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**Article** *in* Revue de médecine vétérinaire · August 2008

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# Paratuberculosis in sheep: an emerging disease in southern Iran

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**SUMMARY**

Based on gross and histopathologic lesions, paratuberculosis was diagnosed in sheep in Shiraz slaughter house, North of Iran. Ileocecal junction, ileum, jejunum, cecum, colon and associated mesenteric lymph nodes (MLNs) of 5000 carcasses were grossly inspected for wall thickness and/or corrugation of the intestine and enlargement of the associated lymph nodes. Of these, 171 animals showed intestinal thickening and/or corrugation and MLNs enlargement and were suspected to paratuberculosis. However, at histopathological level only six animals exhibited moderate to severe lepromatous or tuberculoid granulomatous enteritis in cecum, colon, jejunum and particularly in the terminal ileum, ileocecal junction and the related lymph nodes. High numbers of acid fast organisms were present in terminal ileum and related MLNs and to a lesser extent in cecum, colon and jejunum. The rectal content of five of them were watery and soft and one of them had normal intestinal contents. The wall of ileum and to a lesser extent cecum, jejunum and colon were thicker and corrugated and the MLNs of ileocecal valve, ileum, jejunum and cecum were larger than normal. The fat deposit surrounding the intestinal wall was either absent or had a jelly shape or liquid appearance. Five sheep showed diffused or lepromatous type granulomatous reactions in their intestine and MLNs with diffused infiltration of lymphocytes, plasma cells, macrophages and rarely giant cells in the lamina propria and submucosa of the intestines and MLNs. The last one had follicular lymphoid infiltrates, with multifocal granulomas in the areas adjacent to payer patches in the ileum and to a lesser extent jejunum and cecum. The ileocecal, jejunal and cecal MLNs of this animal had few focal granulomatous reaction containing acid fast bacilli in the interfollicular areas of cortex and paracortical areas.

In a second attempt normal looking samples of intestines and associated lymph nodes of 200 sheep were randomly selected and studied at histipathological level and non of the samples proved to be affected with paratuberculosis.

Therefore it could be concluded that Johne's disease might be present in adult sheep having suffered a chronic wasting disease, with or without diarrhea. Consequently, the intestinal tract and mesenteric lymph nodes of these sheep should always be examined at histological level. This is also true even when more evident problems such as caseous lymphadenitis or gastrointestinal parasitism are present.

**Keywords: Paratuberculosis, Johne's disease, sheep, histopathology, Iran.**

**RÉSUMÉ**

**Paratuberculose ovine : une maladie émergente dans le sud de l'Iran**

La paratuberculose des ovins a été diagnostiquée à l'abattoir de Shiraz (Nord de l'Iran) par l'aspect des lésions macroscopiques et microscopiques. La jonction iléo-cœcale, l'iléon, le jejunum , le cœcum, le colon et les nœuds lymphatiques mésentériques associés de 5000 carcasses ont été macroscopiquement inspectés quant à l'épaisseur, avec ou sans aspect encéphaloïde de la muqueuse de l'intestin et quant à l'augmentation de volume des nœuds lymphatiques associés. 171 animaux ont montré un intestin épaissi, d'aspect encéphaloïde, avec augmentation du volume des nœuds lymphatiques et furent ainsi suspects de paratuberculose. L'examen histopathologique n'a révélé que six animaux porteurs de lésions sévères ou modérées d'entérite lépromateuse ou granulomateuse tuberculoïde sur le cœcum, le colon, le jejunum et en particulier sur la portion terminale de l'iléon, la jonction iléo-cœcale et les nœuds lymphatiques. Un grand nombre de bactéries acido-résistantes est présent dans la portion terminale de l'iléon et sur une petite portion du cœcum, du colon et du jejunum. Le contenu du rectum de cinq de ces animaux était aqueux et mou et un animal présentait un contenu intestinal normal. La paroi de l'iléon et d'une petite portion du cœcum, du jejunum et du colon était épaissie et d'aspect encéphaloïde. Les nœuds lymphatiques de la valvule iléo-cœcale, de l'iléon, du jejunum et du cœcum étaient augmentés de volume. Les dépôts graisseux de l'intestin étaient absents ou remplacés par une substance d'aspect gélatineux ou liquide. Cinq ovins montrèrent des lésions diffuses, granulomateuses ou de type lépromateux dans leur intestin et les nœuds lymphatiques avec une infiltration diffuse par des lymphocytes, des plasmocytes, des macrophages et de rares cellules géantes dans la lamina propria et la sous-muqueuse. Le dernier animal présentait des infiltrats lymphoïdes folliculaires avec de multiples granulomes dans les zones voisines des plaques de Payer dans l'iléon et dans une petite portion du jejunum et du cœcum. Dans certains nœuds lymphatiques se trouvaient un petit nombre de réactions focales granulomateuses contenant des bacilles acido-résistants dans les zones interfolliculaires du cortex et dans les zones paracorticales.

Dans un second temps, les intestins et les nœuds lymphatiques d'aspect normal furent examinés histologiquement chez 200 ovins choisis au hasard, alors qu'ils ne semblaient pas atteints de paratuberculose.

Donc on peut conclure que la maladie de Johne peut être présente chez des moutons adultes présentant un amaigrissement avec ou sans diarrhée. En conséquence, le tractus intestinal et les nœuds lymphatiques de ces moutons doivent être examinés histologiquement. Il doit en être de même lorsque les animaux présentent une lymphadénite caséeuse ou un parasitisme intestinal.

**Mots-clés : Paratuberculose, maladie de Johne, ovins, histopathologie, Iran.**

## Introduction

Paratuberculosis or Johne's disease is a severe debilitating and chronic contagious infection with the acid-fast-staining bacillus *Mycobacterium Paratuberculosis* in sheep with the clinical signs of emaciation, cachexia and ultimately death. It is a major disease of cattle, sheep, goats, deer and camels[1]. Infection takes place soon after birth and neonatal and Juvenile animals are at the highest risk for acquiring an infection of *M. paratuberculosis* [8, 12]. However, clinical

signs may be noticed some years later; thus a flock with clinical cases may harbor many subclinical carriers that shed infective organisms in the feces and contaminate feed or water supplies with the causative organisms [8, 12]. Contamination of the environment by manure from the infected animals is the most common mode of transmission. *M. paratuberculosis* resists destruction in the environment and its ability to survive 250 days or longer in water, feces and cattle slurry makes prevention and control of Johne's disease more difficult [4, 47]. Young animals are most commonly infected through the fecal–oral route either by ingesting the organism through contaminated milk and colostrums [48] or food products or by accidental ingestion of the microorganism from contaminated surfaces [12, 3]. Intrauterine transmission to the fetus also occur [40, 43, 49, 51].

*M. paratuberculosis* targets the mucosa-associated lymphoid tissue of the host [32]. As the infective organisms enter the ileum, cecum, and colon they penetrate the epithelial membrane and enter the lamina propria. The organisms multiply slowly in the payer patches, mucosa, submucosa, lamina propria and mesenteric lymph nodes and may eventually be disseminated into the intestinal tract and shed in the feces [2, 18, 32, 45]. *M. paratuberculosis* bacilli are taken up by macrophages and with the passage of time cause severe granulomatous inflammation in the intestine and in the draining lymph nodes [11, 27]. Cytokine production and the initiation of a cellular immune response by the host cause the appearance of an intestinal granuloma and a cellular response is initiated in the nearby lymph nodes in an attempt to clear the infection [12, 29]. This inflammatory process leads to the clinical manifestations of a corrugated intestinal epithelium and the corresponding characteristic malnutrition syndrome associated with Johne's disease. Presence of the organism in this mesenchymal tissue provokes a series of changes. Initially, neutrophils accumulate around and among the bacteria. Later macrophages and lymphocytes assemble. Cellular proliferation and some fibroplasias eventually occur. The macrophages phagocytize the slowly proliferating bacteria and eventually enter the afferent lymph vessels and pass to the mesenteric lymph nodes, where they accumulate and cause increased cellular development. These changes slowly thicken the infected mucosa and enlarge the lymph nodes. In most natural infections it is presumed that the host response eliminates the organism, but a proportion of animals remain infected and, following a long subclinical phase, develop clinical disease in adulthood [11, 28].

Histopathologic features have been proposed as a good parameter of paratuberculosis diagnosis in sheep [9, 21, 22], being more sensitive than bacteriological, immunodiagnostic and molecular methods, if appropriate tissue specimen collection is performed [15, 20, 26, 35]. The best tissues for diagnosis are the ileocecal junction, terminal ileum and ileocecal and ileal mesenteric lymph nodes. It is stated that acid-fast smears of feces identified only 33 percent of clinically affected sheep and 57 percent of the sheep with histopathological lesions and the PCR test detected 64 percent of the sheep having gross and histopathologic lesions [13]. Although bacteriological culture of feces or intestinal tissue has been commonly used to evaluate serologic test results in cattle [28] this approach does not seem reliable in small ruminants, because the causative organism can be difficult to isolate [25, 44].

Two distinct forms of intestinal pathology have been recognized in clinical cases and this indicates that different immunopathological mechanisms may play significant roles in the development of inflammatory lesions [6, 9, 16]. In the more common multibacillary or "lepromatous" form, sheets of large macrophages, each containing numerous acid-fast organisms, infiltrate into the mucosa and submucosa of the intestine. In the tuberculoid form the cellular infiltrate is predominantly lymphocytic, and macrophages within scattered small granulomata containing very few organisms. The immune response is thought to play an important role in determining the type of histopathological response in mycobacterial infections [52]. In ovine paratuberculosis, "tuberculoid" type is associated with a strong peripheral cellular immune response [37], whereas "lepromatous" form is associated with a marked humoral immune response [10].

In Iran, paratuberculosis is widely prevalent in cattle, but there are very few reports of paratuberculosis occurrence in sheep which are often reared side by side with cattle [33]. Convincing studies comprising histologic evaluation and/or confirmation of the infection by bacterial culture or bacterial genome detection have not been undertaken in sheep despite their economic importance. Therefore this work was undertaken firstly to confirm the presence of the paratuberculosis in sheep of this area and secondly to study the prevalence of paratuberculosis based on gross and histopathological findings in sheep of Shiraz area, Fars Province, South of Iran.

# Materials and Methods

Five thousand sheep of different ages brought for slaughter at Fars Slaughter house, Marvdasht city, Fars Province, Iran, in a six months time period, were examined for visible abnormalities in the ileocecal junctions, ileum, jejunum, cecum, colon and the associated MLNs. Sheep brought to the slaughterhouse generally belonged to small sheep units in the villages of Marvdasht and Shiraz cities where they grazed freely in local pastures. Tissue samples were collected from 171 animals showing any gross abnormalities such as thickening and/or corrugation of the ileum, ileocecal junction, jejunum and cecum or enlargement of the associated ileocecal and MLNs. Similar samples from another 200 grossly normal looking intestines and associated lymph nodes were also processed for histopathological studies. Three sections of approximately 2 cm pieces of the ileocecal junction, ileum, jejunum, cecum and the associated MLN were collected. The excess fat and mesenteric attachments were trimmed off, and the intestinal contents were flushed out for examination of intestinal mucosa. The samples were then preserved in 10% neutral buffered formalin, dehydrated through graded ethanols and embedded in paraffin blocks. Sections of 5 μm in thickness were cut and routinely stained with hematoxylin and eosin and an acid-fast Ziehl-Neelsen stain.

# Results

## LESIONS AT MACROSCOPIC LEVEL

As is shown in table 1 from the 5000 inspected gastrointestinal tracts, the intestinal wall of the ileocecal junction and/or ileum, jejunum and cecum of 171 carcasses were thicker

or corrugated and the associated MLNs of 115 sheep (67.3%) showed moderate to severe enlargement. The rectal contents of sixty six (38.6%) animals were soft or watery and dysenteric. The wall of ileum and jejunum of all suspected carcasses (100%) showed mild to severe thickness then followed by ileocecal junction (90.64%), cecum (8.77%) and colon (3.51%).

## MICROSCOPIC LESIONS

At histopathological level, from the 171 suspected animals, 36 sheep (21.05%) were infected with *Ornithobilharzia tukistanicum* and the parasite were seen in the mesenteric blood vessels, lymphatics and associated lymph nodes of duodenum, jejunum, ileum and cecum of the infected animals. Eggs of this parasite were also observed in the mesenteric lymph nodes of the small intestine. The ileocecal and mesenteric lymph nodes of some of these animals were also infected with larva of *Linguatula serrata*.

From the 171 suspected animals only six sheep were infected with paratuberculosis. Five of the infected animals had watery to pasty or soft rectal contents and the last one had normal feces. The ileum and ileocecal junction and the associated lymph nodes were the most frequently and most severely affected sites; then followed by cecum, colon, jejunum and the related lymph nodes. As is shown in table 2, two animals (cases numbers 1 and 6) showed severe thickness and corrugation of the ileum and the mucosa was formed into transverse ridges that could not be reduced by stretching. Four animals (cases numbers 2, 3, 4 and 5) showed mild thickness of the ileum. In all cases lesions extended with milder changes into the cecum, colon and jejunum. The MLNs of ileal, ileocecal-junction and jejunal areas of all infected animals were markedly enlarged, edematous and congested. On the serosal surface of the thickened intestines the lymph vessels were often prominent, dilated, firm, corded, tortuous and ran an irregular course towards the mesenteric border. The fat deposit of the mesentery was reduced or it was watery and showed serous atrophy of fat.

The lesions of the intestine and mesenteric lymph nodes of five sheep were of lepromatous granulomatous type. Two sheep of this category (cases numbers 3 and 5) showed mild changes. In these cases few epitheloid cells were usually infiltrated in the tips of villi, accompanied by heavy infiltration of the lamina propria with lymphocytes and variable numbers of eosinophils. Mild villous atrophy without involvement of submucosal and serosal areas was evident in these animals. As is shown in Table 3 numerous acid-fast organisms packed the cytoplasm of the large macrophages that infiltrated the mucosa of the ileocecal junction of case number 3. Ileum, jejunum and cecum of this animal showed moderate contamination with these acid-fast bacteria. In case number 5 the acid-fast organisms were only mildly present in the macrophages that infiltrated in the lymph nodes of ileo-cecal junction and other inspected organs were negative for *M. paratuberculosis*. Three sheep (cases numbers 1, 2 and 6) showed more severe changes in the terminal ileum and ileocecal junction. The mucosa and lamina propria were heavily infiltrated with epitheloid cells resulting in severe villous atrophy with bridging of villi and obliteration of the crypts of Lieberkuhn (Fig. 1) and distension of some of the remaining crypts with cellular debris and/or edematous fluid. The submucosa was edematous and loosely infiltrated with lymphocytes,

| Macroscopic lesions | No of animals | Percentage |
|---|---|---|
| **thickening of the jejunum** | | |
| a. mild thickening | 7 | 4.09 |
| b. moderate thickening | 152 | 88.89 |
| c. severe thickening | 12 | 7.02 |
| **thichening of the ileum** | | |
| a. mild thickening | 8 | 4.68 |
| b. moderate thickening | 141 | 82.46 |
| c. severe thickening | 22 | 12.87 |
| **thickening of the ileocecal junction** | 155 | 90.64 |
| **thickening of the cecum** | 15 | 8.77 |
| **thickening of the colon** | 6 | 3.51 |
| **lymphadenomegally of the jejunum LNS** | | |
| a. moderate | 88 | 51.46 |
| b. severe | 27 | 15.79 |
| **lymphadenomegally of ileocecal LNS** | | |
| a. moderate | 83 | 48.54 |
| b. sever | 25 | 14.62 |
| **Rectal content** | | |
| a. soft (pasty) | 40 | 23.39 |
| b. watery | 26 | 15.20 |

TABLE I : Macroscopic changes of the intestines of the suspected sheep to paratuberculosis (n=171).

| Macroscopic changes | No of animals | Percentage |
|---|---|---|
| **1-Thickening of the ileum** | | |
| a- moderate thickening | 4 | 66.67 |
| b- sever thickening | 2 | 33.33 |
| **2- Thickening of the jejunum** | | |
| a- moderate thickening | 6 | 100 |
| b- severe thickening | - | - |
| **3- Severe thickening of ileocecal junction** | 6 | 100 |
| **4- lymphadenomegaly of the jujenum LNS** | 6 | 100 |
| **5- lymphadenomegally of the ileocecal LNS** | 6 | 100 |

TABLE II : Macroscopic changes of the infected sheep (n=6).

| Case number | Jejunum | Ileum | Ileocecal junction | Cecum | Lymph node | |
|---|---|---|---|---|---|---|
| | | | | | Jejunum | Ieoceeal junction |
| Case 1 | ++ | ++ | +++ | ++ | + | + |
| Case 2 | ++ | ++ | +++ | ++ | - | - |
| Case 3 | ++ | ++ | +++ | ++ | - | + |
| Case 4 | + | ++ | ++ | - | + | + |
| Case 5 | - | - | - | - | - | + |
| Case 6 | +++ | +++ | +++ | ++ | + | + |

- no acid-fast organism was found
+ few acid-fast organisms were present in the macrophages
++ acid-fast organism were present moderately in the macrophages
+++ many acid-fast organism were present in the macrophages

TABLE III : Presence of the acid fast organisms in different portions of the intestines and associated lymph nodes (n=6).

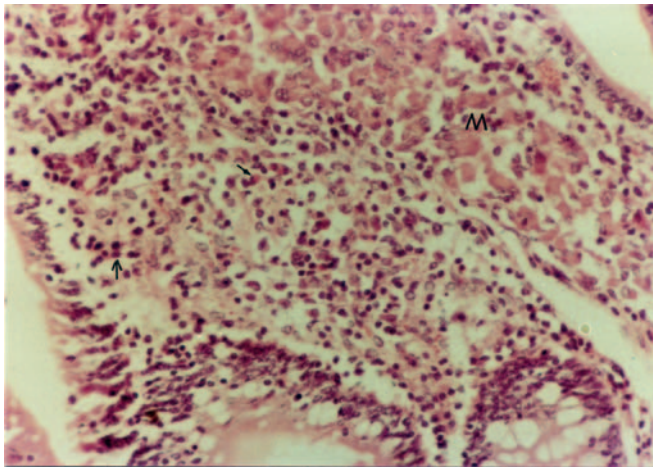

FIGURE 1 : Terminal ileum of sheep with lepromatous paratuberculosis. Atrophic villi, with sloughed lining and disappearance of the Lieberkuhn glands. lymphocytes and eosinophils are diffusely infiltrated in the submucosa and lamina propria. Numerous large epitheloid cells with foamy or vacuolated cytoplasm are infiltrated in the lamina propria (H. and E, X320).

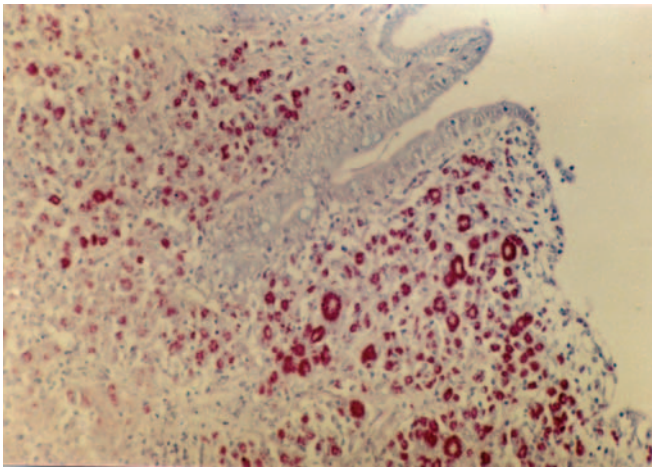

FIGURE 2 : Terminal ileum from sheep with paratuberculosis. The epitheloid cells in the submucosa and lamina propria contain large numbers of acid-fast bacilli (ZN, X205).

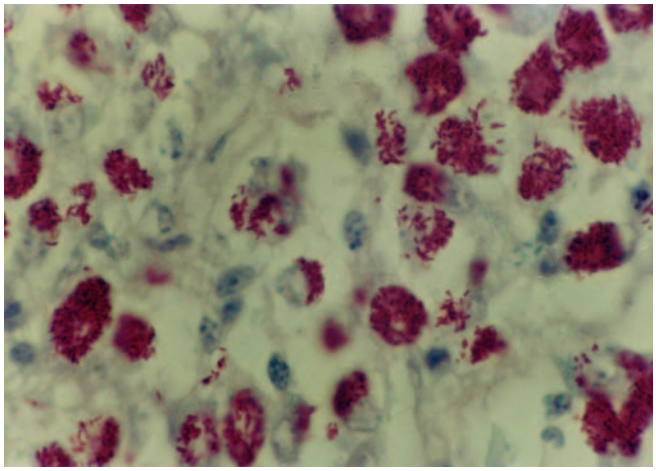

FIGURE 3 : Submucos of the ileum from sheep with paratuberculosis. The epitheloid macrophages contain large numbers of acid-fast bacilli (ZN, X1280).

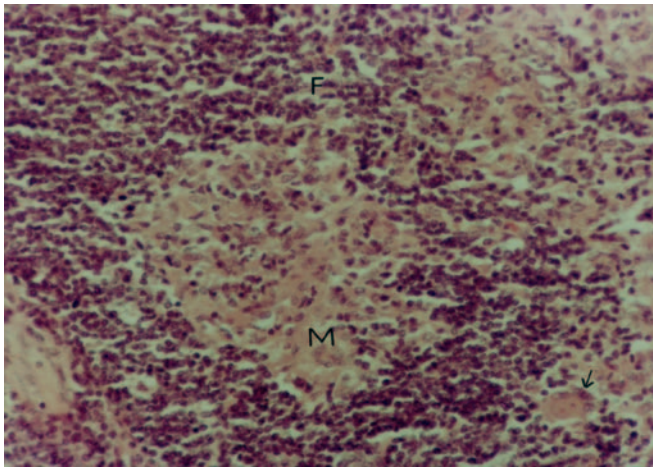


FIGURE 4 : Paratuberculosis in sheep. Various size epitheloid macrophage microgranuloma (M) in the cortical zone of mesenteric lymph node. A langhans giant cell (arrow) is present in bottom right corner (H and E, X320).

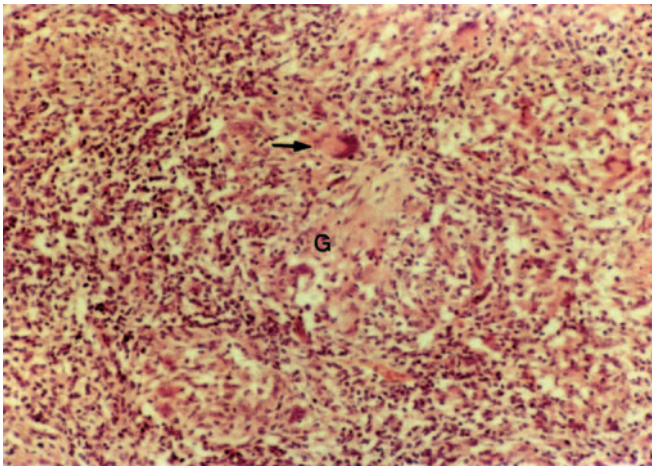


FIGURE 5 : Paratuberculosis in sheep. Confluent aggregate of epitheloid macrophages and Langhans' giant cells replaced the cortical areas of the mesenteric lymph node (H and E, X205).

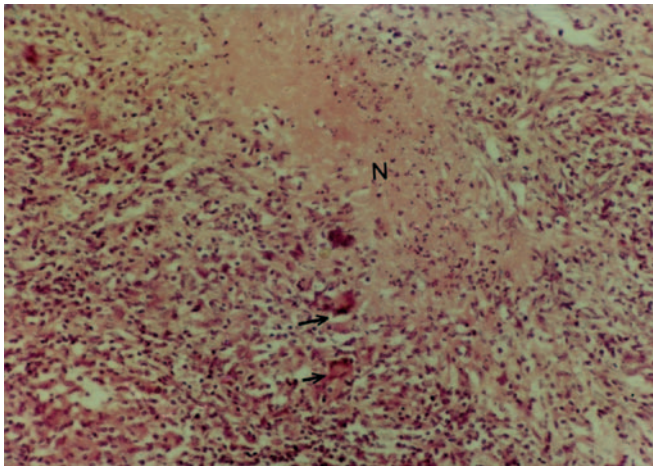


FIGURE 6 : Paratuberculosis in sheep. A caseous necrosis foci (N) is present in this section. Giant cells (arrow) and macrophages are infiltrated in the cortical areas of the mesenteric lymph node (H and E, X205).

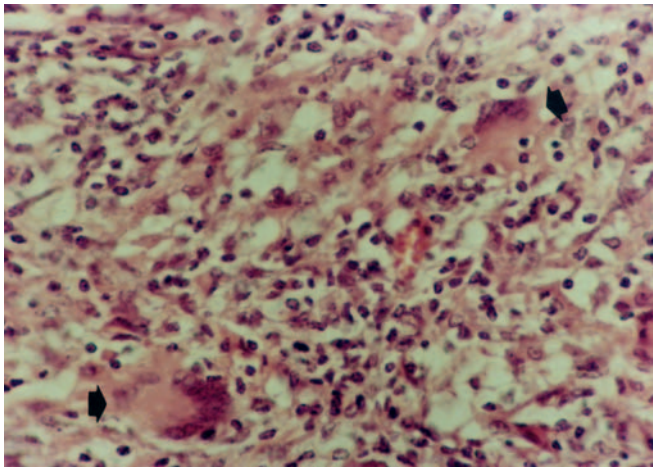

FIGURE 7 : Paratuberculosis in sheep. Langhans giant cells are shown with thick arrows. The epitheloid cells are infiltrated in thwe cortical regions of the ileocecal lymph node. A capillary showing hyperemia is seen in the central area of the microphotograph (H and E, X512).

plasma cells, aggregates of macrophages and eosinophils. Acid-fast organisms were heavily present in the cytoplasm of the epitheloid macrophages (Figs. 2 and 3). The mesenteric lymph nodes of ileum, cecum and ileocecal lymph nodes showed lymphadenomegally and the cut surface of these nodes were edematous and there was no distinction between the cortex and the medulla. The histopathological changes included sinus histiocytosis with infiltration of epitheloid cells in all three cases (Fig. 4) and presence of acid-fast organisms in the cytoplasm of the macrophages of two of these animals and presence of multinucleated giant cells in one of the cases. These epitheloid cells had abundant, pale eosinophilic cytoplasms and formed extensive diffuse sheets. Multinucleated, Langhans-type giant cells were seen in one of these cases.

Lesions of the mesenteric lymph nodes of one of the sheep (case number 4) comprised of caseous necrosis (Fig. 5) and tuberculoid type of granulomatous reaction with fewer epitheloid cells and marked lymphocyte infiltration in the intestinal mucosa and the associated lymph nodes. Few giant cells

were infiltrated around these lesions (Figs. 6 and 7). The prominent granulomatous changes consisting of multiple granulomas and multinucleated giant cells were observed in the mesenteric lymph nodes of the terminal ileum and ileo-cecal junction. This animal showed follicular lymphoid infiltration with multifocal granulomas in the areas adjacent to the payer patches in the ileum and to a lesser extent jejunum and cecum. In this tuberculoid form the intestinal wall thickening, lymph node enlargement and lymphangitis was less prominent compared to those of the lepromatous form. The lesions of jejunum, cecum and colon of this sheep were segmental with thickened portion of small intestine being separated by normal intestine. This case showed lower numbers of acid-fast organisms in macrophages compared to those of the lepromatous form.

The intestine and associated lymph nodes of all 200 normal looking intestines were free of paratuberculosis. However, 44 sheep (22%) of this group showed infection with *Ornithobilharzia tukistanicum* and the parasite was seen in their mesenteric lymphatics, lymph nodes or blood vessels. In these animals the eosinophils were infiltrated in the mucosa, submucosa and lamina propria of the intestine and rarely in the cortical portion of the associated lymph nodes.

# Discussion

The present study describes the pathology of paratuberculosis in sheep and conclusively confirms the infection in these animals. The disease was confirmed in six sheep (0.12%) slaughtered at Shiraz Slaughterhouse. Because the aim of the present study was to diagnose existence or absence of clinically or subclinically infected paratuberculosis in sheep of this area and to explain the gross and histopathologic lesions of the infected animals, only morphologic study of the small and large intestine of the slaughtered animals was performed. Because fecal shedding and touch smear of the rectal area of the living animals is not consistent feature of subclinical infection and fecal culture or rectal smears examination result in a large number of false negative results and are less productive, these tests were not taken into consideration in the present study. However, now that the disease is confirmed in this region, performing fecal sample and touch smear tests are more feasible and economic to do the differential diagnosis of the clinically paratuberculosis infected animals with other clinically similar diseases. Most of the diseased animals present symptoms and may be detected more easily by fecal sample or rectal touch smears than by histological examination that is not so easy to perform.

Histopathologic features have been proposed as a good parameter for diagnosis of paratuberculosis infection in sheep, being more sensitive than bacteriologic culture, immunodiagnostic and molecular methods if appropriate tissue specimen collection is performed [10, 25, 36, 37]. Quantitative assay of *M. paratuberculosis* was not performed in the affected sheep, however, because of the extensive intestinal lesions and massive numbers of acid-fast bacteria demonstrated by histology there is little doubt that the affected animals were passing large numbers of bacteria in the feces. While clinical cases may be detected with reasonable accuracy, subclinically affected animals are not so readily identified. Because fecal shedding is not a consistent feature of subclinical infection, fecal culture or smear examination result in a large number of false negative tests. Most of the serological tests used to detect antibodies to *M. paratuberculosis* are not satisfactory in detecting infected animals at all stages of the disease

Two different forms of the disease were seen at histopathological level: a lepromatous form accompanied by lesions consisting of epitheloid cells full of large numbers of mycobacteria and a tuberculoid form with lesions characterized by small granuloma with few bacilli. Absence of acid-fast organisms in all tested tissues except the lymph nodes of ileocecal junction of case number 5 may reflect sparsity of organisms and is probably a reason that this case is subclinically infected, because absence of acid-fast bacteria in most intestinal organs and associated lymph nodes has already been observed in subclinical paratuberculosis [5, 11, 46]. Prevalence of subclinical infection, however, cannot mitigate the importance of sheep in the epidemiology of paratuberculosis infection. It is likely that the prevalence of the paratuberculosis is more than the observed cases of this project and further work is needed to define the prevalence of the disease throughout the district. The effect on overall flock's production and economic importance of Johne's disease also needs to be determined.

The gross changes might easily be overlooked and the emaciation attributed to another cause. Many other etiologies such as parasites may end to similar lesions; so that many of the suspected animals of our experiment were affected by parasitic infection such as *Ornithobilharzia turkistanicum* and/or *Linguatula serrata*. The submucosa and lamina propria of the intestines of most of the sheep were infiltrated with eosinophils. However, the parasites were not present in the inspected tissues of some of these sheep but eosinophilic infiltration could be due to parasitic infection. Because minor lesions could easily be missed and many affected sheep die of concurrent disease it would be advisable routinely to search the terminal ileum, ileocecal junction and the associated lymph nodes microscopically for acid-fast organisms by smear or histopathology. This is usually a post mortem procedure although biopsy techniques have also been advocated.

Similar to previous studies, infiltration of few epitheloid cells among numerous lymphocytes in cases 3 and 5 marked the lesions in preclinical stage of paratuberculosis [9, 35]. Previous studies on experimental and natural cases of paratuberculosis in sheep and goat have indicated preponderance of lymphocytes to epitheloid macrophages and giant cells in the early paucibacillary cases, in which bacteria could not generally be demonstrated [9, 34, 35, 50]. Diffuse lymphocytic infiltration has been reported in the early stages of other mycobacterial infections and correlated with strong cell mediated immune response mediated by different subpopulation of lymphocytes such as helper, cytotoxic and suppressor cells [17, 46].

While mineralization was not seen, caseous necrosis was observed in areas of dense cellular infiltration of ileal and ileocecal lymph nodes of one of the cases that showed tuberculoid granulomatous type of lesions. It is not clear that necrosis is an outcome of the parasitic infection such as *Ornithobilharzia tukistanicum* and *Linguatula serrata* that are

common in this area [42] or as it is stated, *M. paratuberculosis* is the causative agent of necrosis in its susceptible hosts [30].

Domestic animals can be infected with *M. paratuberculosis* and disseminate large numbers of organisms by the feces to the environment for months prior to developing clinical signs. The persistence and pathogenecity of *M. paratuberculosis* in poor pasteurs ensure that the incidence of Johne's disease will continue to increase in this area. Of greater concern is the ability of infected animals to infect other domestic and wild ruminants with *M. paratuberculosis*. It is showed that an *M. paratuberculosis* strain originally isolated from a bighorn sheep was able to infect other species of wild and domestic ruminants, implicating wild animal populations as a natural reservoir for this pathogen [7, 51].

The soil and pastures of this area are light in texture, deficient in trace elements and have a low cationic exchange capacity. They are inherently low in calcium, magnesium, molybdenum, selenium and copper and require considerable inputs of chemical fertilizers to improve pastures growth. There is strong evidence that soil alkalinity, excess of Iron, and molybdenum and deficiencies of copper and selenium have a role in the diseae process [23, 23, 31, 37, 38]. It is reported that an increase in soil pH of 0.1 was associated with a 5% decrease in the number of ELISA test-positive cattle, and that the application of lime to pastures to reduce soil pH was associated with a herd being only 10% as likely to be paratuberculosis positive and to have a 72% reduction in the number of seropositive cattle as farms that did not apply lime [24]. For every 1 ppm increase in soil iron content, a 1.4% increase in the risk of a herd being paratuberculosis positive is estimated [31, 39]. Following tests on samples of blood, serum, faeces, drinking water and pasture, it is concluded that a deficiency of selenium and primary or induced copper deficiency may predispose animals to paratuberculosis [38].

Because treatment of the disease is ineffective and control and eradication are difficult, the most important management control practices such as overall cleanliness of the farm, manure handling, newborn care, restricting of contact between lambs and mature sheep, herd testing and removal of infected animals [14, 19, 41] are not taken to consideration. Johne's disease in sheep will provide a challenge to veterinarians. We tend to think of paratuberculosis in our cattle industry and ignore other livestock categories. Paratuberculosis is not a disease limited to cattle; it is also a serious problem in sheep, goats and wildlife as well as the exotic animals.

Rather, this species may be more important in the propagation of infection in the environment and yet be escaping detection, possibly because of harboring sub clinical infection and being an intermittent or non fecal shedder. From these findings it could be concluded that the diagnostic pathologists should not forget that Johne's disease might be present in adult sheep having suffered a chronic wasting disease, with or without diarrhea. Consequently, the intestinal tract and mesenteric lymph nodes of these sheep should always be examined histologically, even in the absence of gross lesions suggesting the disease. This is also true even when more evident problems such as caseous lymphadenitis or gastrointestinal parasitism are present

## Acknowledgements

The authors wish to thank Mr. B. Shourangiz and Mr. M. Golrizkhatami for their technical assistance. We are also indebted to the Fars Slaughter house authorities for their helpful cooperation.

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