A comparative evaluation of hematological, biochemical and pathological changes among infected sheep with *Cysticercus tenuicollis* and non-infected control group

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Abstract *Cysticercus tenuicollis*, the metacestode stage of *Taenia hydatigena* are responsible for a high degree of morbidity and mortality in livestock. This study was performed in order to investigate the variations of blood parameters (hematological and biochemical) and pathological changes in 50 sheep infected with *C. tenuicollis* in comparison with 50 non-infected control group. The blood samples were taken from the sheep that were slaughtered in the Kerman slaughterhouse. Blood and sera samples were analyzed for hematology and biochemical parameters and infected livers, were transported to the pathology laboratory for further examinations. According to the analyses performed on the animals blood, a significant increase was detected in number of white blood cells, activities of AST, ALT and levels of total bilirubin in animals with cysticercosis ($p < 0.05$). Also in infected animals, a significant reduction was observed in number of red blood cells, hemoglobin concentration and hematocrit values ($p < 0.05$). In histopathological examination, hepatocellular degeneration and necrosis, fibrosis, mucus gland and biliary

hyperplasia, mild lymphocytic hepatitis, granuloma and telangiectasis were observed. It seems that the increased and reduction of significant blood parameters, may be due to liver failure and pathological changes following larval migration and stimulating of immune responses.

Keywords *Cysticercus tenuicollis* · Hematology · Biochemical · Pathology · Sheep

Introduction

Cysticercus tenuicollis is the larval stage (metacestode) of the canine tapeworm, *Taenia hydatigena* (Soulsby 1986). The intermediate hosts, most commonly sheep and goats, become infected by ingesting proglottids or eggs passed in the feces of the dog in pastures or feeding areas (Kaufmann 1996). Pathogenicity of adult parasites is not high for definitive hosts. However, large numbers of developing cysticerci migrate contemporaneously in the liver of intermediate hosts, producing hemorrhagic and fibrotic tracts known as “hepatitis cysticercosa”, a condition whose gross pathology resembles acute fasciolosis, that is often fatal (Soulsby 1986; Kara and Doganay 2005). *C. tenuicollis* causes considerable economic losses in livestock that suffering from cysticercosis (Abidi et al. 1989). Migration of cysticerci and cyst formation in the liver may result in condemnation of the liver at slaughter (Jepson and Hinton 1986). Diagnosis of infection in animals is based on the finding the cysts during meat inspection where the other methods such as serological (ELISA), biochemical and hematological tests can be useful for diagnosis of live animals (Berezhko 1989). Iran is considered as one of the endemic areas of *Taenia hydatigena*. The infection rate of *Cysticercus tenuicollis* in sheep in Iran and the city of Kerman was 12.8 and 33 %, respectively (Radfar et al. 2005).

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The purpose of this study is to compare haematological and biochemical values of blood and histopathological change in liver among naturally infected sheep by *Cysticercus tenuicollis* and non-infected control group. This values may be useful in further studies of cysticercosis as well as for diagnostic purposes in sheep, especially, in areas where fascioliasis not endemic.

Materials and methods

Animal

The study was conducted in Kerman province, southeast of Iran during April 2009 to March 2010. In this study two groups of 50 sheep, were randomly selected. The first group was consisted of sheep infected with *Cysticercus tenuicollis*. The presence of disease condition was confirmed by inspection of carcasses at Kerman slaughterhouse. Digestive system, respiratory system, circulatory system and liver were inspected for related parasitic and microbial infection macroscopically. Animals with infections were excluded. A second arm was included as non-infected control group. In both groups, the sheep were free from other parasites and clinically normal.

Hematology

Blood samples were taken from the jugular vein using EDTA free tubes, stored at +4 °C and analyzed within 8 h. Total white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and number of platelets (PLT) were analyzed using a fully automated hematology analyzer Sysmex Kx-21 (Sysmex Corporation, Japan).

Biochemical analysis

Sera were separated from blood samples by centrifugation at 971×g. Serum samples were frozen in plastic tubes at −20 °C until use. Serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and concentration of glucose, creatinine, calcium, total protein and total bilirubin were measured according to standard procedures using an automatic analyzer Hitachi 911 (Roche Company, Swiss) with Pars-Azmoon commercial kits (Pars-Azmoon Laboratories LTD, Tehran, Iran).

Pathology

At slaughter, carcasses were carefully inspected and infected livers with *Cysticercus tenuicollis* were transported to the pathology laboratory at Faculty of Veterinary Medicine in Shahid Bahonar University for further examinations. The anatomical location of each cyst was recorded.

Parasite migration routes were identified and 1 * 1 * 1 cm dimensions slices were prepared from the parasites and surrounding tissue. Samples were fixed in 10 % formalin and embedded in paraffin. Four μm thick tissue sections were stained with the haematoxylin and eosin (H&E) for histopathological study.

Statistical analysis

Data were analyzed using SPSS software. Results are expressed as mean ± standard deviation (SD). Significance of difference between groups was determined by Student's *t* test. Values of *p* < 0.05 were considered significant.

Results

Hematology

Hematological parameters of infected and non-infected sheep are summarized in Table 1. The number of red blood cells, Hb concentration and hematocrit were reduced (*p* < 0.05) in infected group, compared with control group. In contrast, the WBC count, was significantly higher than that in control group (*p* < 0.05). However, there were no significant changes in MCV, MCH and MCHC values.

Table 1 Mean ± SD of hematological parameters in infected sheep with *Cysticercus tenuicollis* and non-infected control group

Parameters	Infected sheep	Non-infected sheep
WBC* (10 ³ /μl)	14.589 ± 1.850	8.668 ± 2.855
RBC* (10 ⁶ /μl)	8.03 ± 1.63	10.02 ± 2.08
Hb* (g/dl)	8.32 ± 0.77	10.39 ± 1.68
Hct* (%)	25.17 ± 2.19	31.40 ± 5.08
MCV (fl)	34.02 ± 15.38	31.29 ± 3.55
MCH (Pg)	11.24 ± 5.14	10.47 ± 1.15
MCHC (g/dl)	23.07 ± 1.4	33 ± 0.81

* Significant differences among infected sheep with *Cysticercus tenuicollis* and non-infected control group (*p* < 0.05)

SD standard deviation

Biochemistry

Biochemical results are presented in Table 2. AST, ALT and total bilirubin were increased significantly ($p < 0.05$) in infected sheep sera in comparison with the non-infected animals, while no significant changes were observed in serum levels of alkaline phosphatase and serum concentrations of glucose, creatinine, calcium and total protein.

Pathology

In the exteriorizing investigation of infected livers, enlargement and congestion were observed on liver surface and parenchyma. In chronic cases, different size and different shape cysts was found on the liver. Anatomical position of cysts were mainly in the left hepatic lobe of visceral surface, right and left hepatic lobes of partial surface (Fig. 1) and right renal impression.

Histopathology investigation showed, biliary hyperplasia (Fig. 2) and hyperplasia of mucous glands with dilation of central veins. In addition, cysts were observed in liver slices without inflammatory reactions.

Lipofuscin pigments were detected in larval migration route on a background of fibrotic tissues. Also, telangiectasis, squamous metaplasia of the bile duct, accumulation of mononuclear cells, dilation of central veins, separation of hepatocytes and necrosis, granulomatous hepatitis and mild lymphocytic hepatitis were observed.

Discussion

The cysticerci of *Taenia hydatigena* are responsible for a high degree of morbidity and mortality in livestock (Kara and Doganay 2005). There is little information available concerning haematological and biochemical values for

Fig. 1 Anatomical position of *Cysticercus tenuicollis* in the right and left hepatic lobes of partial surface

sheep infected with *Cysticercus tenuicollis*. Similar to the larvae of *Fasciola*, migration of cysticerci in the liver may cause haemorrhagic and fibrotic tracts and serofibrinous peritonitis (Soulsby 1986; Balzek et al. 1985; Abdorahman et al. 1988). In the present study, evaluation of haematological parameters showed that mean number of WBCs in infected sheep was approximately 2 times higher than non-infected sheep, whereas mean number of RBCs and Hb concentration were reduced significantly ($p < 0.05$). Likewise, hematologic investigations in naturally infected pigs by *Cysticercus tenuicollis* compared with non-infected pigs showed that the number of WBCs increased, on the contrary the number of RBCs and Hb concentration were reduced (Pathak et al. 1984).

It seems that the increased number of WBCs may be due to liver failure, inflammation following larval migration and stimulating of immune responses. Also, bleeding and

Table 2 Mean $\pm$ SD of biochemical parameters in infected sheep with *Cysticercus tenuicollis* and non-infected control group

Parameters	Infected sheep	Non-infected sheep
Glucose (mg/dl)	71 $\pm$ 17.1	64 $\pm$ 19.6
Cr (mg/dl)	1.7 $\pm$ 0.94	2 $\pm$ 0.8
Ca (mg/dl)	10.4 $\pm$ 1.23	10.1 $\pm$ 1.16
AST* (μ l/l)	471 $\pm$ 14.54	243 $\pm$ 7.88
ALT* (μ l/l)	65.7 $\pm$ 57.92	22.6 $\pm$ 6.87
AIP* (μ l/l)	360 $\pm$ 466.96	101 $\pm$ 24.35
Total bil (mg/dl)	0.6 $\pm$ 0.33	0.3 $\pm$ 0.23
TP (g/dl)	6.29 $\pm$ 1.11	7 $\pm$ 1.16

* Significant differences among infected sheep with *Cysticercus tenuicollis* and non-infected control group ($p < 0.05$)

SD standard deviation

Fig. 2 Biliary hyperplasia and fibrosis caused by migration of *Cysticercus tenuicollis* (haematoxylin and eosin, 400 $\times$)

congestions are caused by larval migration in the peritoneum and liver parenchyma cause reduction of RBCs and Hb concentration in infected animals.

Our study revealed that biochemical parameters including liver enzymes (AST, ALT) and total bilirubin were increased significantly in infected animals compared with control group. The present findings are consistent with those found by Pathak and Gaur (1981) who showed a significant increase in enzyme levels in infected goats with *Cysticercus tenuicollis*. Doaa et al. (2007) showed that levels of liver enzymes, total bilirubin, gammaglobulins and creatinine were increased in serum of infected sheep with *Fasciola hepatica*. Metanovic et al. (2007) reported similar results in infected cattle with *Fasciola gigantica*. Singh et al. (2004) reported an increase of AST levels 2 weeks post-infection synchronizing with the migratory phase of *Fasciola hepatica* in the liver parenchyma. They reported that liver damage is the most important cause of the increase in serum ALT activity in the infected sheep.

Larval migration in the liver, especially in young animals, leads to serious consequences. This migration causes hemorrhage and fibrosis in liver parenchyma. Morbidity of infection in ruminants is high and mortality in lambs is reported (Soulsby 1986).

The condition of hepatitis cysticercosis, caused by *Cysticercus tenuicollis* in sucking pigs, was described by Bertullo (1943) and Lloyds (1964). Pullin (1955) and Karasev et al. (1978) described the pathological features of *Cysticercus tenuicollis* infection in sheep. Deorani (1967) observed hepatitis cysticercosis in sheep and deer. Deodhar and Narsapur (1968) described “pneumonitis cysticercosa”, a new disease condition in the lungs of goats. However, these reports did not include a description of the development of lesions, as the studies were largely made on natural cases, as well as Pathak et al. (1982) and Balzek et al. (1985) studied the pathology of *Cysticercus tenuicollis* in goats on successive days.

In another study Darzi et al. (2002) on pathological investigation concluded that, the liver showed haemorrhagic tunnels/pools, fibroplasia, and thickening of the Glisson's capsule. These researchers reported brown and red areas with blood spots on liver surface and liver parenchyma at necropsy observations. Their histopathology findings showed that cavities filled with fibrin, erythrocytes and degenerated and necrotic tissues.

In the present study, histopathologic changes were included degeneration, fibrosis, necrosis, mild lymphocytic hepatitis, mucous gland hyperplasia, bile duct hyperplasia, dilation of central veins, telangiectasis and formation of granuloma and pseudolobule observed. Degeneration, necrosis and fibrosis observed in the most histopathology sections were undeniable complications, caused by larval

migration. It is noteworthy that the bleeding, a sign of acute infection, was not observed.

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