Original Article

A study on histopathological changes due to zoonotic nematodes in sheep in Ilam province, Iran

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Summary

Livestock has important role in the human life cycle. Humans along with livestock over the years have been accompanied by undesirable consequences like zoonotic diseases. Zoonotic infectious agents such as helminths are among the most prevalent causes for the major human emerging and re-emerging infections. So, this study was performed to identify the zoonotic gastrointestinal nematodes and determine histopathological changes occurred on gastrointestinal tract in Kurdish sheep slaughtered in Ilam industrial abattoir. For this purpose, tissue samples of 375 above mentioned sheep were checked strictly. As a result, identified nematode species were *Ostertagia ostertagi*, *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Trichuris globulosa*, *Oesophagostomum* sp., *Bunostomum* sp. Histopathological findings of infected tissues, particularly lymph nodes and mesentery have been described in results detailed. Briefly, presence of black dot or black streak on serosa of intestine, mesentery, paleness and enlargement of mesenteric lymph node, decrease in the number and size of the lymphatic follicles, decrease in the lymphoid cell population in the medulla tissue, increase of lymph node capsule thickness and sever depletion in secondary lymph follicles associated with lymphadenitis which represented by proliferation of lymphocytes and infiltration of macrophage as well as hyperemia and congestion in cortex and medulla were considerable histopathological changes. Accordingly, given that these parasites have a direct life cycle, thus, lack of hygiene in farms where animals are kept leads to increased infection and it is necessary to pay more attention to this field.

Key words: Zoonotic Nematodes, Histopathological changes, Sheep, Ilam.

Introduction

Livestock has undeniable role in the human life cycle and ecosystem balance. At times, coexistence of humans alongside livestock over the years has been accompanied by undesirable consequences like zoonotic diseases. Zoonotic infectious agents are among the most prevalent on earth and are thought to be responsible for more than 60% of all human infections and 75% of emerging human infectious. The success and widespread epidemiology of these infections can be attributed to a range of human factors including social and dietary changes as well as an increased mobility of the human population. As the human population continues to grow there is an ever increasing need to develop and

maintain food products with a high protein content (particularly livestock and fish) under intensive farming situations, which is inevitably leading to a greater spread of animal diseases and their transmission to humans. Improved diagnosis and/or recognition of neglected human infections can account for some diseases apparently emerging or re-emerging in recent times. Climate change has also been suggested as a cause for disease spread and is a concern for the future. Human infections caused by parasitic helminths are of particular importance given the relatively recent acknowledgement of a number of species as important human pathogens. Humans can develop patent infection with a wide range of helminth parasites, whose natural host is another vertebrate such as ruminants (Robinson and Dalton, 2009; Mc Carthy and Moore, 2000). Parasitic infections are generally regarded as the most prevalent and important health problems in human and in ruminants due to economic losses associated with endo and/or ectoparasites including decreased production, cost of prevention, cost of treatment and death of infected animals causing a combined annual loss of approximately a billion dollars (McLeod et al., 1995; Barger, 1982; Donald and Waller, 1982).

Generally, human infections due to gastrointestinal helminths, except for a few ones, are usually asymptomatic clinically. However, in the most severe cases, the hematophagism of the L_4 larvae and adult forms can cause inflammation of the bowel mucosa and tissue damage. The clinical symptoms are considered as general signs such as cramping, rash, abdominal pain, flatulence, nausea, loose feces or diarrhea, emaciation, weight loss, fatigue, headache, anorexia, eosinophilia and anemia (Watthanakulpanicha et al., 2013; Boreham et al., 1995).

Parasitism by helminthes is one of the major causes of disease in developing countries. In Iran, parasitic diseases in human and domestic animals result in ill health and considerable economic loss in poultry and livestock production. The present study was designed to determine certain nematodes spp. that are of zoonotic importance, and to characterize histopathological changes in naturally infected Kurdish sheep in Ilam province, Iran. It is necessary to note that there are only case reports of infection with nematodes determined in this study both from Iran and the worldwide and also there are not any histopathological findings as characterized to the main zoonotic helminthes in humans.

Materials and Methods

From April to August 2011 in Ilam industrial abattoir, a total of 70 randomly selected sheep were subjected to this study and total intestines accompanied with abomasum were transferred to Veterinary Faculty of Ilam University. At first, intestines of each animal were opened longitudinally in their whole length separately for any part in order to explore parasites existence. Thereafter, 375 tissue samples of various glands (including internal, external iliac and mesenteric lymph node) were obtained for histopathological study.

In this stage different lymph nodes from infected sheep were separated and preserved in 5% formalin. Tissues were prepared for microtome, cut in 8 µm and stained with Haematoxylin and Eosin. Slides were studied on Olympus camera attached microscope. Observations were recorded and micropathography was done for projection slides and photographs.

After washing contains of the different parts of intestines under tap water into the

wire mesh, parasites then were removed under stereomicroscope and identification were followed by parasitological methods as described by reliable references (Soulsby, 1982).

Results

The type of the parasites in the different parts of gastrointestinal tract of the sheep studied presented in Table 1. For example a number of macroscopic and microscopic images of parasites on and/or into the gastrointestinal tract of infected animals were shown (Fig. 8, 9, 10).

The gross pathological lesions of mesentery were evident by the presence of black dot or black streak on serosa of intestine, mesentery, paleness and enlargement of mesenteric lymph node. Sever adhesion have also been seen between mesentery, intestine and abdominal muscles. Histopathological section of lymph nodes showed decrease in the number and size of the lymphatic follicles, decrease in the lymphoid cell population in the medulla tissue, increase of lymph node capsule thickness and sever depletion in secondary lymph follicles associated with lymphadenitis which was represented by proliferation of lymphocytes and infiltration of macrophage as well as hyperemia and congestion in cortex and medulla (Fig.1, 2, 3).

Histopathological section of mesentery tissue revealed decrease in cell population in the cortical follicles, deformation of lymphatic follicles, necrosis of the cortical tissue and presence of severe hyperemia and congestion, thickness in the wall of arterioles due to presence of vacuole in all layers of arterioles and infiltration of mononuclear inflammatory cells in mesentery (Fig. 4, 5, 6, 7).

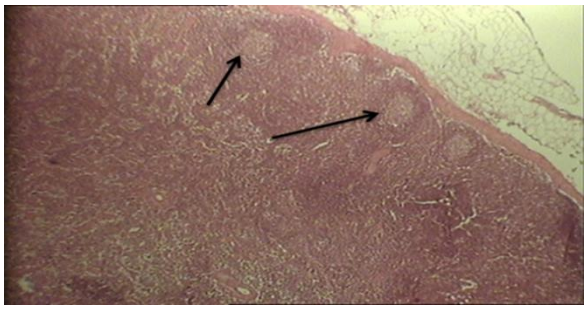

Fig 1: External Iliac, decrease in the number and size of the lymphatic follicles (H&E staining, ×10)

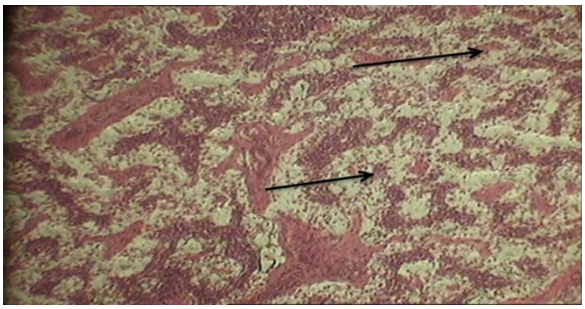

Fig 2: Internal Iliac, decrease in the lymphoid cell population in the medulla tissue (H&E staining, ×40)

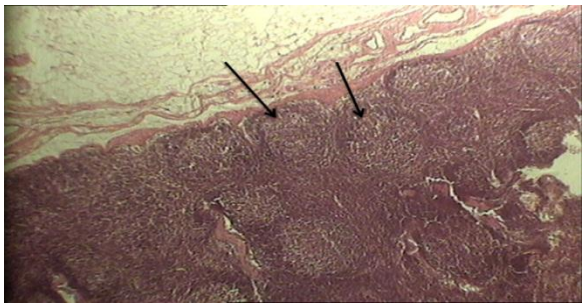

Fig 3: Internal Iliac, increase of lymph node capsule thickness (H&E staining, ×10)

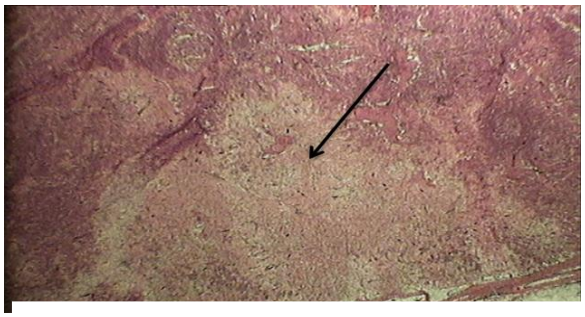

Fig 4: Internal Iliac, necrosis of the cortical tissue (H&E staining, ×10)

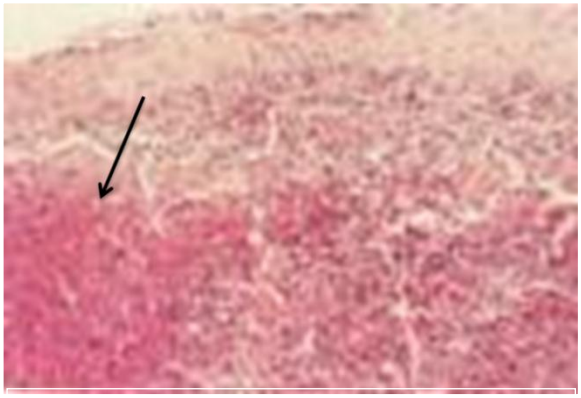

Fig 5: Internal Iliac, necrosis of the cortical tissue ((H&E staining, ×40)

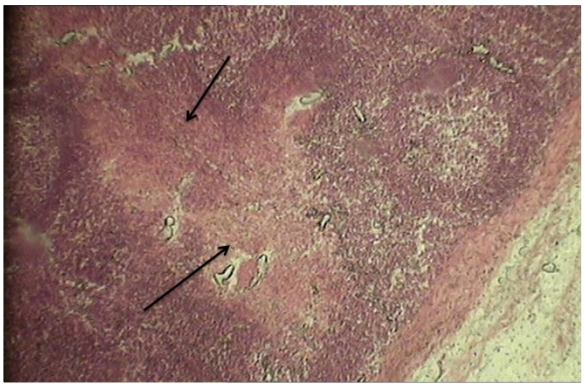

Fig 6: Mesenteric lymph node, congestion and Hyperemia (H&E staining, ×40)

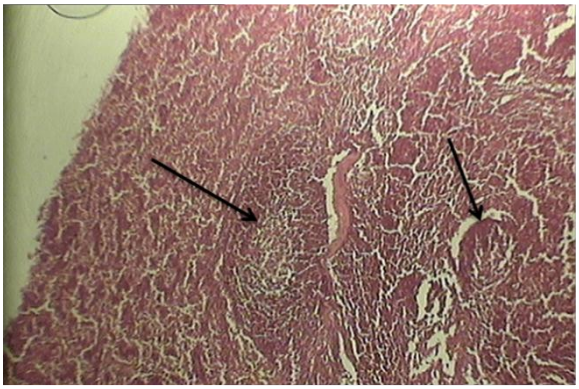

Fig 7: Mesenteric lymph node, deformation of lymphatic follicles (H&E staining, ×10)

Fig 8: *Haemonchus contortus* on stomach surface showing areas of hemorrhage

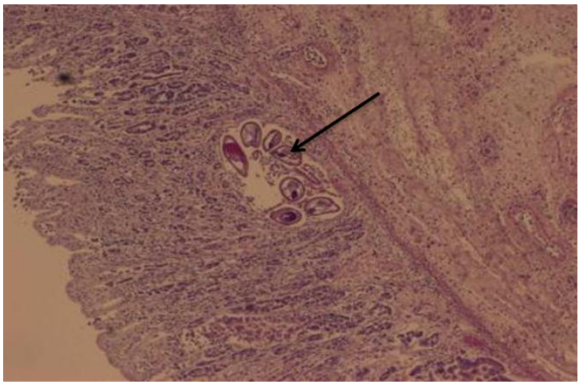

Fig 10: Mesentery of sheep infected with parasite revealed thickening in wall of artery due to sever vacuolation in wall of arteries (A) associated with congestion and thrombus(B)(H&E, ×250)

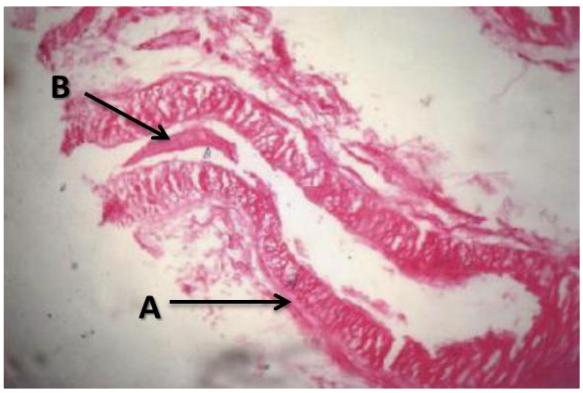

Fig 9: *Ostertagia ostertagi* larvae inside an abomasal gastric gland of sheep

Table1. The distribution of nematode spp. in different parts of sheep gastrointestinal tract

Type of parasite	AB	SI	CO	CAE	RE
Ostertagia ostertagi	5	0	1	0	1
Haemonchus contortus	18	38	8	11	8
Trichostrongylus colubriformis	0	6	1	0	0
Trichuris globulosa	0	1	2	3	0
Bunostomum sp.	0	8	0	0	0
Oesophagostomum sp.	5	7	7	9	1

AB: Abomasum; SI: Small Intestine; CO: Colon; CAE: Caecum; RE: Rectum

Discussion

In the present study, we identified nematode helminths namely, *Ostertagia ostertagi*, *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Trichuris globulosa*, *Oesophagostomum* sp., *Bunostomum* sp. and accompanied with histopathological changes in Kurdish sheep slaughtered in Ilam industrial abattoir. These parasites are an important part of ruminant pathogens, and some are extremely pathogenic and deadly, and thus vitally important from veterinary viewpoint. Although they are the most important nematodes in ruminants with relatively distinct clinical and histopathological signs, but the species from these helminths have reported as case reports sometimes with nonpathgnomonic symptoms and/or as asymptomatic infections in Iran (Ghadirian and Arfaa, 1973; Gadirian et al., 1968; Eslami, 1997; Pestechian et al., 2014; Ashrafi et al., 2015) and the other parts of the world (Polderman and Blotkamp, 1995; Nolan, 1998; Polderman et al. 1999; Tligui et al., 2005; Bradbury R, 2006). However, this study was performed to determine the latest status of zoonotic gastrointestinal nematodes in sheep in Ilam province, western of Iran.

Histopathologically, as the main aim, histopathological section of lymph node showed decrease in the number and size of the lymphatic follicles, decrease in the lymphoid cell population in the medulla tissue, increase of lymph node of the capsule thickness. Histopathological section of mesentery tissue revealed decrease of cell population in the cortical follicles, necrosis of the cortical tissue and presence of severe hyperemia and congestion, thickness in the wall of arterioles due to presence of vacuole in all layers of arterioles had been observed. Oliveira-Sequeira et al. (2000) reported the same observations of infected sheep with nematode.

The present study has further demonstrated that the accumulation of granulocytes particularly granulocytes in glands is matched by morphological changes within the organ and it is in accordance with Scott et al. (2000). Abnormal cells in either the presence or absence of parasites often had dilated canaliculi and other cells were swollen with pale cytoplasm, swollen pale nuclei and changes in mitochondrial structure. These latter features are suggestive of necrosis, the normal process of parietal cell death (Karam, 1993). The acute response to adult parasites involved large numbers of eosinophils and neutrophils, the oxidative bursts of both cells are known to be damaging to host tissues (Sasayama et al., 1997).

Accordingly, given that these parasites have a direct life cycle, thus, lack of hygiene in farms where animals are kept leads to increased infection. To reduce infection,

protect livestock, and increase production in endemic areas, it is recommended that drug resistance in the found species should be evaluated and livestock should be periodically treated with anthelmintic medications. Additionally, because of zoonotic nature of some species of the parasites, measures must be taken to protect the health of people who are directly engaged with these animals.

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