HYDATID CYSTS FERTILITY AND *ECHINOCOCCUS* PROTOSCOLECES VIABILITY IN BUFFALOS, CATTLE AND SHEEP CARCASSES AT A SLAUGHTERHOUSE, TABRIZ, IRAN

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Abstract. *Echinococcus* hydatid cysts are a public health and veterinary concern. In order to determine hydatidosis prevalence and the effects of age, sex and season, lung and liver of animals (n = 10,355) at a slaughterhouse, Tabriz, Iran were inspected between April 2017 and March 2018. Hydatid cysts were found in 325/4,000 (8.1%) cattle, 35/335 (10.4%) buffalos and 1,580/6,000 (26.3%) sheep. The prevalence of hydatid cysts increased with age of cattle (p-value <0.05) and was higher in female compared to male sheep and cattle (p-value <0.05). The season of the year only affected prevalence in sheep, being highest in winter (31.4%) (p-value <0.05) possibly due to differences in grazing patterns compared to cattle and buffalo. The presence of protoscoleces in cyst aspirate was used to assess its fertility, being 34/65 (52%), 33/175 (19%) and 114/196 (58%) in buffalos, cattle and sheep, respectively. Based on an eosin exclusion assay, viable protoscoleces from buffalos, cattle and sheep were 18, 24 and 42%, respectively. The results indicate in Tabriz, Iran cattle and buffalos played a major role in disease transmission, but sheep were the more important intermediate host based on prevalence, cyst fertility and protoscolex viability.

Keywords: buffalo, cattle, hydatidosis, protoscolex, sheep

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INTRODUCTION

Cystic echinococcosis (hydatidosis) caused by the larval stage of *Echinococcus* spp, especially *Echinococcus granulosus sensu lato*, is one of the most important parasitic diseases of livestock causing economic loss and posing a public health issue, particularly in developing countries including Iran (Rokni, 2009; Miambo *et al*, 2020; Borhani *et al*, 2021). The ability of this cestode to adapt to a wide variety of domestic and wild intermediate hosts has resulted in its distribution worldwide (Torgerson and Budke, 2003; Manterola *et al*, 2022). In the life cycle of *Echinococcus*, dog serves as final host and livestock, such as sheep, cattle, goat, and buffalo, as intermediate host. After ingestion of eggs, hydatid cysts form in the organs of livestock, resulting in rejection at slaughter. Hydatid cysts are characterized by lesions with clear boundaries, grow 1-30 mm in diameter annually and contain numerous tiny protoscoleces. Hydatid cysts most often develop in the liver and lungs, although they can be found in other organs, such as the spleen, heart, kidneys, bones, eyes, and the central nervous system (Cardona and Carmena, 2013). Adult *Echinococcus* develops in dogs after consumption of infected organs, but dogs do not tend to have any clinical signs of infection.

An important factor in the epidemiology of hydatidosis is the variation in fertility (*ie*, presence of protoscoleces), which depends on the intermediate hosts and geographical situations (Khan *et al*, 2001; Kouidri *et al*, 2012). In addition to fertility, the viability (live or dead) of protoscoleces in intermediate hosts is an important index for the transmission of *Echinococcus* to dogs. Studies on the prevalence and fertility of hydatid cysts and protoscolex viability have been carried out in some parts of Iran (Dalimi *et al*, 2002; Pour *et al*, 2012). However, due to the effect of geographical locations, changes over time in the pattern of infection and fertility of cysts and protoscolex viability, regional and updated studies are needed to assess the role of these indicators in the stability of the parasite lifecycle and spread and persistence of infection in humans.

Thus, the current study was conducted to investigate the prevalence of hydatid cysts as well as the fertility of cysts and viability of protoscolex in livestock slaughtered in an industrial slaughterhouse, Tabriz, Iran. The results will enable a better understanding of which animals to focus on for meat inspection to decrease human infections.

MATERIALS AND METHODS

Sample collection

During April 2017 - March 2018, buffalos, cattle and sheep at an industrial abattoir of Tabriz in the northwest Iran were examined for hydatid cysts. Sampling occurred for ten days of each month on all animals slaughtered unless there were too many to process in one day, in which case the excess carcasses were not examined. The days selected, based on slaughterhouse records, were considered representative of all days as regards the source and age of animals. The month of slaughter, sex, and age (<3 or ≥3 years of age based on dentition) were recorded for each animal. The liver and lungs of the slaughtered animals were carefully inspected visually, palpated and cut as needed into sections to reveal hydatid cysts and the number observed in each animal recorded.

Due to slaughterhouse regulations and the permit under which the study was undertaken, a limited number of infected livers and lungs could be removed for further analysis. These were randomly selected and representative of the age groups on day of collection. Cysts in these organs were isolated and stored on ice packs for transport to the laboratory.

Laboratory investigations

Under aseptic condition, cyst fluid was aspirated, centrifuged at 2000 g for 3 minutes and the sediment examined under a light microscope (40× magnification) for protoscoleces. A cyst was categorized as fertile if protoscoleces were present. For assay of viability, protoscoleces were stained with 0.1% aqueous eosin solution and movement of flame cells were observed under a light microscope (1,000× magnification). Then, one drop of 1% aqueous eosin solution was added and the viability of protoscoleces assessed, those stained red categorized as dead and those that remained colorless categorized as viable (eosin exclusion test) (Smyth and Barrett, 1980). Within each animal species, the presence of cysts, number (<5 or ≥5) and fertility status, and age and sex of the animal, organ origin of cyst, and season (spring: April - June; summer: July - September; autumn: October - December; winter: January - March) were recorded.

Data analysis

The fertility rate, calculated as percent cysts with protoscoleces, was compared between organs and hosts using a Chi-square test. For each fertile cyst, the percent viable protoscoleces was calculated and the percent and mean were compared across animal species and between the liver and lungs within an animal species. A Chi-square test (with a Yates correction when a cell in a

2x2 table contained <10 parameters) was used to compare the number of infections according to age, sex, season, and organs. Due to a non-normal distribution of the data (based on a Kolmogorov-Smirnov test), a Kruskal-Wallis test was used for all comparisons with a Bonferroni correction for multiple comparisons. A p-value <0.05 is considered significant. All statistical analyses were performed using a VassarStats website for statistical computation (vassarstats.net) or Minitab® 21.1 software package (Minitab LLC, State College, PA).

RESULTS

During the one year of study (April 2017 - March 2018), slaughtered animals (n = 10,355), consisting of buffalos (n = 335), cattle (n = 4,000) and sheep (n = 6,000), were inspected. Highest prevalence of hydatid cysts were in sheep (26.3%) and lowest in cattle (8.1%) (Table 1), with all positive animals having at least 5 cysts. Only cattle (not buffalos or sheep) ≥3 years of age had higher rate of infection than younger cattle (<3 years of age). These two age categories were used to define young and old animals according to a previous study conducted in the same region (Mirzaei *et al*, 2015) and all animals in the ccurrent study were 2-5 years of age. A significantly higher prevalence in female than male animals were observed in cattle and sheep (p-value = 0.044 and <0.001 respectively). Seasonal changes in cyst prevalence affected only sheep, being highest (31.4%) in winter and lowest (21.9%) in autumn (p-value <0.001). In all three animal species, hydatid cysts were most frequently found in the lungs, with a significant difference between lung and liver infections in cattle (p-value <0.001) and sheep (p-value <0.001).

The highest number of cysts (n = 196) were isolated from sheep liver and lungs and lowest from buffalo organs (n = 65), highest cyst fertility (58.2%) from sheep and lowest (18.9%) from cattle, highest proportion of viable protoscoleces from fertile cysts (41.7%) obtained from sheep and lowest (18.0%) from buffalos, and highest number of viable protoscoleces per fertile cyst (69.2%) from sheep and lowest (27.7%) from buffalos (Table 2).

DISCUSSION

In studies carried out in different geographical regions of Iran, varied prevalence of hydatidosis in sheep and other livestock has been reported (Vaisi-Raygani *et al*, 2021). In the current study, hydatidosis was more prevalent in sheep compared to buffalo and cattle in agreement with most previous reports (Daryani *et al*, 2007, Fakhar and Sadjjadim 2007; Azami *et al*, 2013) and in agreement

Table 1

Hydatid cysts (*Echinococcus* spp) in animal carcasses according to age, sex, season, and organ at a slaughterhouse, Tabriz, Iran (April 2017 to March 2018)

Variable	Buffalo			Cattle			Sheep		
	Number examined	Number infected *n* (%)	*p*-value*	Number examined	Number infected *n* (%)	*p*-value*	Number examined	Number infected *n* (%)	*p*-value*
Total	335	35 (10.4)		4,000	325 (8.1)		6,000	1,580 (26.3)	
Age									
<3 years	99	5 (5.1)	0.058	1,500	72 (4.8)	<0.001	4,500	1,160 (25.8)	0.091
≥3 years	236	30 (12.7)		2,500	253 (10.1)		1,500	420 (28.0)	
Sex									
Male	85	7 (8.2)	0.572	1,500	105 (7.0)	0.044	3,500	700 (20.0)	<0.001
Female	250	28 (11.2)		2,500	220 (8.8)		2,500	880 (35.2)	
Season									
Spring	100	12 (12.0)	0.247	1,000	85 (8.5)	0.499	1,500	400 (26.7)	<0.001
Summer	100	8 (8.0)		1,000	77 (7.7)		1,500	380 (25.3)	
Autumn	80	12 (15.0)		1,000	90 (9.0)		1,500	329 (21.9)	
Winter	55	3 (5.5)		1,000	73 (7.3)		1,500	471 (31.4)	
Organ									
Liver	335	30 (9.0)	0.603	4,000	197 (4.9)	<0.001	6,000	1,313 (21.9)	<0.001
Lung	335	35 (10.4)		4,000	325 (8.1)		6,000	1,580 (26.3)	

Note: Spring: April - June; Summer: July - September; Autumn: October - December; Winter: January – March

*$p<0.05$ considered significant, based on Chi-square test with a Yates correction when a 2x2 cell contained <10 variables

Table 2

Number of cysts per organ, cyst fertility and viability of protoscoloces from animal carcasses, Tabriz slaughterhouse, Iran (April 2017 to March 2018)

Animal	Organ	Number of cysts examined	Number of organs with ≥5 cysts *n* (%)[†]	Fertile cysts *n* (%)	Protoscoleces in fertile cysts	
					% viable protoscoleces (mean ± SD)	Number of viable protoscoleces per cyst (mean ± SD)
Cattle	Liver	77	27 (35.5)	15 (19.5)	18.2 ± 10.1	30.5 ± 12.5
	Lung	98	32 (32.7)	18 (18.4)	28.6 ± 5.5	38.5 ± 9.4
	Total	175	59 (33.7)	33 (18.9)*	23.9 ± 9.4	34.9 ± 11.5
Buffalo	Liver	30	13 (43.3)	17 (56.7)	13.8 ± 4.8	24.5 ± 11.6
	Lung	35	18 (51.4)	17 (48.6)	22.1 ± 19.3	30.9 ± 10.2
	Total	65	31 (47.7)	34 (52.3)	18.0 ± 14.5	27.7 ± 11.3
Sheep	Liver	102	32 (31.4)	69 (67.6)**	40.3 ± 12.2	66.4 ± 22.0
	Lung	94	20 (21.3)	45 (47.9)	43.9 ± 15.3	73.6 ± 25.5
	Total	196	52 (26.5)	114 (58.2)	41.7 ± 13.6***	69.2 ± 23.6***

*p-value <0.001 compared between cattle and the other two animal species (Kruskal-Wallis test)

**p-value = 0.005 compared between organs (Chi-square test)

***p-value <0.001 compared between sheep and the other two animal species (Kruskal-Wallis test).

[†]All animals with cysts had ≥5 cysts, but not all individual organs had ≥5 cysts

with the life cycle of *Echinococcus* that is better adapted to sheep (Cardona and Carmena, 2013). Previous reports from Iran also indicated that sheep are the most common and important intermediate host of *Echinococcus* and with the highest rates of cyst infection and fertility (Akhlaghi *et al*, 2005; Rokni, 2009; Daryani *et al*, 2007). The prevalence of hydatid cysts in sheep in Iran is estimated to be 4.3% (Vaisi-Raygani *et al*, 2021), although much higher prevalence has previously been reported in specific regions, such as Kurdistan (51.9%) and Ardabil (74.4%) provinces (Akhlaghi *et al*, 2005; Daryani *et al*, 2007). As regards hydatid cysts in buffalos and cattle, other regions of Iran have reported both lower and higher prevalence (Dalimi *et al*, 2002; Daryani *et al*, 2007; Khanmohammadi *et al*, 2008; Pour *et al*, 2012; Azami *et al*, 2013; Vaisi-Raygani *et al*, 2021), with no indication of trends in prevalence based on the year of the study with both recent and older studies indicating high and low prevelance based on the animal and region.

The variations and differences in prevalence reported in previous studies compared to the current study could be due to differences in management practices, sample size and age of animals. The current study noted an increasing trend of hydatidosis in animals ≥3 years of age, in agreement with the findings of other researchers (Craig *et al*, 2015). The main reason for this is that older animals have a longer exposure time to *Echinococcus* eggs.

The finding that hydatidosis prevalence was higher in female than male animals was in agreement with Pour *et al* (2012) and Daryani *et al* (2007) findings in carcasses at slaughter houses. A possible association was observed between female sex hormones and immune system responses that results in a higher infection in female animals (sheep, goats, cattle and buffalo) has also been found by other authors (Bortoletti *et al*, 1990; Mehmood *et al*, 2020). The higher infections in females also could also be due to the higher age of females compared to males at slaughter as females are often kept for a longer duration to benefit from milk production and reproduction.

The seasonal difference was only seen in sheep in agreement with the findings of Almalki *et al* (2017). The higher number of infections in sheep during the winter season could be attributed to the seasonal migration of sheep, which are moved to other grazing areas during spring and summer and are returned in mid-autumn to Tabriz Province. The spring and summer grazing areas might result in different exposures to different populations of the final hosts. Also, older animals and those with low fecundicity are culled and

sent to slaughter upon return to Tabriz. On the other hand, buffalo and cattle are usually kept in houses and stables, hence, older animals with an accumulation of cysts and weaker and thinner sheep, with potentially lower immunity, are sent to slaughter in the winter.

As regards infected organs, the number of cysts detected is significantly higher in the lungs than liver of cattle, in agreement with previous studies (Khan *et al*, 2001; Dalimi *et al*, 2002; Daryani *et al*, 2007). The difference in lung and liver infections are the presence of greater capillary beds in the lungs, dilation of liver capillary with animal leading to oncospheres passing directly to the lungs, and hexacanth embryo entering the lymphatic circulation and carried via the thoracic duct to the lungs (Arene, 1985).

The definition used to distinguish between low and high cyst infection per animal and per organ in the current study differed from several previous studies that categorized low as <10 and high as ≥10 cysts/animal, as most animals had a cyst load ≥10 (Daryani *et al*, 2007; Azami *et al*, 2013). In the current study, because of low cyst infection rate, a low cyst infection is defined as <5/animal or organ similar to that of Amer *et al* (2018). Intensity of infection could be attributed to several factors, *viz* different susceptibility of host animals, dispersion density, infectivity rate of eggs (Bortoletti *et al*, 1990), role of the immune system in preventing the establishment of new cysts, and suppression of formation of new cysts by parasite aggregation through a density-dependent constraint mechanism acting in a limited volume (Lahmar *et al*, 1999). In the latter situation, Lahmar *et al* (1999) explained that as larger cysts form in older animals, competition for space could be a reason for a lower number of multiple cysts.

In the current study, the majority of cysts in cattle were sterile while those in sheep had a much higher rate of fertility, with fertility rate of hepatic cysts being statictically higher than that of pulmonary cysts in sheep, in keeping with other studies in Iran and elsewhere (Arbabi and Houshyar, 2006; Daryani *et al*, 2007; Rokni, 2009). The viability of protoscoleces from fertile cysts also was highest in sheep as observed by Dalimi *et al* (2002) in western Iran where viability in sheep is 82% compared to 75% in cattle.

The role of buffalo and cattle in the epidemiology of *Echinococcus* compared to sheep, has been studied less in Iran (Vaisi-Raygani *et al*, 2021). While there is a significant relationship between the fertility rate of hydatid cysts and the rate of protoscolex viability, overall, these

levels were lower in buffalo and cattle than in sheep; nevertheless, these animals still have a potential role in the disease transmission cycle and hence should be included in future prevention and control programs.

A limitation of this study was the lack of nucleotide sequencing to identify the species and genotype of *Echinococcus* in the different intermediate hosts examined. Differences in genotypes could impact infection rates in the intermediate hosts as well as the importance of different final hosts (dogs, jackals, and wolves). Even with this limitation, our results support previous findings that sheep are an important intermediate host for *Echinococcus* not only due to the high prevalence of hydatid cysts but also the high fertility of the cysts and high viability of the protoscoleces (Daryani *et al*, 2007; Elmajdoub and Rahman, 2015; Amer *et al*, 2018).

In conclusion, the study emphasizes sheep as a major focus when considering strategies to control *Echinococcus*, a parasite of public health importance. However, the roles of buffalos and cattle should not be neglected. In addition, safe disposal of infected offal of these livestocks will be needed to reduce the transmission of cysts from slaughterhouses to the final hosts in this region of Iran.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

Akhlaghi L, Massoud J, Housaini A. Observation on hydatid cyst infection in Kordestan Province (West of Iran) using epidemiological and seroepidemiological criteria. *Iran J Public Health* 2005; 34: 73-5.

Almalki E, Al-Quarishy S, Abdel-Baki AS. Assessment of prevalence of hydatidosis in slaughtered Sawakny sheep in Riyadh city, Saudi Arabia. *Saudi J Biol Sci* 2017; 24: 1534-7.

Amer OH, Haouas N, Al-hathal EAA, El-shikh I, Ashankyty I. Cystic echinococcosis in slaughtered animals in Ha'il, Northwestern Saudi Arabia. *Jpn J Vet Res* 2018; 66: 289-96.

Arbabi M, Houshyar H. Survey of echinococcosis and hydatidosis in Kashan region, central Iran. *Iran J Public Health* 2006; 35: 75-81.

Arene FOI. Prevalence of hydatid cysts in domestic livestock in the Niger Delta. *Trop Anim Health Prod* 1985; 17: 3-5.

Azami M, Anvarinejad M, Ezatpour

B, Alirezaei M. Prevalence of hydatidosis in slaughtered animals in Iran. *Turkiye Parazitol Derg* 2013; 37: 102-6.

Borhani M, Fathi S, Darabi E, *et al*. Echinococcoses in Iran, Turkey, and Pakistan: old diseases in the new millennium. *Clin Microbiol Rev* 2021; 34: e0029020.

Bortoletti G, Gabriele F, Seu V, Palmas C. Epidemiology of hydatid disease in Sardinia: a study of fertility of cysts in sheep. *J Helminthol* 1990; 64: 212-6.

Cardona GA, Carmena D. A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. *Vet Parasitol* 2013; 192: 10-32.

Craig P, Mastin A, van Kesteren F, Boufana B. *Echinococcus granulosus*: epidemiology and state-of-the-art of diagnostics in animals. *Vet Parasitol* 2015; 213: 132-48.

Dalimi A, Motamedi GH, Hosseini M, *et al*. Echinococcosis/hydatidosis in western Iran. *Vet Parasitol* 2002; 105: 161-71.

Daryani A, Alaei R, Arab R, Sharif M, Dehghan MH, Ziaei H. The prevalence, intensity and viability of hydatid cysts in slaughtered animals in the Ardabil province of Northwest Iran. *J Helminthol* 2007; 81: 13-7.

Elmajdoub LO, Rahman WA. Prevalence of hydatid cysts in slaughtered animals from different areas of Libya. *Open J Vet Med* 2015; 5: 1-10.

Fakhar M, Sadjjadi SM. Prevalence of hydatidosis in slaughtered herbivores in Qom Province, central part of Iran. *Vet Res Commun* 2007; 31: 993-7.

Khan AH, El-Buni AA, Ali MY. Fertility of the cysts of *Echinococcus granulosus* in domestic herbivores from Benghazi, Libya, and the reactivity of antigens produced from them. *Ann Trop Med Parasitol* 2001; 95: 337-42.

Khanmohammadi M, Gayem Maghami S, Zakaria Zadeh M. The prevalence of hydatidosis by sex, season and location in slaughtered buffaloes at the Tabriz abattoir in 2006-2007, 2008 [cited 2022 Mar 02]. Available from: URL: https://print.ispub.com/api/0/ispub-article/3572

Kouidri M, Benchaib Khoudja F, Boulkaboul A, Selles M. Prevalence, fertility and viability of cystic echinococcosis in sheep and cattle of Algeria. *Bulg J Vet Med* 2012; 15: 191-7.

Lahmar S, Kilani M, Torgerson PR, Gemmell MA. *Echinococcus granulosus* larvae in the livers of sheep in Tunisia: the effects of host age. *Ann Trop Med Parasitol* 1999; 93: 75-81.

Manterola C, Totomoch-Serra A, Rojas C, Riff-Campos ÁL, García-Méndenz N. *Echinococcus granulosus sensu lato* genotypes in different hosts worldwide: a systematic review. *Acta Parasitol* 2022; 67: 161-85.

Mehmood N, Arshad M, Ahmed H, Simsek S, Muqaddas H. Comprehensive

account on prevalence and characteristics of hydatid cysts in livestock from Pakistan. *Korean J Parasitol* 2020; 58: 121-7.

Miambo RD, Afonso SMS, Noormahomed EV, Pondja A, Mukaratirwa S. Echinococcosis in humans and animals in Southern Africa Development Community countries: a systematic review. *Food Waterborne Parasitol* 2020; 20: e00087.

Mirzaei M, Rezaei H, Nematollahi A. Role of ruminants in the epidemiology of *Echinococcus* granulosus in Tabriz area, Northwest of Iran. *Trop Biomed* 2015; 32: 269-75.

Pour AA, Hosseini SH, Shayan P. The prevalence and fertility of hydatid cysts in buffaloes from Iran. *J Helminthol* 2012; 86: 373-7.

Rokni MB. Echinococcosis/hydatidosis in Iran. *Iran J Parasitol* 2009; 4(2): 1-16.

Smyth JD, Barrett NJ. Procedure for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. *Trans R Soc Trop Med Hyg* 1980; 74: 649-52.

Torgerson P, Budke CM. Echinococcosis – an international public health challenge. *Res Vet Sci* 2003; 74: 191-202.

Vaisi-Raygani A, Mohammadi M, Jalali R, Salari N, Hosseinian-Far M. Prevalence of cystic echinococcosis in slaughtered livestock in Iran: a systematic review and meta-analysis. *BMC Infect Dis* 2021; 21: 429.