Egypt. J. Vet. Sci. **Vol. 51,** No.3, pp. 399-403 (2020)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Investigation of The Fertility Prevalence of Hydatid Cysts and Protoscoleces Viability in Livestock Slaughtered in Ilam Industrial Slaughterhouse, Ilam Province, Iran During 2016

Ozra Bahmani[1] and Ali Anisian[2]
[1]*Department of Pathophysiology, Islamic Azad University, Abhar Branch, Abhar, Iran.*
[2]*Ebn-e Sina Apllied and Science and Technology Institute, Tehran, Iran.*

HYDATID cysts are the main cause of human-animal disease transmission, so that we witness substantial economic and health damage due to this every year. Fertile cysts and their protoscoleces viability are important factors for the stability of the parasite cycle and the survival of infection in humans and animals. The aim of this study was to investigate the fertility rate of hydatid cysts and protoscoleces viability in livestock slaughtered in Ilam industrial slaughterhouse. In this study, organs (liver and lung) infected with hydatid cysts in sheep and cows were collected during intermittent periods during June to September 2016 and transferred to the parasitology laboratory of Ilam Veterinary Network. In the laboratory, cysts were first classified based on their type and microscopic characteristics (calcified, purulent, and active). The active cysts were then prepared for the next steps. Of the 175 cysts examined, 86 were obtained from cows and 89 from sheep. Of these, 94 cysts were diagnosed as hepatic and 81 as pulmonary. Average viability of fertile cyst protoscoleces was obtained (73.73±7.23) in cows and (56.10±19.81) in sheep, with a statistically significant difference ($P<0.05$). There was a weak relationship between animal species and the development rate of hydatid cysts (p=0.05). The prevalence of hydatid cysts in sheep was higher than in cows, which may be due to a specific time division of the study. The highest percentage was obtained for fertile liver cysts (54.85%) and the lowest percentage for sterile cysts (14.85%). Moreover, calcified cysts had the highest rate following fertile cysts (30.28%). The significant fertility rate of liver cysts in sheep and lung cysts in cows, and protoscoleces viability of sheep indicates the importance of these two animals in terms of epidemiology and spread of hydatidosis in the region. The findings show that the prevalence of this disease is high in the region. It is worth mentioning that the authorities can largely eliminate the infected organs in the slaughterhouses by taking preventive measures, which will help reduce the disease development process.

Keywords: Hydatid cyst, Viability, Protoscoleces, Slaughterhouse, Ilam, Iran.

Introduction

Hydatidosis is an important health and economic issue. Hydatidosis is a common disease between humans and animals that is caused by the larval form of *Echinococcus granulosus* [1]. *E. granulosus* is the causative agent of cystic hydatidosis. The adult worm lives in the intestines of dogs and their babies live in the human body and in domestic ruminants [1]. The disease is widespread worldwide and is prevalent in countries where animal husbandry is prevalent, and is common in the Middle East, India, South America, and Australia [1]. In Iran, infection rate in dogs and herbivores such as sheep, cattle, and goats is substantial, and human patients have been frequently reported from most parts of the country, especially important medical centers [2]. Living protoscoleces in intermediate hosts are an important index for the transmission of

*Corresponding auther: Ali Anisian, E.mail: ali.anisian@ymail.com, Tel. 00989119182429
(*Received* 04/07/2020, *accepted* 11/08/2020)
DOI. 10.21608/ejvs.2020.34633.1180

the disease, and stray dogs that feed around the slaughterhouse to feed can spread the infection by eating the organs infected with the cyst. The most common site of hydatid cyst, in the first place, is the liver (70%) followed by the lung (20-30%) [3,4], while the involvement of other organs, including the brain, spinal cord, kidneys, etc. has been reported as well. In cows and horses, more than 90% of cysts are usually found in the liver. The frequency of fertile hydatid cysts varies in cattle and sheep, from 59% in sheep and 10% in cows [5]. Various studies on fertility and protoscoleces viability of hydatid cysts have been performed in some parts of Iran [6]. However, due to the effect of geographical location and time changes in the pattern of infection and fertility of cysts and protoscoleces viability [7], and the role of these indicators in the stability of parasite cycle and the spread and persistence of infection in humans, the current study was conducted to investigate the fertility prevalence of hydatid cysts and protoscoleces viability in livestock slaughtered in Ilam industrial slaughterhouse, Iran

Materials and Methods

Sampling

During several consecutive visits to the slaughterhouse of Ilam city (Ilam province, western Iran) in intermittent times from June to September 2016, while inspecting the slaughterhouse, the cysts were examined based on the type and microscopic characteristics into calcified, purulent and active. The contents of the active cysts were aspirated under sterile conditions by a syringe and poured into separate test tubes. Then the origin and characteristics of each sample, including the type of infected organ and the type, sex, age, and area from which the animal came were recorded on the test tubes and the samples are transferred to the laboratory of the General Veterinary Administration of Ilam Province.

The contents of the tubes were rinsed several times with physiological serum and then centrifuged at a rate of 500 rpm for 1 min. The precipitates were separated and then expanded and examined under an optical microscope. Cysts without protoscoleces were considered sterile and fertility of the cysts was confirmed and diagnosed by examining the protoscoleces. The precipitates of the fertile cysts were stained with 0.1% eosin solution for 15 minutes and after lam preparation, the viability of the protoscoleces was examined under an optical microscope. In this method, at least 100 protoscoleces were counted, and stained protoscoleces were considered non-living and those that were unstained were considered as living.

Hydatid sheep cyst fluid and primary preparation of protoscoleces

The active cysts were prepared for the next steps in such a way that first the supernatant of the cysts was sterilized with iodine alcohol and hot blade in a sterile hood environment, and then evacuated using 5 and 10 ml syringes. After opening the cysts, their reproductive membranes, were placed in a PBS buffer to completely remove the protoscoleces inside them. Washing and concentrating of protoscoleces were performed three to five times by centrifugation with PBS solution (pH=2-2.7) at 1500 g at 2-3 minutes. The origin and characteristics of each cyst, including the type of infected organ and the type of animal, were recorded on the test tubes to determine the fertility and sterility of the cysts at this stage.

If the fluid was transparent and did not contain protococcus, the cyst was considered sterile.

In the next step, using open scissors, the residual fluid inside the germination layer was tested for the presence of protococcus and finally fertile and non-fertile cysts were recorded.

In the next step, using a dropper, one or two drops of the precipitate from the bottom of the tube obtained from the centrifuge were transferred to a clean slide, and a drop of eosin 0.01% was poured on it and examined under a microscope at 40% magnification after putting the under the microscope.

If the protoscoleces were living, the dye would not be able to penetrate, and the protoscoleces would be seen as a natural bright green color, and if it were dead, the dye would easily penetrate into it, and as a result, the protoscoleces would turn red. All protoscoleces samples were examined. A total of 100 protoscoleces were counted, of which 30 were not stained or had viability.

Results

According to the results, out of a total of 175 cysts examined, 86 were obtained from cows and 89 from sheep. Of these, 94 cysts were diagnosed as hepatic and 81 as pulmonary. The frequency of liver and lung cysts in slaughtered animals is shown in Table 1.

TABLE 1. Frequency of liver and lung cysts of slaughtered animals.

Animal species	Organ type		Total Number (percent)
	Lungs Number (percent)	Liver Number (percent)	
Cow	50	36	86 (100)
Sheep	31	58	89 (100)
Total	81	94	175 (100)

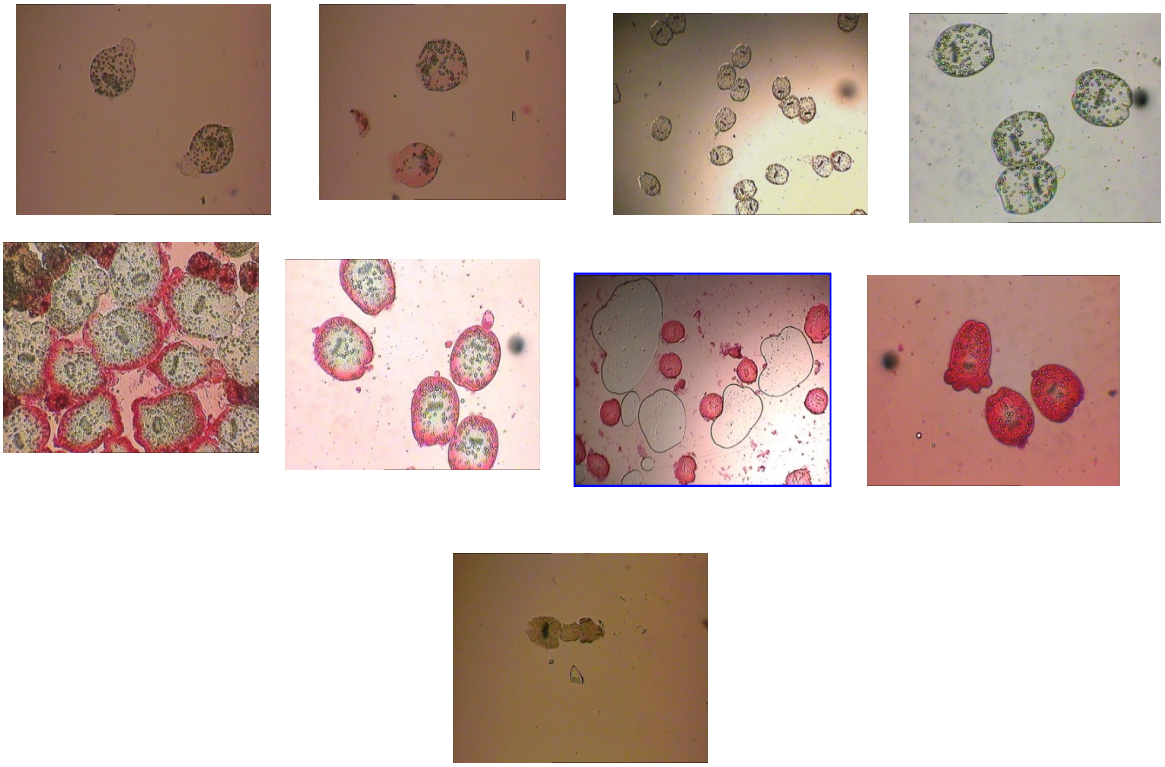

Fig. 1. Determination of fertility and viability of protoscolices during the process of 0.1% eosin staining.

Table 2 shows the frequency of hydatid cysts in livestock slaughtered in Ilam slaughterhouse according to the type of animal and the type of organ. Of the total hydatid cysts of sheep lungs examined, fertile cysts accounted for 64.51% of the cysts, calcified cysts accounted for 32.25%, and sterile cysts accounted for 3.22%.

Findings from the sheep liver section also showed that 58.62% of the cysts were fertile, 13.79% were calcified, and 27.58% were sterile. Moreover, in a study of hydatid cysts in the lungs of cows, 50% of cysts were fertile, 40% were calcified, and 10% were sterile.

In samples of cow liver, 47.22% contained fertile cysts, 41.66% contained calcified cysts, and 11.11% contained sterile cysts.

Average viability of fertile cyst protoscoleces was obtained (73.73±7.23) in cows and (56.10±19.81) in sheep, with a statistically significant difference ($P<0.05$).

Discussion

This study showed that there was a significant relationship between geographical location and quarterly temporal and environmental changes from June to September 2016 in the spread pattern of cyst hydatid infection ($P<0.05$).

TABLE 2. Frequency of hydatid cysts in livestock slaughtered in Ilam slaughterhouse based on organ type.

Animal species	Organ type	Type of cyst			Total
		Sterile	Caliphate	Fertile	
Sheep	Lung	3.2%	32.25%	64.51%	100%
	Liver	27.58%	13.79%	58.62%	100%
Cow	Lung	10%	40%	50%	100%
	Liver	11.11%	41.66%	47.22%	100%
Total		14.85%	30.28%	54.85%	100%

Since the study was conducted in the summer, most of the slaughtered animals were young, and therefore the prevalence of hydatid cysts was lower than in other seasons.

From geographical perspective, the results of this study are consistent with the studies of Scala *et al*. [3], Dalimi *et al*. [6], Kebede *et al*. [8] and Daryani *et al*. [9].

In these studies, conducted in different countries, the researchers identified one type of cyst as the most recurrent cyst. In this regard, we can point to the results of the study of Kebede *et al*. [8] in northwestern Ethiopia, which shows the high prevalence of sterile hydatid cysts.

However, in the study of Scala *et al*. [3], the number of calcified cysts in Sardinia, Italy was much higher.

Therefore, it can be seen that the geographical location can be significantly related to the spread pattern of hydatid cyst infection. The study also found a relationship between the prevalence of hydatid cysts and the type of sacrificed animals.

According to the findings of this study, there is a weak relationship between animal species and the development rate of hydatid cysts (p=0.05). In this study, the prevalence of hydatid cysts in sheep was higher than in cows, which may have been due to the specific time division of the study. The highest percentage was associated with fertile liver cysts (54.85%) and the lowest percentage was associated with sterile cysts (14.85%). Moreover, calcified cysts had the highest rate following fertile cysts (30.28%).

The results of Shahnazi and Barikani's study [10] showed that there is a significant relationship of the type of cysts with the type of animal and the type of organ.

Our results were consistent with the results of studies of Shahnazi and Barikani [10], Dailami *et al.* [6], Hebadi *et al.* [8] and Daryani *et al.* Because they also came up with different statistics on sheep and cattle cysts in their studies. Fallah *et al.* [11] also concluded in their study that there was a relationship between the type of livestock and the infection with hydatid cysts, but the results of this study showed higher statistics than the cysts detected in cows with the results of the hypothesis. We are not in agreement on this.

In our study, it was found that there was a relationship between the prevalence of hydatid cysts and the sex of slaughtered animals, because most of the examined cysts based on the number of the first studied sample were found in female cows, female sheep, male cows and finally male sheep. The results of this study are consistent with the investigations of Fallah *et al.* In their study, Fallah *et al.* [11] reported that the prevalence of hydatid cysts was 15.5% in female livestock and 6.7% in male livestock. This result is consistent with the studies of many researchers. This is probably due to a weakened immune system in female livestock during pregnancy, as well as the greater economic value of female livestock, which leads to their sacrifice at older ages. Average viability of fertile cyst protoscoleces was obtained (73.73±7.23) in cows and (56.10±19.81) in sheep, with a statistically significant difference ($P<0.05$). The study of Shahbazi and Barikani study [10] showed that the highest and lowest fertility rates were obtained in sheep liver cysts (81.05%) and cow liver cysts (1.27%), respectively, which is in agreement with the results of our study because in this study also the highest and lowest fertility rates were observed in sheep liver (63.6%) and cow liver (15.27%) cysts, respectively. Moreover, in a study conducted in Qazvin, the highest viability was obtained in cow's lungs and the lowest in sheep's liver. In western Iran and Saudi

Arabia, lung and sheep liver cysts were the most prevalent, respectively.

Conclusion

According to the results, the fertility rate of hepatic and pulmonary hydatid cysts in sheep and their protoscoleces viability in the region is considerable and is important from epidemiological perspective and for the spread of hydatid disease. In addition, although the fertility of cysts in sheep's organs was low, they had viability. Therefore, it is necessary to take preventive measures such as exterminating infected organs infected with animal cysts, keeping dogs away from the slaughterhouse, and controlling stray dogs in an efficient manner. Finally, we will see the promotion of public health in the region and similar areas.

Acknowledgements

The authors wish to thank the Islamic Azad University, Abhar Branch, Abhar, Iran for their financial support.

Conflicts of interest

The authors declare no conflict of interests of the manuscript.

Funds statement

The authors wish to thank the Islamic Azad University, Abhar Branch, Abhar, Iran for their financial support.

References

1. Mohebali, M. and Samak, E. Investigation of human hydatidosis and infection with hydatid cysts in animal husbandry slaughtered in Arak slaughterhouse, *Kerman Uni. Med . Sci. J.,* **1**, 46-48. (1995).
2. Moayedi, A. and Faisahi Harandi, M. Assessing the fertility rate of human and viable prostate cystatoid cysts. *J Qazvin Uni. Med. Sc. Health Serv.*, **17**, 61-67 (2011).
3. Scala, A, Garippa, G, Varcasia, A, Tranquillo, V.M. and Genchi, C. Cystic echinoccosis in slaughtered sheep in Sardina (Italy). *Vet. Parasitol.,* **135**(1), 33-38 (2006).
4. Meymandinejad, M.H. Epidemiology of cyst hydatid in Iran and the world. Tehran University Press, 2005.
5. Islami, A. Veterinary Criminology. Sastudha, vol. 2. Tehran: University of Tehran. **1991**; 117-167.
6. Dalimi, A., Motamedi, G.H., Hosseini, M. and Emameini, M. Echinococcosis hydatidosis in western Iran. *Vet. Parasitol*., **105** (2), 161-171 (2002).
7. Dueger, E.L. and Gilman, R.H. Prevalence, intensity and fertility of ovine cystic echinococcsis in the central Peruvian Anders. *Trans. R. Soc. Trop. Med. Hyg.*, **95**, 379-383 (2001).
8. Kebede, N., Abebe, M. and Tilahum, G. Hydatidosis of slaughtered animals in Bahir Dar Abattoir, Northwestern Ethiopa. *Trop. Anim. Health Prod*., **410**, 50-53 (2009).
9. Daryani, A., Sharif, M.M., Amouei, A. and Nasrolahei, M. Fertility and viability rates of hydatid cysts in slaughtered animals in the Mazandaran Province, Northern Iran. *Trop. Anim. Health Prod*., **41**(8),1701-1705 (2009).
10. Shahbazi, M., Barikani, A. and Jafari, A. Evaluation of fertility rate of hydatid and vitiligo cysts of protoasculxes, *Scien. J. Percep*., **20**, 15-12 (2010).
11. Fallah, M., Ghassemi, M. and Shahbazi, G.H. Frequency of Hydatid cysts in domestic ruminants of Hamadan, west of Iran. *J. Hamadan Uni. Med. Sci*., **9**(4), 50-55 (2002).