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Study Pathophysiological of sheep glands infected with helminthes

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ABSTRACT

Gastrointestinal nematode parasitism is arguably the most serious constraint affecting sheep production worldwide. The nematode of particular concern is Haemonchus contortus, which can cause severe blood loss resulting in anemia, anorexia, depression, loss of condition, and eventual death. In this study the haematology and histopathology of different glands randomly selected sheep was studied after collection of blood and tissue sample from industrial slaughter houses in Ilam. Different regions of alimentary tract of the 375 animals were checked for the type of parasites present. Those animal with parasite consider as infected and without parasite concerned as a control The present study researched to know the species of parasite affected sheep at Ilam province having 465 Km border-line with Iraq country, in addition to study the pathological lesions in some immune system tissue glands. Dominent infection found in sheep were Haemonchus contourtus Trichurous, globulosa,Tristrongylus, colubriformis, Bunostomom trigonocephalum, Ostertagia ostertagi and sometime trematodes were also found .Erythrocyte number were significantly reduced in infected sheep. Haemoglobin concentration was also found droped in infected animal. Mean cell haemoglobin and Mean cell haemoglobin concentration did not show significant decrease while packed cell volume was significantly reduced in infected animal. Total leukocyte showed consistent pattern of increase in infected sheep percentage count of Neutrophil appear to be reduced with an evident increase in Lymphocytes. Results of this study illustrated that clinical signs of infected animals showed emaciation, sunken eye, and diarrhea, due to infection with Nematode which was identified based on the morphology of the adult worms. Histopathological findings of tissues of infected sheep particularly lymph node, revealed lymphadenitis, while in mesentery cross section of adult parasites in lumen of vein associated with thrombophelebitis has been seen, liver also showed minute granulomas around central vein. These results signify immunopathological response of the host.

Key words: Parasites, Tissue gland, Haematology, Histology, gastrointestinal helminthes,

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INTRODUCTION

Several studies have been conducted to elucidate the mechanisms by which the hosts develop resistance to gastrointestinal parasites. Tissue histopathological changes of the glands have received special attention as indicators of the immune response since they are frequently associated with gastrointestinal parasitic diseases [1]. Several investigators consider that the increase in the numbers of these cells in the mucosa are a strong indicator of their functional importance in the immune response associated with resistance to parasitism [2, 3]. Parasitic infections are generally regarded as the most prevalent and important health problems of *grazing* ruminants in Australia, with losses associated with nematodes, and ectoparasites causing a combined annual loss of approximately a billion dollars[4].

Infections with gastrointestinal nematodes (GIN) can negatively affect the health and the overall productivity of infected animals [5]. Economic losses are caused by decreased production, cost of prevention, cost of treatment, and the death of infected animals (6, 7). The common clinical signs of an infection with GIN are anorexia, diarrhea, emaciation and anaemia [8] The high level infections with nematodes may lead to the death of the infected animals. Under field conditions, most infections are usually mixed and include different species of nematodes. However, the impact of nematode infections on the animal is dependent on the intensity of infection as well as the physiological and immunological status of the host. Growing lambs and periparturient ewes are most susceptible to the infection by nematodes [9]. Today, the control of GIN in sheep has become less effective due to the development of anthelmintic resistance [10]. Furthermore, the climate change will probably lead to changes in epidemiology and intensity of parasite infections [11]. Due to these facts, the economic impact of nematode infections will possibly increase in sheep farms in the future.

In recent years, much attention has been paid on pathophysiological and immunological aspects of nematode infections all over the world. Parasitism by helminthes is one of the major causes of disease in developing countries .In Iran, parasitic disease in human and domestic animals result in ill health and considerable economic loss in poultry and livestock production. In a study carried out in shahr-e kord city it, s indicated that 54 percent of sheep slaughtered has nematode infection [12]. Endemic parasites are a major source of economic loss in animal husbandry, especially in tropical areas and developing countries, but as discussed later, the extent of those losses has yet to be accurately specified, and knowledge about the economics of treatment of these diseases is inadequate, mostly because the damage functions, and in addition, the response functions to treatment are imperfectly known. The present study was designed to determine the association between the tissue response of the glands , lymph nodes and Hematolog parasite burden, *nematodes* spp. in kurdish sheep submitted to natural infection.

MATERIALS AND METHODS

The pathology of glands and hematological randomly selected sheep was studied after collection of blood samples and tissues from industrial slaughter house in Ilam during the period April to August 2011.A total number of 375 samples of blood ,tissues from various gland including (Scapular, popliteal, internal and external iliac, mesenteric lymph node and Liver) were obtained from sheep ,previously(3 month before slaughtering). treated several time with anthelmintic Ivermectin and Albendazole .GIT of each animal was opened with entrotome longitudinally for its entire length different region were checked for the types of parasites present ,animal without any parasites calculate as a control and for the animal if any parasite(nematodes) were found as infected animal. Blood was collected directly in a wide mouth test tube coated with EDTA, at a rate of 4mg /ml of the blood received, as an anticoagulant. Erythrocytes and Leukocytes were counted using Neubauer heamatocytometer .Counting was done only from one dilution.

Haemoglobin concentration was estimated with commercial kit based on reaction of haemoglobin with Drabkins cyanide-ferricyanide solution.Packed cell volum was determined with microhaematocrit procedure. Mean cell volum,Mean cell haemoglobin.mean cell haemoglobin concentration were determined statistically . Blood smear was prepared, fixed in methanol, and differentiated for differential leukocytes count (DLC) with Giemsa stain.

Proceeding Tissue

Tissue of different Lymph node include of (Scapular, popliteal, internal and external iliac, mesenteric lymph node and Liver) infected and non-infected sheep separated and preserve in 5% formalin.Tisue were prepared for microtome, cut in 8 µm and stained in haematoxylin and eosin. Slides were studied on Olympus camera attached microscope. Observation was recorded and micropathography was done for projection slides and photographs.

Statistical analysis :

The computer software ,SPSS Version 9.0 for window (SPSS INC., Chicago, LL,USA) and Chisquare tests were used for statistical analysis.

RESULTS

Incidence of the infection

The type of the parasites with the percentages of their prevalence in the different region of gastrointestinal tract (GIT)of the sheep studied presented in Table 1. *Heamonchus contorts* was the most prevalent species in ovine of Ilam province.

Table 1.percentages incidence of different type of Nematode infection in different region of GIT in sheep

Name of parasites	AB	OM	RT	RU	DU	JEU	IL	CO	CAE	RE
Haemonchus cotortus	18	16	2	1	9	13	16	8	11	8
Oesophagostomium venulosum	5	3	0	0	2	0	3	4	4	0
O.columbianum	0	0	0	0	1	0	1	3	5	1
Trichuris globulosa	0	0	0	0	0	1	0	2	3	0
Ostertagi aostertagi	5	7	1	1	0	0	0	1	0	1
Bunostomum trigonocephalum	0	0	0	0	4	3	1	0	0	0
Tricostrongylus colubriformis	0	0	0	0	2	2	2	1	0	0
Trematodes	2	2	10	16	0	0	0	0	0	0

Ab, abomasums ;OM ,omasum; RT, reticulum; RU , rumen; DU ,duodenum; JEU ,jujeunum ; IL , ileum; CO, colon ;CAE;caecum;RE,rectum.

Red blood cell counts (RBC)
Counts were significantly reduced in infected sheep compared to non-infected ones. It was 23.14% lower in infected sheep in comparison with non infected.

Haemoglobin concentration (HB %)
HB concentration was significantly lowered in the infected group compared to non-infected group. It was 22.52% lower in the infected sheep in comparison with non infected ,the analysis shows significance $p<0.001$.

Packed cell volume
It was significantly reduced in infected cases compared to non-infected ones. It was14.81 reduced in infected sheep. reduction was $p<0.01$respecively.

Mean cell haemoglobin (MCH)
Infected sheep(7.47 pg) did not exhibit noticeable differences compared to non-infected sheep(7.48 pg).

Mean cell volume (MCV)
No noticeable difference was found between infected and non-infected sheep.

Mean cell haemoglobin concentration (MCHC)
It was significantly reduced in infected sheep compared to non-infected. The value were 14.71% lower in infected animals in comparison to non infected group.

Total leukocytes counts (TLC)
It was appreciably increased in infected group. The increase was 17.99% in infected sheep in comparison to control animal.

Differential leukocyte count (DLC)
Neutrophil count was markedly lower in infected group compared to non-infected animals. It was 21.83% lower in infected group of the sheep.

Clinical signs findings: The most sign showed on infected animals are anorexia, Diarrhea and some time hemorrhagic diarrhea, sunken eye and emaciation.

Pathological findings: The gross pathological lesions of mesentery represented by Presence of black dot or black streak on serosa of intestine , mesentery, paleness,and enlargement of mesenteric lymph node. Hepatomegaly and Sever adhesion also have been seen between mesentery, intestine and abdominal muscles.

Lymph node: 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 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 congestion of blood vessel in cortex and medulla.(Fig.1,2,3,4,5,6).

Liver tissue: liver section of sheep infected with nematode showed free space in the parenchyma and sever congestion of blood vessels per vascular cuffing of lymphocytes (minute granuloma) vascular degeneration and fibrosis in portal area associated with infiltration of mononuclear Inflammatory cells (Fig. 7).

Mesentery: Histopathological section of mesentery tissue revealed decrease of cell population in the cortical follicles , necrosis of the cortical tissue and presence of sever congestion in arterioles and veinioles , thickness in the wall of arterioles due to presence of vacuole in all layers of arterioles. Infiltration of mononuclear inflammatory cells in mesentery (Fig.8, 9)

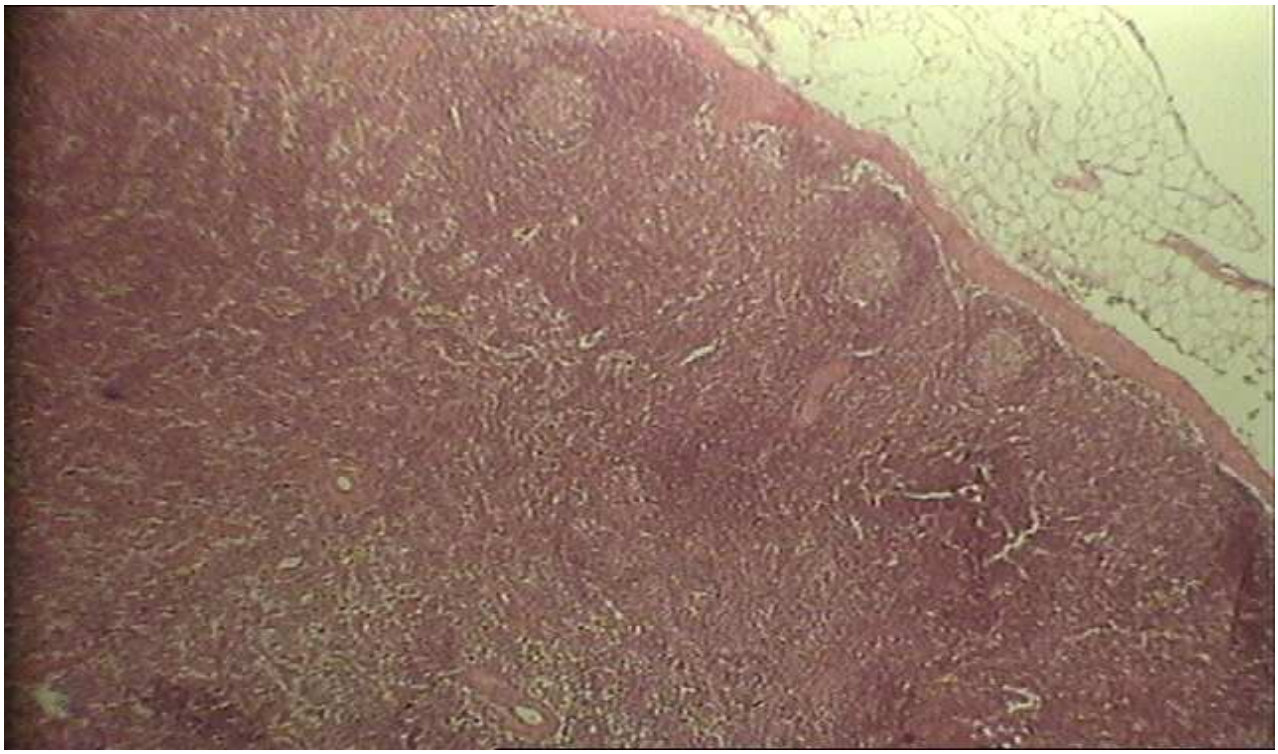

Fig1. (External iliac) Decrease in the number and size of the lymphatic follicles

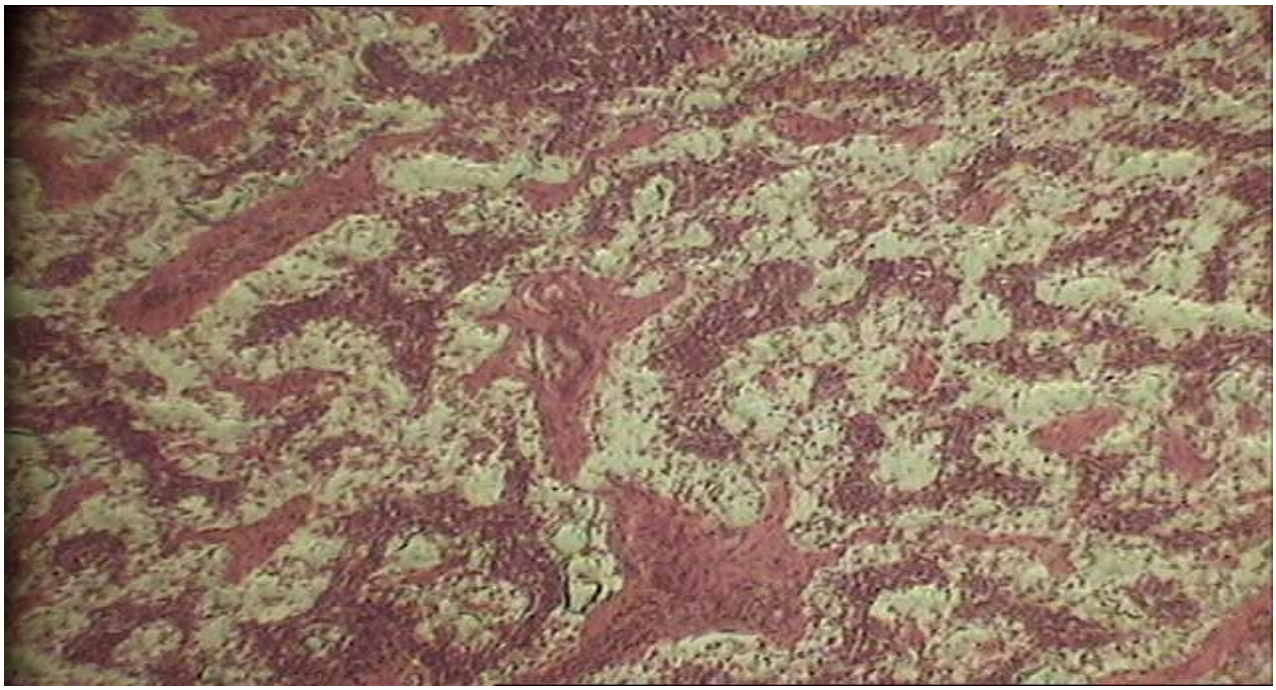

Fig2. (Internal iliac) Decrease in the lymphoid cell population in the medulla tissue

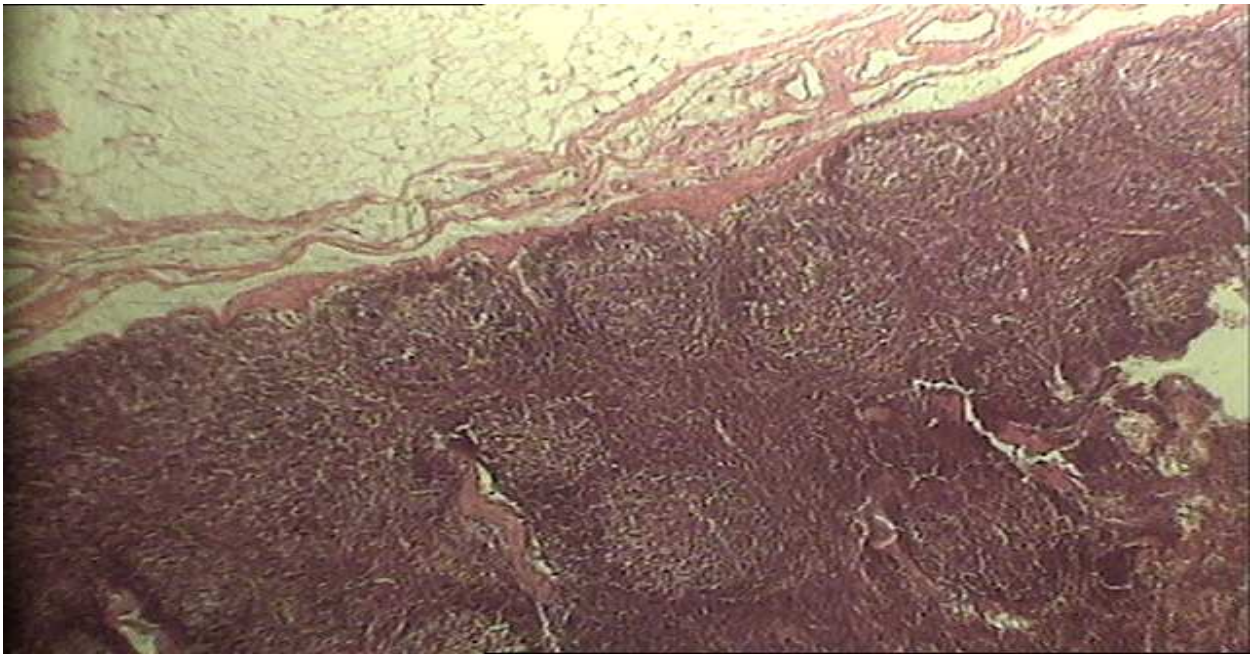

Fig3.(Internal iliac)Increase of lymph node capsule thickness

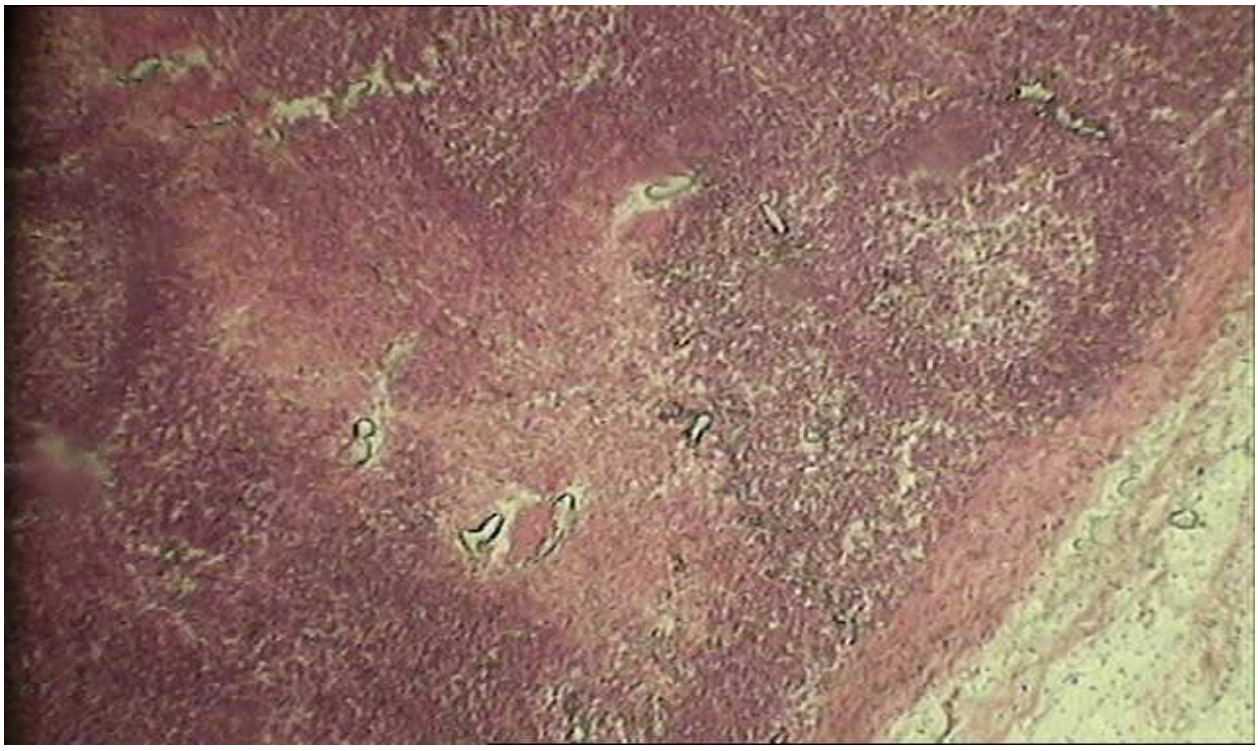

Fig.4.(Internal iliac) necrosis of the cortical tissue 40

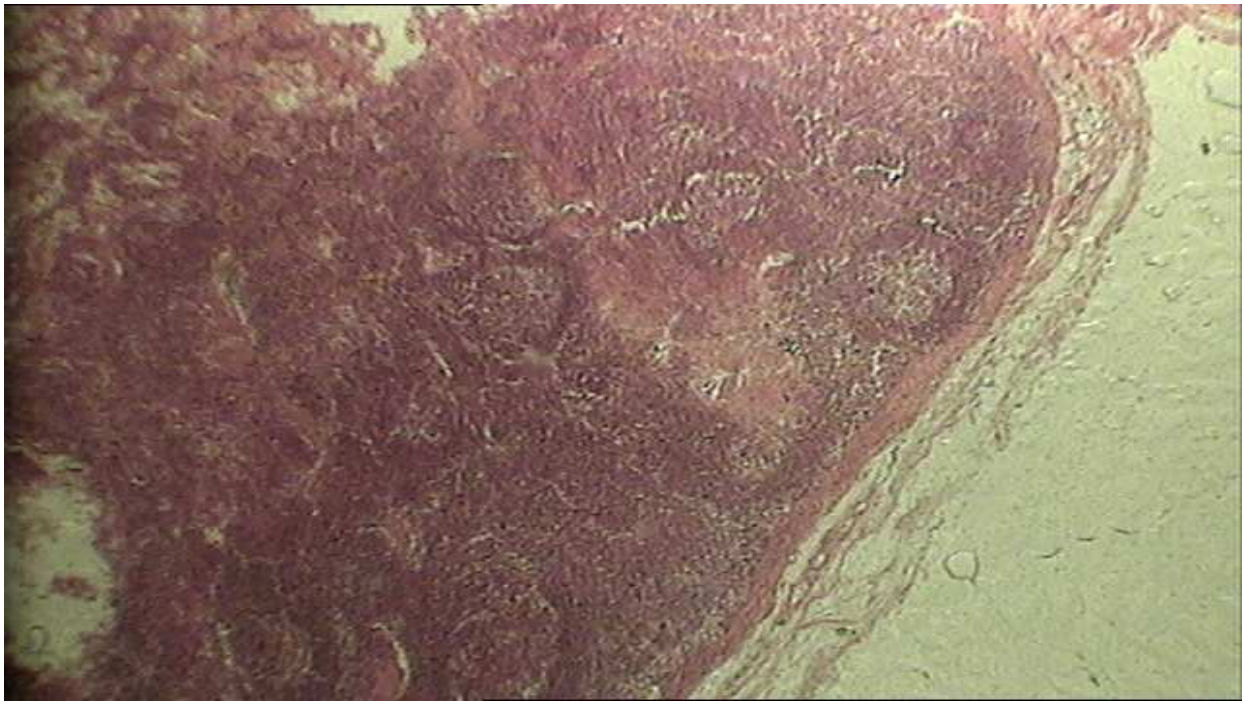

Fig.5.(Internal iliac) necrosis of the cortical tissue 40

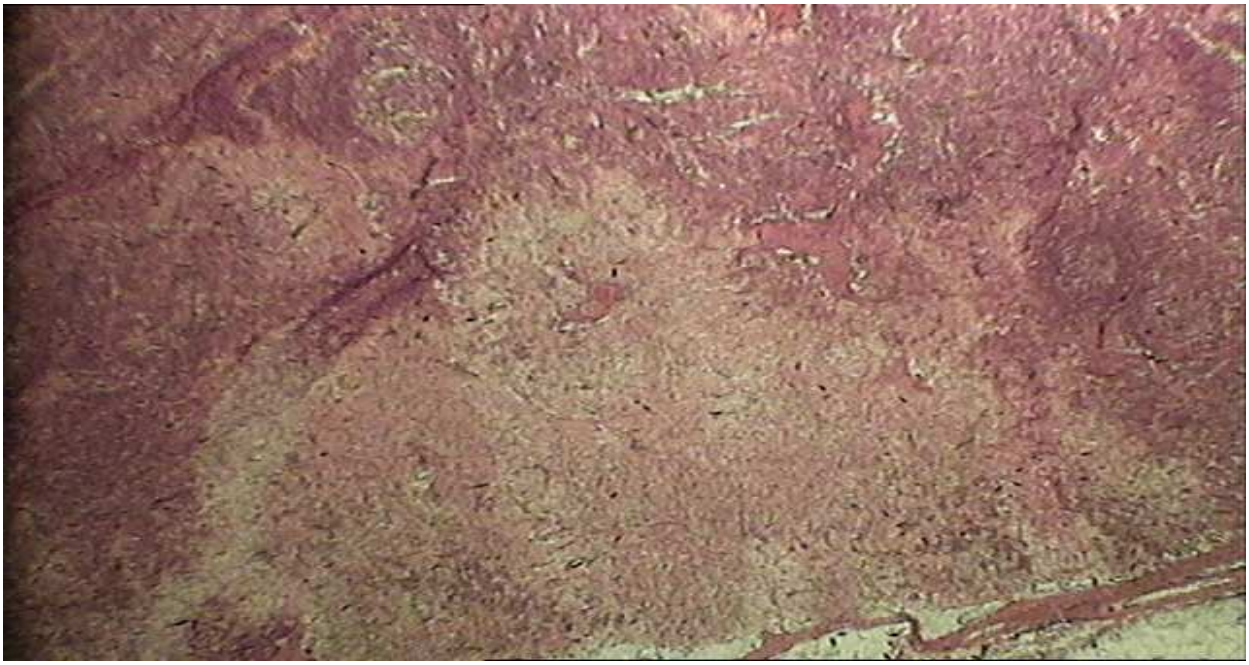

Fig.6(Internal iliac) necrosis of the cortical tissue 40

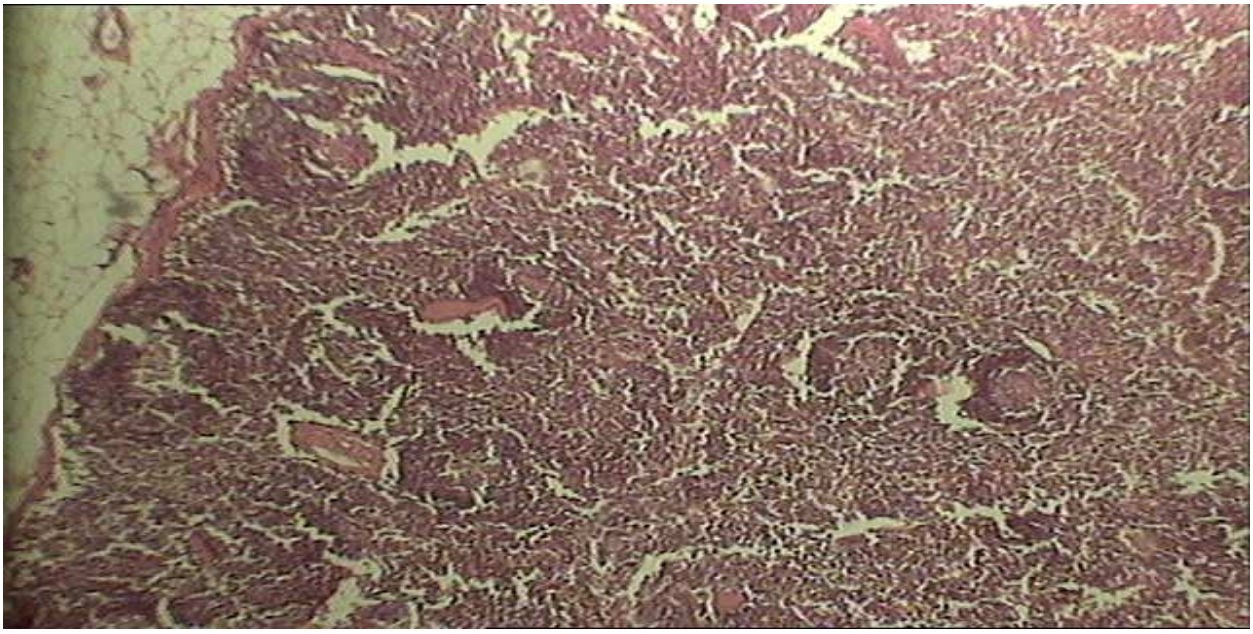

FIG7. (Liver) free space in the paranchyme1

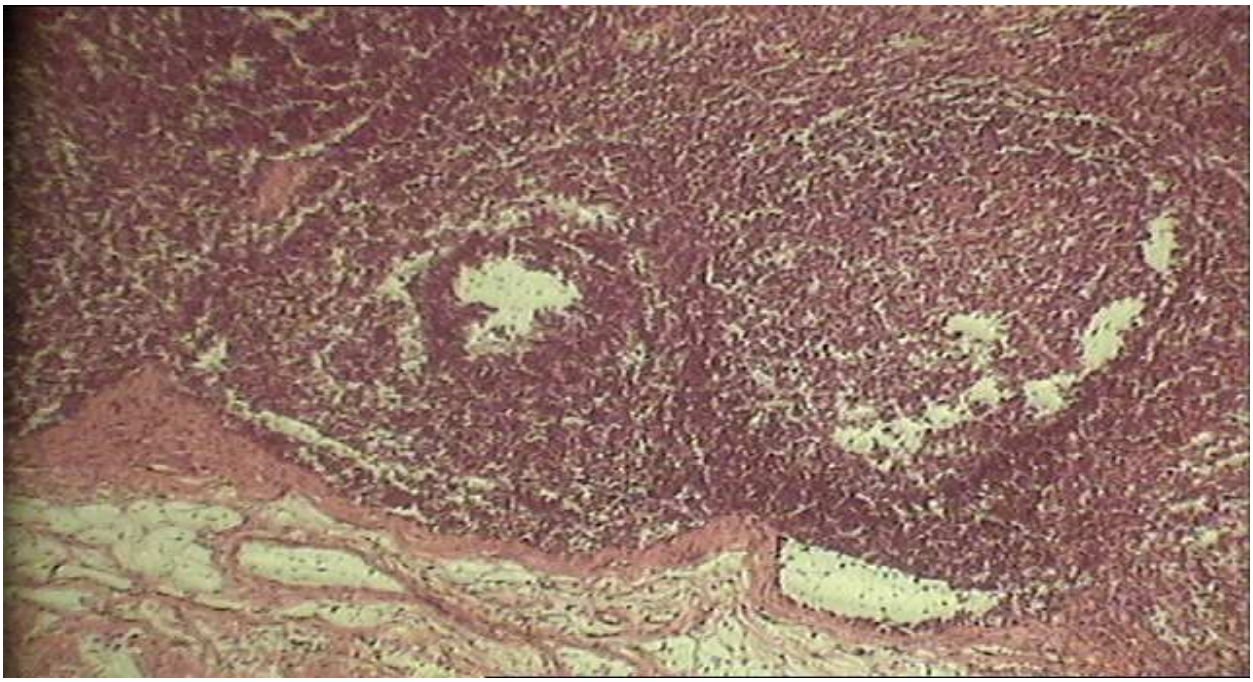

FIG.8 (mesenteric lymph node) decrease of cell population in the cortical 40follicles

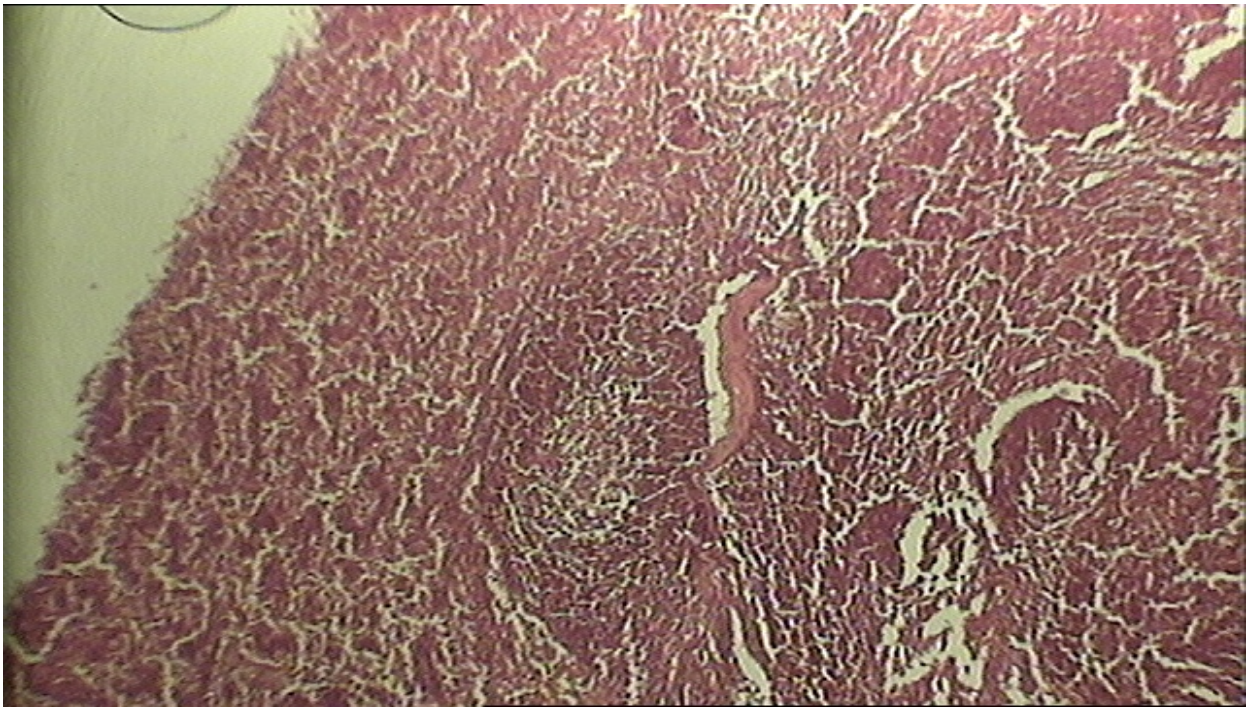

FIG.9. (mesenteric lymph node)deformation of lymphatic follicles

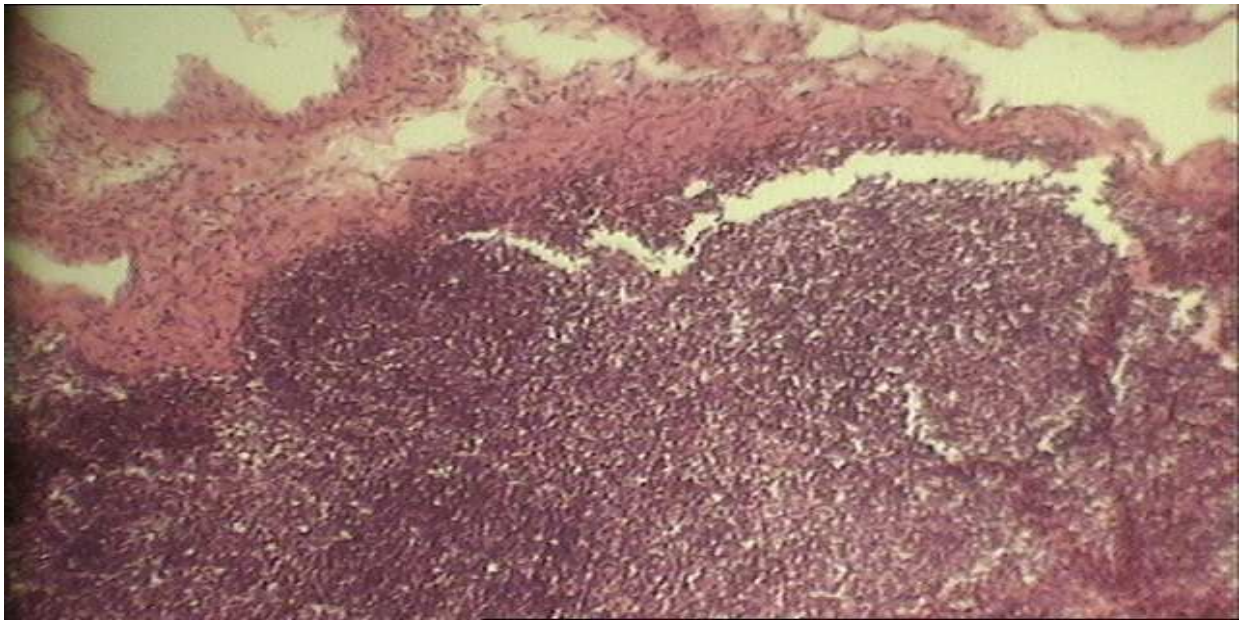

FIG.10.(popliteal Lymph node)increase capsular thickness

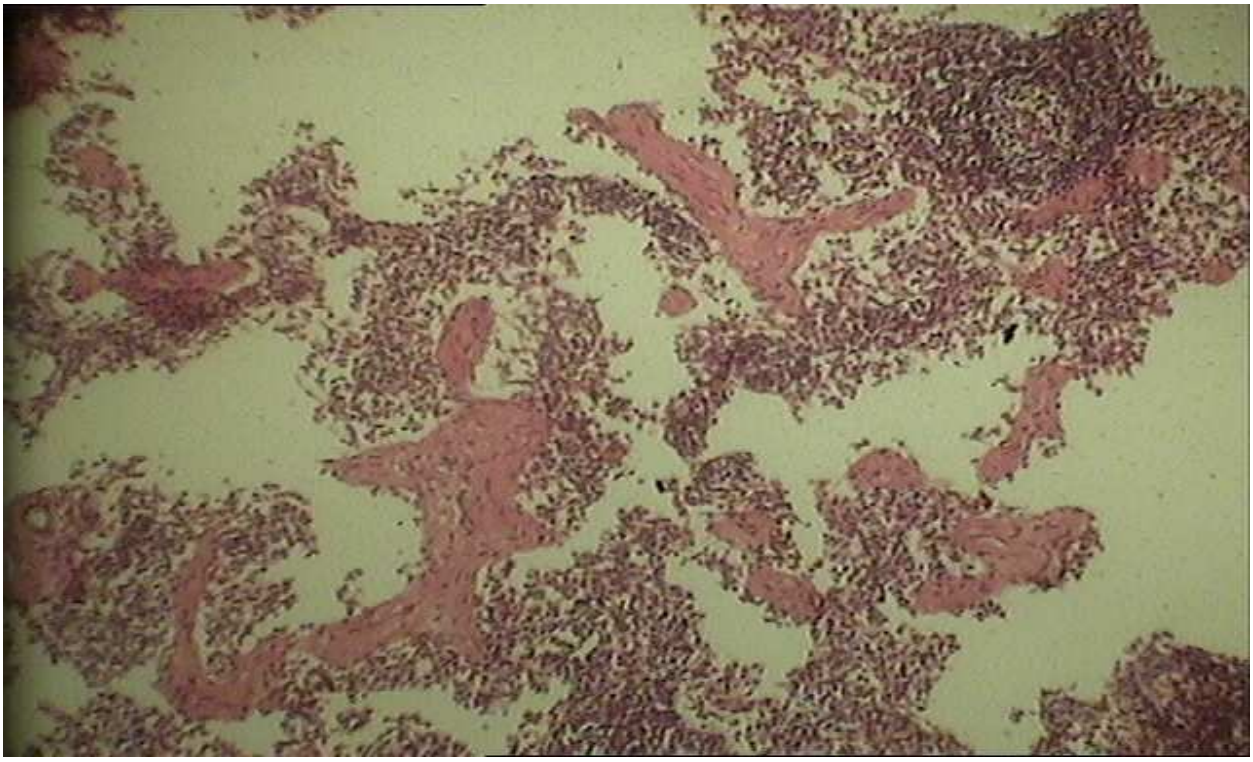

Fig.11 (scapular) free space in the paranchyme of gland

DISCUSSION

In the present study 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 sever congestion in arterioles and veinioles , thickness in the wall of arterioles due to presence of vacuole in all layers of arterioles had been observed. Oliveira-Sequeira and et al reported same observation of infected sheep with nematode [3].

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 [13]. 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 (14). 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 [15]. In present study ,liver section of sheep infected with nematode showed free space in the parenchyma and sever congestion of blood vessels per vascular cuffing of lymphocytes (minute granuloma) vascular degeneration and fibrosis in portal area associated with infiltration of mononuclear Inflammatory cells . Oliveira-Sequeira reported Vascular changes were limited to interstitial edema and, in some cases, to small focal points of hemorrhage in the sheep infected with nematode [3]. Cell mediated immunity mainly through lymphoid cells have been demonstrated in nematode infection [16].Lymphocytes and neutrophils are also involved in inflammatory response [16].

Nematodes common in this study were in abomasum and omasum are *Haemonchus contortus* (Barber pole worm) and Ostertagi ostertagi (Brown stomach worm). Nematodes common in the small intestine are *Haemonchus contortus* (Barber pole worm) *Trichostrongylus colubriformis*, (Bankrupt worm), *Bunostomum trigonocephalum*, *Tricostrongylus colubriformis*.The nematode common in the large intestine is *Oesophagostumum* spp (Nodular worm) and *Trichuris globulosa* .The nematode of particular concern worldwide especially in tropical and subtropical climatic regions is *H. contortus.* Presence of various species and numbers of helminthes in gastrointestinal tracts of sheep has been reported by many workers from different geographical regions and from various countries .Sudies on infected sheep with *Ostertagia ostertagi* shows significant reduction in plasma Total

free amino acide and total plasma protein and significant increase in alkoline phosphatase and acid phosphatase in infected animals[17] .

Comparison of the results for sheep parasites obtained in the present study to those in other surveys in Iran showed no marked differences.

In the present study, the infected animals showed signs of varying degree of anemia. Anemia in infected animals is accordance with the result of Reduction in RBC, Hb% and PCV indicate the loss of blood and consequently the anemia caused by infection [18]. Development of anemia is reported in sheep infected with Nematodes [19]. Lowering of MCH and MCHC also show the reduced formation of haemoglobin in infected animals. This may be due to reduce availability of proteins due to loss during infection. Stear and his coworkers reported a loss of proteins in to intestine in various nematode infections[20].TLC was found significantly elevated in infected animals and the increase was mainly due to greater count of lymphocytes, otherwise neutrophils count was noticeably reduced in infected compared to non-infected animals. The changes in TLC in the infected animals clearly show immunopathological response of the host. Ultimately, infection produces a relative protein deficiency with poor growth. The relative protein deficiency has four causes. Infected animals eat less. Ingested protein is digested less efficiently. There is a leakage of protein into the gastrointestinal tract and infection increases protein demand as protein is diverted into repair processes and immune and inflammatory responses [21].

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