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Comparison of Eosin and Trypan Blue Staining in Viability of Hydatid Cyst Protoscoleces

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Abstract: The viability assessment in cystic echinococcosis is important for *in vitro* and *in vivo* studies and also drug experiments. Variable stains like eosin, methylene blue, Giemsa, Ziehl-Neelsen, Toluidine blue and trypan blue have been used to assess viability of protoscoleces in fertile cysts. In this study, the viability of fertile cysts were analyzed and comparison by trypan blue and eosin staining techniques. A total of 206 sheep and cattle hydatid cyst were examined by two staining techniques. The obtained results were statistically analyzed by ANOVA test. The mean numbers of viable protoscoleces in examined cysts according to eosin and trypan blue staining techniques were The viability of protoscoleces recovered from sheep liver (94% ± 3%) and sheep lung (85% ± 4%) that was higher than those from cattle liver (79% ± 3%) and cattle lung (67% ± 5%). No statistically significant differences were observed among the staining techniques. As a result, it was found that all two staining methods have similar results in viability assessment of hydatid cysts.

Key words: Echinococcosis · Sheep · Cattle · Protoscoleces · Viability

INTRODUCTION

Echinococcus granulosus lives as a small intestinal tapeworm of dogs and occasionally other carnivores. The shedding of gravid proglottids or eggs in the feces occurs within 4-6 weeks after infection of the definitive host [1]. Infections with *E. granulosus* occur worldwide, predominantly in countries of South and Central America, the European and African part of the Mediterranean area, the Middle East and some sub-Saharan countries, Russia and China [2]. Fertility rate of hydatid cysts in various animal of the disease are very important and age, geographic region and vary depending on various factors such as parasite strains [2, 9]. Cystic echinococcosis of the epidemiology and drug trial against several in vitro and in vivo studies are determine the viability of such studies are very important [3].

The main aim of the present survey was to determine the viability of liver and lung cysts from sheep and cattle and to compare the efficacy of eosin and trypan blue staining in monitoring the viability of hydatid cyst protoscoleces.

MATERIALS AND METHODS

This cross sectional study was carried out on 206 animals (162 sheep and 44 cattle) in the Amol slaughtered (Mazandaran Province, in Northern Iran) from July to September 2009. During the study, the industrial slaughterhouse was visited periodically to examine the liver and lungs of slaughtered animals for the presence of cystic echinococcosis. Individual cysts were grossly examined for any evidence of degeneration and calcification, some cysts in sheep and cattle were randomly selected for viability studies. After preparation protoscoleces were transferred to a microscope slide to which drop of 0.1% eosin dye [2] and 0.25% trypan blue [4] were added. Viability was established by checking the movement and activity of flame cells of protoscoleces, A protoscoleces with inactive flame cells, disrupted membrane or stained with eosin is considered as dead [3]. Statistical analysis were carried out using the SPSS (ANOVA test) to compare the rate of infections and means of viability of protoscolices.

RESULTS

Among all examined samples (206), 44(21.35%) was from cattle and 162(78.65%) was from sheep. The cysts recovered from liver and lungs of sheep showed higher viability rates than those of cattle. The most viability rate of cysts was found in the liver of sheep, while the highest rate of suppurative /calcified cysts was found in the liver of cattle. The viability rate of cysts recovered from either lungs or liver of different animals is shown in Table 1. The viability of protoscoleces recovered from sheep liver

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Table 1: Evaluation of comparison Eosin and Trypan blue staining in viability of hydatid cysts protoscoleces in sheep and cattle in Northern Iran, 2009

Animals	Infected organ	No. of cyst organ examination (%)	Viability of protoscoleces with eosin staining (Mean ± SD)	Viability of protoscoleces with trypan blue staining (Mean ± SD)
Sheep	Liver	78(48.14%)	93% ± 5%	95% ± 1%
Sheep	Lung	84(51.86%)	88% ± 3%	82% ± 5%
Cattle	Lliver	28(63.63%)	82% ± 4%	76% ± 2%
Cattle	Lung	16(36.37%)	69% ± 7%	65% ± 3%

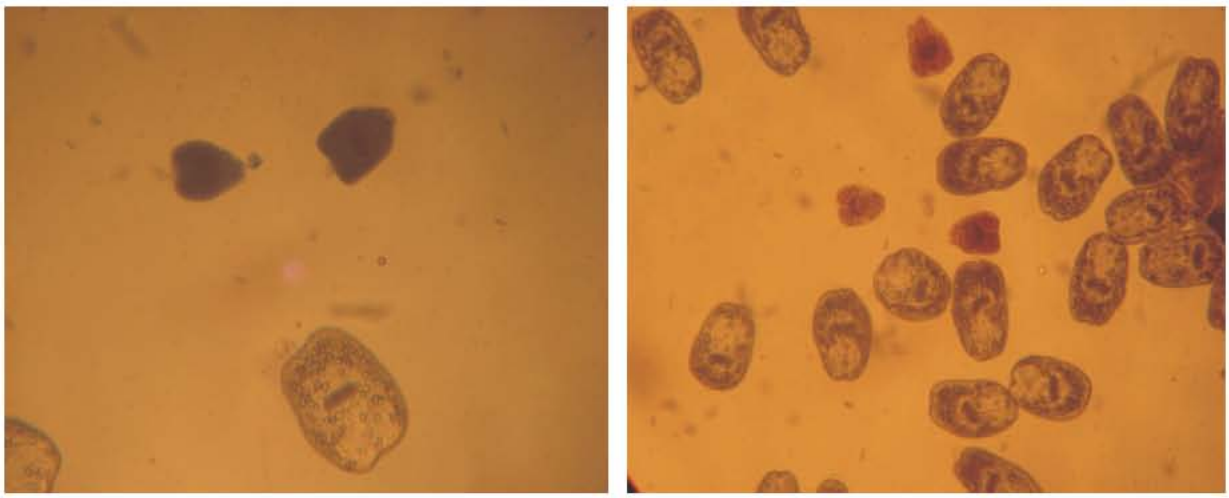

Fig. 1: Viability of Protoscoleces staining with Trypan blue at 0.25 %(left) and eosin 0.1% staining (Right)

(94% ± 0.3%) and lung (85% ± 0.4%) was higher than those from cattle liver (79% ± 0.3%) and lung (67% ± 0.5%).

No statistically significant differences were observed among the two staining techniques eosin and trypan blue. As a result, it was found that all two staining methods have similar results in viability assessment of hydatid cysts protoscoleces in sheep and cattle.

DISCUSSION

Hydatid disease is an important medical and veterinary problem in the world. Domestic intermediate hosts (cattle, sheep, goats and buffaloes) are major reservoirs for the disease in humans. The widespread distribution and nature of the life cycle of *E. granulosus* suggest that there will always be a risk of re-introducing the cestoda as long as live animals are imported [9]. From Iran, based on abattoir survey, the mean prevalence of Hydatidosis of sheep and cattle in different parts of the country has been reported to be 8.1 and 12%, respectively [4]. In other study about hydatidosis in Mazandaran province, has reported an overall infection of 14.7 and 6.7% in sheep and cattle, respectively [11]. Also Youssefi [13] found infection rates of 7 and 5.9% in sheep and cattle of Mazandaran. Data on the prevalence and fertility of cysts in various domestic herbivores provide reliable indicators of the importance of each type of animal as a potential source of infection to dogs. Cysts, depending on the geographical situation, host, site, size and type of cyst may have different rates of viability that studied in many countries [12].

Trypan blue and Eosin staining methods commonly used in the determination of the cystic echinococcosis in viability of the staining method. The proportion of viable protoscoleces from fertile cysts from sheep were 89.5% (lungs, 85% and liver, 94%) and cattle 73% (lungs, 67% and liver, 79%), respectively. On the contrary, Dalimi in western Iran reported that viability rate in sheep 82% was higher than that in cattle 75% [5]. In Ardabil also fertile cysts in sheep 61% was higher than cattle 32%. In our study such as western Iran [7,8] viability rates of protoscoleces in liver and lungs of sheep and also those of cattle were the no same.

Walker *et al.* [13] indicated cysts were detected in sheep trypan blue staining method and were found to be 85% viability rate, Similarly Himonas *et al.* [14] fertile cysts in sheep were determined as 81.3% viability rate

were reported [14]. As fertility rate of hydatid cysts in sheep is higher than those in cattle, probably predominant strain in this area is sheep strain, which do not develop into fertile cysts in horse; and sheep clearly have an important role to play in the contamination of the E. granulosus life cycle in Northern Iran. Therefore As a result, it was found that all two staining methods have similar results in viability assessment of hydatid cysts.

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