



RESEARCH PAPER

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Serum liver enzymes profiles of cattles infected with hydatid cyst at Shahrekord abattoir in Chaharmahal Va Bakhtiari Province, Iran

Abdul-Rasool Namjoo^{1*}, Nabi Arzanipour²

¹*Department of Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, P.O. Box 166, Shahrekord, Iran*

²*Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, P.O. Box 166, Shahrekord, Iran*

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Abstract

Liver is the most important organ that is susceptible to several parasitic and bacterial infections. Hydatidosis is one important parasitic disease in warm blooded vertebrates and occasionally in many other mammal species. We don't have enough information available on serum enzyme activity in cattle with hepatic hydatid cyst. This study aimed to determine antibody titer, serum liver enzyme activity such as alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), serum glutamate-oxal-acetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in cattles having hepatic hydatidosis compared to cattle with healthy livers at abattoir. In current study, blood samples were obtained from the jugular vein from 97 cattle slaughtered in Shahrekord, Iran. In macroscopic examination of 41 cattle observed liver hydatid cysts. The tissue samples were fixed in 10% buffer formalin and stained with hematoxylin and eosin. In this study, histopathological changes in livers with hydatid cyst, serum enzyme concentration and antibody titer of IgG were measured by ordinary light microscope, spectrophotometry and ELISA kit, respectively. Serological tests showed that the concentration of ALP, LDH, SGOT and SGPT in cattle infected with hydatid cysts were significantly lower than the healthy cattle ($p < 0.05$). ELISA results showed that serum antibody immunoglobulin G anti- *Echinococcus* had no significant difference in the infected group compared to the healthy group ($p > 0.05$). In addition, low level of antibody for sterile hydatid cyst is related to, non-stimulated cellular, humoral defense and low levels of IgG and IgE against proteins of hydatid cyst. It is clear that activity of the specific enzymes of serum can be helpful in diagnosis of hydatid cyst infection.

* **Corresponding Author:** Abdul-Rasool Namjoo ✉ ar.namjo72@gmail.com

Introduction

Cystic echinococcosis is a zoonotic parasite belonging to the genus *Echinococcus* (Family Taeniidae). The final host are carnivores, and intermediate hosts include sheep, goats, cattle, small rodent, wild herbivores and humans. This parasite might inhabit especially in liver and lung than other organs (e.g., kidneys, spleen, brain, bones, and heart) (Bakır *et al.*, 2012). Hydatidosis is an economically important disease as it causes severe problems in different species animals especially in the industrial and semi-industrial cattle farms by close contact with the final host via domestic and wildlife reservoirs (Umesh *et al.*, 2010).

Echinococcosis can be accurately evaluated in Definitive Hosts and Intermediate Hosts (humans), respectively based on the patient's history, clinical findings, hematological serum biochemical profiles and serological testing, (Elshazly *et al.*, 2009). Ultrasonography (US), computed tomography (CT), (Kalinov, 2007), and magnetic resonance (MR) imaging (Mortele *et al.*, 2004). Abdominal palpation, x-rays and echography (Heier *et al.*, 2007).

The liver is one of the essential organs and largest gland of the body (Schmucker, 2005), sensitive to different parasites and disease conditions which affect the total health state of the animal (Ahmedullah *et al.*, 2007). The liver, which plays a serious role in lipid, carbohydrate and protein metabolism, performs tasks such as bile construction, vitamin storage and the biotransformation of drugs and toxins. In addition, the liver plays a role in immune functions (Schmucker, 2005).

The hepatic enzymes such as alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), serum glutamate-oxal-acetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT) are principal enzymes that primarily represent hepatocellular necrosis and cholestasis, respectively. So, there are of special usefulness in the diagnosis of serious hepatic diseases (Kim *et al.*, 2008). It is clear that activity of the specific enzymes of serum in the

infected of serum cattle help to diagnosis of hydatid cyst. In farm animals infested by hydatid cysts may not show any signs and symptoms hydatid infection may be detected at slaughter or at post mortem examination and recognized as round swelling in the liver and lungs in of infected food animals and may be more common in older animals. The purpose of the present study has been performed to evaluation activity of the specific enzymes of serum in the infected of serum cattle help to diagnosis of hydatid cyst.

Material and methods

Study area

This study was performed in Chaharmahal Va Bakhtiari province, Iran, from may 2012 to June 2012. The province of Chaharmahal Va Bakhtiari with 16532 square kilometer (sq. km.) is situated between 31° 09' North latitude and also to 32° 48' East longitude. The geographical latitude and longitude of this area is 31° 09' 'N and 32° 48' 'E, respectively. This Province is located at the center of Zagros Mountains. About %45 to %50 of the country water sources are in this province (Fasahat *et al.*, 2013).

In this descriptive-cross sectional study, ninety-seven Holstein cattle at Juneghan abattoir in Chaharmahal Va Bakhtiari province of Iran. They included 70 females and 27 male with age range between, 2 to 8 years. None of the cattles weren pregnant (by palpation through rectal method) or in first month of lactation. Under clinical examination, body temperature, heart rate and respiratory rate were normal and the body condition scoring was determined ($BCS \geq 3$) (Ferguson *et al.*, 1994).

Before slaughter, an ante mortem examination was done on all cattle during which their age and gender were evaluated. The age of the cattle was determined by examination of the teeth as described by Kelly (Kelly, 1975). Blood samples were collected at 5:00 to 6:30 h am before slaughter, from the jugular vein into vacuoliner tubes, without anticoagulant.

After clotting for 3 hours at 4°C and centrifugation

(1500G, 10 min, 4°C), sera were carefully harvested and stored at -20°C until analysis (Djokovic *et al.*, 2013).

After slaughtering the cattle, post-mortem examination was carried out by meat inspectors using standard procedures recommended by FAO/UNEP/WHO (FAO/UNEP/WHO, 1994; Swai and Schoonman, 2012). Post mortem examination procedure employed visual inspection, palpation and systematic incision of each carcass, visceral organs particularly heart, kidney, liver, lung, and spleen and targeted lesions consistent with hydatidosis (Swai and Schoonman, 2012). A total of ninety-seven livers were examined for gross pathological hydatid cyst. Livers were cut into about 1cm pieces and fixed promptly in 10 percent buffered neutral formalin (pH 7.4). Following a routine histological tissue-processing procedure, samples were embedded in paraffin blocks and serial sections were cut at a thickness of 4-5 microm and stained with haematoxylin & eosin, also cleared in xylene. In the next stage mounted in Canada balsam (Kul and Yildiz, 2010). Hepatic lesion in cattle infected with hydatid cyst were definitive diagnosed based on the study of histological section. The blood samples were centrifuged at 3000 rpm for 10 min within 2 h of collection and the obtained sera were separated, stored at -20 °C, and analyzed within 1 week. GGT, LDH, SGOT, and SGPT were measured with commercially available kits (Parsazmoon, Tehran, Iran) using an automated biochemical analyzer (Biotechnica, Targa 3000, Roma, Italy) in accordance with the instructions provided via the

company. Also, ELISA test was performed by Vircell-G1006 kit (Granada, Spain) to determine IgG titer in cattle serum. The SPSS version 16 statistical package was used for data analysis. The independent sample T-Test was used for comparing the groups, and $p < 0.05$ was considered to be significant.

Results

In the present study 42.26% of livers were affected with hydatidosis. Multiple variable-sized cystic lesions were seen scattered in hepatic lobes from 2 to 6 cm. Hydatid cysts were usually irregular or distorted in shape, inflated and fluid-filled without larve (protoscolex) and were often enclosed by a fibrous capsule. Histopathological changes in liver of cattle infected with hydatid cyst were characterized by well-developed fibrous layer, laminated layer and germinal layer. Around the hydatid cyst there was marked cellular reaction characterized by proliferation of fibroblasts, infiltration of chronic inflammatory cells and eosinophils. The fibrous layer contained giant cells and macrophages. Some liver cysts showed calcification in vicinity to the liver parenchyma (Figure 1), which there was hydropic degeneration, with moderate or severe cell swelling in the surroundings of hydatid cyst. The histopathologic figure of immature sterile hydatid cyst (*Echinococcosis*), in liver of cattle showed in figure 2. Analysis of the liver enzyme revealed that SGOT, SGPT, LDH and the ALP in cattle infected with hydatid cysts, were significantly lower than the health cattle ($p < 0.05$) (Table 1).

Table 1. Enzyme activity SGOT, SGPT, LDH and GGT in blood serum of holstein cattle (Ref: Pearson and Smith, 1996; The merck veterinary manual, 1998).

Groups	SGOT (U/L) M±SE	SGPT (U/L) M±SE	LDH (U/L) M±SE	ALP (U/L) M±SE	GGT (U/I) M±SE
Group1(N:56) Healthy control group	98.40±34.91	35.44±10.25	3649.33±1017.26	470.02±229.49	18.87±19.89
Group2(N:41) Infected with liver hydatid cysts	83.00±27.03	30.48±12.42	3118.41±856.95	254.75 ± 153.25	18.89±11.37
P Value	0.024	0.041	0.010	0.000	0.996
Ref Value	45.3- 110.2	6.9- 35.3	308.6- 938.1	17.5-152.7	4.9- 25.7

The independent sample T-Test was used to compare the groups and $p < 0.05$ was considered to be significant.

Discussion

Hydatidosis is a chronic parasitic disease that primarily can affect any organs such as liver, lung, kidney, spleen, bone and brain (Pedrosa *et al.*, 2000). This disease is caused by *Echinococcus* spp in various domestic and wild mammals (Ahmadi and Badi, 2011).

Echinococcosis is of major economic importance in Iran (Dalimi *et al.*, 2002; Daryani *et al.*, 2007; Garedaghi, 2013; Oryan *et al.*, 2012).

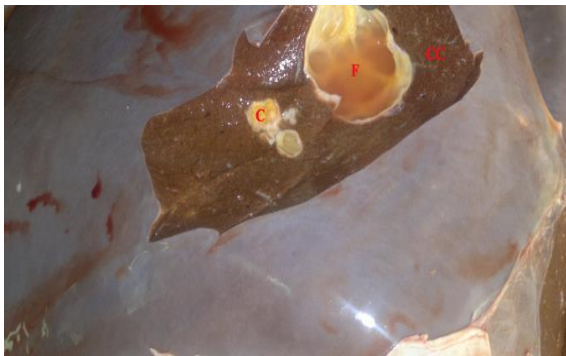


Fig. 1. liver of a cattle infected with hydatid cyst. The cyst contain fluid and Surrounded by a fibrous capsule. C: classification; F: fluid; CC: capsular cyst.

Hydatid disease can exist undetected in livestock animals for years and only diagnosed incidentally during meat inspection in abattoirs for use of food animals (Oryan *et al.*, 2012). Clinical hydatid disease is uncommon in animals, but hydatid cysts in liver and other tissues at slaughter are widespread and cause condemnation and economic loss. Hydatidosis is a common disease in the Middle East, especially in Iran and the neighboring countries (Dalimi *et al.*, 2002; Daryani *et al.*, 2007).

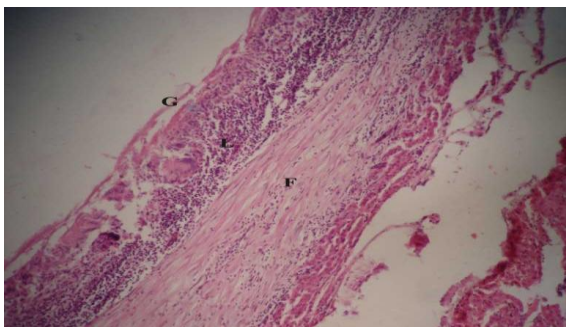


Fig. 2. The histopathologic figure of immature sterile hydatid cyst (*echinococcosis*). in liver of cattle., germinal layer (G), inner portion of the fibrous capsule (L) and fibrous layer (F) (H&E×100).

Study by Luka *et al.*, (2008), was carried out to investigate antigenic characteristics of hydatid cyst fluid in goat and sheep via Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE), to evaluate the specificity and sensitivity of ELISA and Western blotting for the determination and diagnosis of seroprevalence of hydatidosis in sheep and goats slaughtered in Kano abattoir, Northern Nigeria. The sensitivity and specificity of ELISA were determined as 66% and 86% in sheep and 54% and 73% in goats. Where as rates for western blotting were determined as 71% and 65% in sheep and 69% and 72% in goats (Luka *et al.*, 2008). But, in the current study with aiming to determine serum antibody immunoglobulin G anti-*Echinococcus granulosus* there was no significant difference in the cattle infected group compared to the cattle healthy group ($p > 0.05$). Also, study by Akyıldız *et al* (2009) show that serodiagnosis of cystic Echinococcosis may be negative in 10–15% of cases, especially in well-encapsulated cyst (Akyıldız *et al.*, 2009), which simillary this study.

Hydatidosis is one of the major zoonotic diseases in Iran, especially in rural regions. With infection of cow, sheep, goat, water buffalo, camel, elk, llama, pig and horse (Soulsby, 1982; Oryan *et al.*, 2012), hydatid disease happens in herbivores (domestic animals) when they ingest the ovum while grazing on contaminated ground (vegetation contaminated with tapeworm eggs is ingested by the intermediate hosts). *E. granulosus* small (3-5mm long) tapeworm that inhabits the small intestine of the canines and wild carnivores (definitive host) and produces eggs that are Trapped in the feces. Carnivores are mainly infected by eating the raw or uncooked viscera of domestic animals. When environment contaminated with eggs is ingested by herbivores and accidentally humans. Humans are random host and do not play a role in the biological cycle. The ovum loses its protective hard layer in the duodenum and released hexacanth embryo, pass through the intestinal mucosa into the portal circulation and develops into a cyst within the liver and visceral organs (Soulsby, 1982).

Hydatid cysts usually irregular or distorted in shape, due to the variable resistance of liver parenchyma and portal tracts. In this study cysts in cattle livers were 1 to 7 cm across. They commonly measure 5-10 cm in diameter in domestic animals; rarely, cysts in animals may be large, but in humans, hydatid cysts can become huge (Jubb *et al.*, 1993).

Immature hydatid cysts are surrounded by an infiltrate of mixed inflammatory cells including giant cells and eosinophils. As they develop, a layer of granulation tissue, which may contain round cells and eosinophils, invests the cysts, and this evolves so that the inner portion of the fibrous capsule is composed of mature collagenous connective tissue, which is relatively a cellular. Within this, and in close apposition, is the lamellar hyaline outer layer of the hydatid cyst wall, which with time may become hundreds of micrometers thick. The cyst is lined by the thin, cellular germinal layer, from which the brood capsule forms on fine pedicles (Jubb *et al.*, 1993).

The present study showed that the frequency of hydatid cyst in cattle was 42.26%. This finding is more than the reports from other places in Iran. Throughout the country, the mean prevalence of hydatidosis of intermediate hosts in various cities has been reported from 1.5 to 70%.

Oryan *et al.* (2012) reported a mean prevalence of 45.52% of hydatidosis in sheep, 10% in goats and 11.6% cattle at Shiraz abattoir, Southern Iran.

High prevalence of *echinococcosis* infestation in the present study could be due to high population of wild carnivores and stray dogs in the pasture area of livestock, vegetables contaminated by definitive host faeces, reduction of control programmes, lack of awareness among farmers and climatologic conditions, (Garedaghi, 2013). Lack of suitable elimination of infectious carcasses (Swai and Schoonman, 2012).

Results of present study showed significant reduction

($P \leq 0.05$) activity of ALP, LDH, SGPT and SGOT in the serum of cattle infected with hydatid cysts when compared to healthy cattle (Table 1). Because of little information exists about serum biochemical profiles of liver hydatid cyst these results considered the important question of whether the changes in aminotransferase activity in vivo are related to (a) chronic granulomatous hepatitis with hydatid cyst in ruminants or (b) metabolic disturbances. that two hypothesis suggests include: (1) the reduction of pyridoxal 5'-phosphate by a direct action of liver hydatid cyst or (2) a defect specifically developed in pyruvate or 2-oxoglutarate metabolism (Dhami *et al.*, 1979).

The histopathological examination of the liver of hydatidosis cattle revealed, fluid filled cysts develops, that differentiates into 3 layers to form hydatid cysts. The wall of the cyst is composed of outer layer formed by host; inner two are formed by parasite. At histopathologic analysis, a hydatid cyst is composed of three layers: (a) the outer pericyst, which composed of modified host cells such as fibroblasts, eosinophils, giant cells and modified hepatocytes (Shaw *et al.*, 2006) and fibrous protective zone (Pedrosa *et al.*, 2000) (b) the middle laminated membrane or endocyst, an inner germinal layer; which is a cellular and allows the passage of nutrients (Mortele *et al.*, 2004) and impermeable to bacteria (Shaw *et al.*, 2006) and (c) the innermost layer, the germinal layer or ectocyst or brood capsule, (Shaw *et al.*, 2006) a thin, translucent interleaved membrane (Mortele *et al.*, 2004). The endocyst and ectocyst form the true wall of the cyst (Pedrosa *et al.*, 2000).

The pathological of hydatidosis in the present study were similar recorded by Mohamadin and Abdelgadir (2011). In this study cysts in cattle livers were 1 to 7 cm across and coarse wall calcifications are present in most cases but daughter cysts were not found. Many livers infected with hydatid cysts, lesions were characterized by central caseous necrosis and calcification. They commonly measure 5-10 cm in diameter in domestic animals; rarely, cysts in animals may be large, but in humans, hydatid cysts can become huge.

Later on a fluid filled cyst develops, that differentiates into 3 layers to form hydatid cysts. The wall of the cyst is composed of outer layer formed by host; inner two are formed by parasite. The inner most parasitic lining gives rise to "hydatid sands," which are brood capsules for daughter cysts in which scolices are formed (Umesh *et al.*, 2010).

Conclusion

The data obtained from this survey can be used as an indicator and groundwork for more extensive biochemical research. These findings demonstrated that hydatid disease can markedly decrease hepatic enzymes.

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