes. Also, differences within immunological responses of each host and calcareous corpuscles in protoscolices might explain the differences in viability rates (Yones et al. 2014; AL-Jobori et al. 2016).

Conclusion

The results of the present study showed that CE is prevalent in domestic animals in Zakho city, Kurdistan region/ Iraq. Sheep play an important role in spreading the disease due to their high susceptibility rates. Furthermore, cysts isolated from sheep are characterized by high rates of fertility and viability of their protoscolices. Also, this district is populated with many stray dogs that gain access to infected organs of animals slaughtered outside abattoirs, thereby contaminating the environment with echinococcal eggs, thus acting as a positive source of infection. This situation necessitates the initiation of prevention and surveillance programs that will be important in reducing CE not only in animals but also in humans.

Author contribution

WMSM and SHA planned the experimental design; ARI collected the samples, conducted the research, and wrote the first draft of the manuscript. WMSM and SHA checked the data and reviewed the manuscript. All authors have finally approved the manuscript for publication.

Ethical Statement

All the experiments were conducted according to animal ethical principles. The University ethical committee approved the experimental layout.

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Consent for Publication

All the authors consented to the publication.

Data Availability

All the data regarding this research can be obtained from the corresponding author.

Competing Interest

The authors declare they have no relevant financial or non-financial interests to disclose.

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