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Effect of Temperature and Salinity on Survival of Protoscoleces of Hydatid Cyst in Liver *In Vitro*

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Abstract

Hydatid cyst is the metacestode stage of *Echinococcus granulosus* that occurs in herbivores and humans as intermediate hosts by consuming parasite eggs through forage and vegetables. Carnivores, as definitive hosts, become infected by consuming infected vesicles of herbivores. The most effective treatment for a hydatid cyst is surgical operation. Inactivating *E. granulosus* protoscoleces through heating, cooling, or chemicals such as sodium chloride can be considered an effective method for controlling hydatidosis in both humans and animals. The main objective of this study was to evaluate the effect of different temperatures and salinity conditions on the survival of *Echinococcus granulosus* protoscoleces. For this purpose, 50 g of infected liver (in triplicate) was separately treated with different temperatures (+10°C, +50°C, +60°C, +72°C, and −20°C) and concentrations of sodium chloride (5%, 10%, 15%, and 20%) for 3, 6, 12, 24, 48, and 72 h. Additionally, 50 g of infected liver was stored separately in the refrigerator (+4°C) as a control group. The survival rate of the protoscoleces was evaluated by staining with 1% eosin under a light microscope. The results showed that the protoscoleces were significantly affected, with 100% mortality at −20°C after 0.5 h, and complete death at +72°C, +60°C, +50°C, and +10°C after 1, 1.5, 3, and 24 h, respectively ($p < 0.005$). Similarly, the protoscoleces in the liver mass survived at 5% NaCl after 3 h but died at 10% after 24 h, at 15% after 12 h, and at 20% after 6 h. It is concluded that exposing the liver infected with protoscoleces hydatid cyst to a temperature of −20°C and a sodium chloride concentration of 10% for 24 h is suitable for inactivating the protoscoleces.

Keywords: *Echinococcus granulosus*, protoscolex, temperature, sodium chloride, liver

Introduction

Echinococcosis, a parasitic disease caused by *Echinococcus* spp., is an emerging zoonotic condition that is widely recognized as one of the most significant helminthic diseases worldwide (Budke et al., 2006). Cystic echinococcosis (CE) not only poses a serious threat to human health, potentially leading to severe illness and even death, but also imposes economic burdens in terms of treatment expenses, lost income, and livestock-related production losses (Hajipour et al., 2021, 2023). In the life cycle of *Echinococcus*, dogs serve as the final host, whereas livestock such as sheep, cattle, goats, and buffalo act as intermediate hosts. Upon ingestion of eggs, hydatid cysts develop in the organs of livestock, often resulting in their rejection during slaughter. Although hydatid cysts mainly form in the liver and lungs, they can also be found in other organs such as the spleen, heart, kidneys, bones, eyes, and the central nervous system (Hajipour et al., 2023). As there is currently no effective drug treatment available for this disease and the rupture of cysts and growth of secondary cysts at the surgical site present challenges, it poses significant problems. Although adult *Echinococcus* develops in dogs after consuming infected organs, dogs typically do not display any clinical signs of infection. Echinococcosis caused by the tapeworm *Echinococcus granulosus* continues to be a significant public health issue in Iran. The country exhibits a high prevalence of *E. granulosus* infections in both domestic dog populations and livestock animals. This has led to a correspondingly high incidence of CE, the disease caused by *E. granulosus* in the human population within Iran (Ahmadi and Meshkehkar, 2011; Ansari-Lari, 2005; Ansari-Lari and Moazzeni, 2006; Borji et al., 2012; Hajipour et al., 2021, 2023). To effectively implement control programs, it is crucial to epidemiologically

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FIG. 1. **(A)** Treatment of livers infected with hydatid cysts: treatment with different temperatures and **(B)** treatment with different concentrations of NaCl.

determine the duration of protoscolex viability or survival in discarded carcasses and their potential to infect carnivore hosts. Although a limited number of studies have explored the impact of temperature and sodium chloride on the survival of *E. granulosus* protoscoleces *in vitro* (Al-Hadithi and AL-Khamesi, 2010; Diker et al., 2007; Hazrati Tappeh et al., 2011; Ohnishi et al., 1984; Moazeni and Alipour-Chaharmahali, 2011), there is currently no research available concerning the effect of temperature and sodium chloride on protoscolex survival in liver tissue. Therefore, this study was designed to investigate the influence of temperature and salinity on the survival of protoscoleces within hydatid cysts in infected livers.

Materials and Methods

Sampling

To conduct this study, 100 livers infected with hydatid cysts were collected from sheep slaughtered at the Tabriz slaughterhouse between November 2018 and June 2019. These livers were immediately transported on ice to the Food and Aquatic Health Laboratory at the Faculty of Veterinary Medicine, University of Tabriz. Sheep livers were chosen for this research owing to previous studies indicating a higher fertility rate of protoscolexes in hydatid cysts compared with cattle. Additionally, it was noted that most hydatid cysts in cattle livers are sterile.

Assessing the fertility or sterility of hydatid cysts in the infected livers

The cyst fluid was extracted under aseptic conditions and subsequently subjected to centrifugation at 2000 × *g* for 3 min (Figure 1). The resulting sediment was examined under a light microscope at 40× magnification to identify protoscoleces. To determine their viability, the protoscoleces were stained with a 0.1% aqueous eosin solution, and the movement of flame cells was observed using a light microscope at 1000× magnification. Following this, one drop of a 1% aqueous eosin solution was added, and the viability of the protoscoleces was evaluated (Figure 2). Those that stained red were classified as dead, whereas those that remained colorless were categorized as viable using the eosin exclusion test (Smyth and Barrett, 1980). Any liver that tested positive for infection was stored in a refrigerator at +4°C for further research procedures.

Evaluation of the effect of temperature on protoscolexes in infected liver

Liver samples weighing 50 g, previously confirmed as infected through the 1% eosin staining method, were subjected to various concentrations (5%, 10%, 15%, and 20%) and temperatures (+10°C, +50°C, +60°C, +72°C, and −20°C) for different time intervals (3, 6, 12, 24, 48, and 72 h). For each time and concentration, a 50 g piece of infected liver, measuring 2–3 cm in thickness, was carefully selected and placed in small plastic bags for treatment, with three repetitions performed. As controls, infected livers that were placed in the refrigerator at

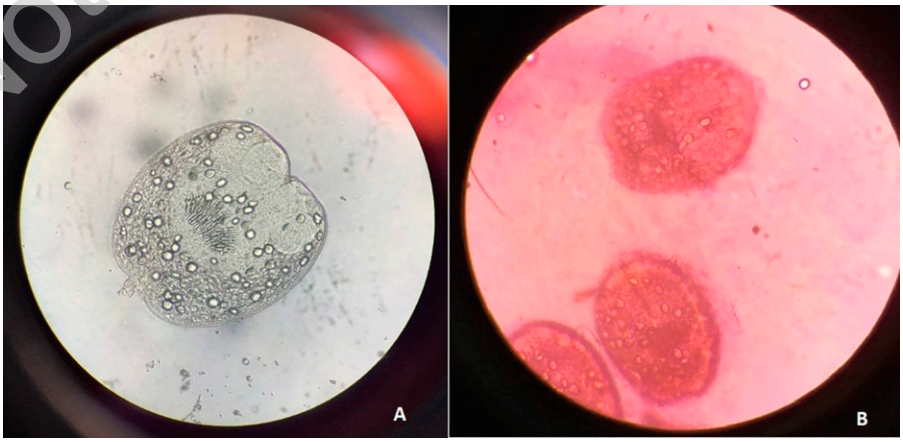

FIG. 2. **(A)** Protoscolex after staining with 1% eosin: live protoscolex and **(B)** dead protoscoleces.

Table 1. Mean Survival Percentage of Hydatid Cyst Protoscoleces at Different Temperatures in Infected Liver ± Standard Error

Te (°C)/Ti (h)	*0.5*	*1*	*1.5*	*3*	*6*	*12*
+10	92.43 ± 0.48^{cg}	92.67 ± 4.129^{cg}	73.79 ± 7.018^{cf}	65.26 ± 6.74^{cf}	55.23 ± 2.39^{be}	38.42 ± 3.24^{bd}
+50	35.50 ± 8.779^{bd}	35.12 ± 9.36^{bd}	23.61 ± 6.05^{bd}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}
+60	30.09 ± 10.88^{bd}	7.00 ± 3.86^{ae}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}
+72	1.587 ± 1.58^{ad}	0.00 ± 0.00^{ad}	0.00 ± 0.00^{ad}	0.00 ± 0.00^{ad}	0.00 ± 0.00^{ad}	0.000 ± 0.00^{ad}
−20	100.0 ± 0.00^{cg}	85.64 ± 4.11^{cf}	75.85 ± 4.09^{cf}	37.48 ± 8.88^{be}	3.80 ± 1.98^{ad}	0.00 ± 0.00^{ad}

Letters a–g in each column and row indicate statistical significance.
Te, temperature; Ti, time.

+4°C were used for each temperature and concentration. The survival rate of protoscolexes was determined by assessing their ability to stain with 1% eosin, indicating viability, or their inability to stain, indicating nonviability.

Statistical analysis

The data collected from the research, which measured the survival percentage of parasites, underwent statistical analysis using one-way analysis of variance. If there was a significant difference in the means ($p < 0.05$), the researchers employed Duncan's *post hoc* test to further investigate the variation between groups. Additionally, to identify differences in concentration and temperature across various time points, the general linear model and repeated measures method were utilized. The data analysis was conducted using SPSS version 21 software.

Results

The results of the present study showed that all hydatid cyst protoscoleces in infected livers were alive (100%) at −20°C after 0.5 h. They were completely dead at +72°C, +60°C, +50°C, and, +10°C after 1, 1.5, 3, and, 24 h, respectively (Table 1) ($p < 0001$). The results showed that all protoscoleces survived in 5% concentration after 3 h, but all of them died in 10% concentration after 24 h, in 15% concentration after 12 h, and in 20% concentration after 6 h (Table 2) ($p < 0001$). The results of the study showed that the average survival percentage of protoscoleces in infected livers at a given time between different temperatures and concentrations was statistically significant (Tables 1 and 2). Also, the survival rate of protoscoleces at the temperatures and concentrations between different times was significantly observed.

Discussion

Hydatidosis, a zoonotic infection, is caused by the larval stage of *E. granulosus*. To prevent this disease, one approach is to render one-step in the parasite's life cycle inactive or eliminate it entirely. Proper heating and the utilization of chemical compounds, such as sodium chloride, are among the methods used for deactivation (Franssen et al., 2019). Our results demonstrated that at −20°C, all protoscoleces in infected livers remained alive after 0.5 h. However, as the temperature increased, the survival rate decreased significantly. At +72°C, +60°C, +50°C, and +10°C, all protoscoleces were completely dead after 1, 1.5, 3, and 24 h, respectively. These results indicated that higher temperatures lead to a rapid decline in protoscolex viability, with longer exposure resulting in complete mortality, which was consistent with other studies (Andersen and Loveless, 1978). They reported that the maximum survival time at each temperature was 2 days at 37°C, 6 days at 0°C and 24°C, 10 days at 4°C, and 16 days at 12°C (Andersen and Loveless, 1978). In another study, the mortality rates of *E. granulosus* protoscolex at temperatures of 25°C, 37°C, and 40°C were reported as 3%, 9.38%, and 01.70%, respectively (Al-Hadithi and AL-Khamesi, 2010). Diker et al. (2007) reported that the survival rate of hydatid cyst protoscolexes was 5% at −10°C, 30% at 0°C, 25% at +10°C, and 11% at +40°C with different relative humidities (Diker et al., 2007). According to a study conducted by Moazeni and Alipour-Chaharmahali (2011), warm water at a temperature of 45°C exhibited the highest scolicidal activity, reaching 40.4% after 15 min. However, warm water at temperatures of 50°C, 55°C, and 60°C achieved the best scolicidal effect, with a complete kill rate (100%), in 5, 2, and 1 min, respectively.

Nematollahi and Ghazi (2014) reported that the mortality rates of hydatid cyst protoscoleces at temperatures of −20°C, 40°C, and 60°C after 5 min were 4.5%, 2.5%, and 5.5%, respectively. After 10 min, the mortality rates were 9.0%, 4.5%, and 11%, respectively. After 15 min, the mortality rates were 15.5%, 7%, and 13.5%, respectively. Finally, after 60 min, the mortality rates were 28.5%, 11%, and 30%, respectively. These studies are in agreement with the results of our research. The reason for this could be attributed to the fact that they studied the effects of different temperatures on protoscoleces isolated from hydatid cysts, whereas we evaluated these effects on protoscoleces within the liver mass.

Table 2. Mean Survival Percentage of Hydatid Cyst Protoscoleces at Different Concentrations of NaCl in Infected Liver ± Standard Error

C (%)/T (h)	*3*	*6*	*12*	*24*	*48*	*72*
5	100.00 ± 0.00^{di}	91.35 ± 1.40^{di}	81.45 ± 4.11^{ch}	59.94 ± 2.52^{bg}	38.25 ± 3.98^{bf}	20.95 ± 3.03^{be}
10	66.53 ± 7.81^{cg}	55.23 ± 2.39^{cg}	36.63 ± 3.91^{bf}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}
15	31.47 ± 4.27^{bg}	12.11 ± 3.94^{bf}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}
20	11.39 ± 1.71^{af}	0.000 ± 0.000^{ae}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}	0.00 ± 0.00^{ae}

Letters a–i in each column and row indicate statistical significance.
C, concentration; T, time.

In the present study, concentrations of 10%, 15%, and 20% sodium chloride caused the death of protoscoleces after 24, 12, and 6 h, respectively, which was consistent with the studies conducted by Hazrati Tappeh et al. (2011). However, our studies were not consistent with the results obtained from the studies of Nematollahi and Ghazi (2014). The reason was that they evaluated different concentrations of sodium chloride on protoscoleces, whereas we evaluated on liver mass infected with hydatid cyst containing protoscoleces.

A study proved that 20% saline is a widely used scolicidal agent. Undiluted form of this substance killed the protoscoleces in both 5 and 10 min of exposures. Ten percent saline could not kill the protoscoleces in 5 min, but when we prolong the exposure time, the protoscoleces were killed at 10 min (Besim et al., 1998). In conclusion, these findings suggest that inactivating *E. granulosus* protoscoleces through temperature manipulation or the use of sodium chloride can be an effective strategy for preventing the recurrence of hydatid cysts during surgical operations. Further research and implementation of these control measures could significantly contribute to the management and reduction of hydatidosis in both humans and animals.

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Statement

We are not employed by the government of Iran, and we are preparing the article in our personal capacity; in other words, we are not working as an official representative or otherwise on behalf of a sanctioned government such as Iran. We are working at an academic institution as researchers.

Authors' Contributions

N.H. made substantial contributions to the conception, design, study implementation, analysis, and interpretation of data and major contribution to writing, and read and approved the final version; P.H. and P.Z. participated in the collection of sample and examination. All authors read and approved the final article.

Disclaimer

The authors confirm that this article has not been published elsewhere and is not under consideration by another journal.

Disclosure Statement

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