Atrophy of Intestinal Epithelial Cells Subsequent Bovine Johne's disease: A Histopathological Study

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Abstract: Johne's disease is chronic enteritis of ruminants caused by the aerobic bacterium Mycobacterium johnei (syn. M. paratuberculosis). The disease is widely distributed and causes substantial economic losses through death and loss of productivity during the prolonged preclinical stage. The purpose of this study was to determine whether M. avium subsp. paratuberculosis infection causes atrophy in bovine intestinal epithelial cell atrophy or not. Samples of ileum or ileocaecal tissue from 10 cows were used. Tissues were fixed in 10% formalin solution then were transferred to pathology laboratory of Islamic Azad University. Compatible lesions and acid fast bacilli were previously confirmed by hematoxylin and eosin and ZN staining performed following routine techniques. This effect appeared to require viable bacilli, and exhibit a significant increase atrophic change in epithelial intestinal cells.
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1. Introduction

Mycobacterium avium subspecies *paratuberculosis* (*M. paratuberculosis*) is an organism first observed by Johne and Frothingham in 1895. *Mycobacterium avium* subsp. *paratuberculosis* causes paratuberculosis or Johne's disease, an intestinal granulomatous infection. First recognised in cattle, then in sheep and later in goats, paratuberculosis is found most often among domestic and wild ruminants and has a global distribution. The disease has also been reported in horses, pigs, deer and alpaca, and recently in rabbits, stoat, fox and weasel (Chiodini et al., 1984; Stabel and Stabel, 1995). Under natural conditions, the disease in cattle spreads by ingestion of *M. paratuberculosis* from the contaminated environment. The disease persists after the introduction of infected animals. Infection can be spread vertically to the fetus (Stevens and Czuprynski, 1996) and semen can be infected with the organism (Sung and Collins, 1998). The primary source of infection in calves is milk from infected cows or milk that is contaminated with the faeces of diseased cattle.

The identification of *M. paratuberculosis* is based on its mycobactin requirement and its pathogenicity in the host. Mycobactin dependence has long been used as a taxonomic characteristic for *M. paratuberculosis* because most mycobacteria are able to make mycobactin for themselves. *Mycobacterium avium* subsp. *paratuberculosis, M. silvaticum* and some primary isolates of *M. avium* lack this capacity, however, and require mycobactin to grow in the laboratory. Thus, the mycobactin requirement is not confined to *M. paratuberculosis;* this characteristic exists to various degrees within the *M. avium* group. Clinical signs of paratuberculosis are a slowly progressive wasting and diarrhoea, which is intermittent at first, becoming progressively more severe until it is constantly present in bovines (Stabel, 1995; Stabel and Goff, 1996). Diarrhoea is less common in small ruminants. Early lesions occur in the walls of the small intestine and the draining mesenteric lymph nodes, and infection is confined to these sites at this stage. As the disease progresses, gross lesions occur in the ileum, jejunum, terminal small intestine, caecum and colon, and in the mesenteric lymph nodes. *Mycobacterium avium* subsp. *paratuberculosis* is present in the lesions and, terminally, throughout the body. The intestinal lesions are responsible for a protein leak and a protein malabsorption syndrome, which lead to muscular wasting (Lambrecht et al., 1988; Laochumroonvorapong et al., 1996; Minshall et al., 1995; Sung and Collins, 1998). Clinical signs usually first appear in young adulthood, but the disease can occur in animals at any age over 1–2 years. Within a few weeks of infection, a phase of multiplication of *M. paratuberculosis* begins in the walls of the small intestine. Depending on the resistance of the individual, this infection is eliminated or the animal remains infected as a healthy carrier. The proportion of animals in these categories is unknown. A later

phase of multiplication of the organisms in a proportion of carriers leads to the extension of lesions, interference with gut metabolism and clinical signs of disease. Subclinical carriers excrete variable numbers of *M. paratuberculosis* in the faeces. In most cases larger numbers of organisms are excreted as clinical disease develops. Delayed-type hypersensitivity (DTH) is detectable early in the infection and remains present in a proportion of the subclinically infected carriers, but as the disease progresses, DTH wanes and may be absent in clinical cases. Serum antibodies are detectable later than DTH. They may also be present in carriers that have recovered from infection. Serum antibodies are present more constantly and are of higher titre as lesions become more extensive, reflecting the amount of antigen present. In sheep, there may be a serological response that is more likely to be detected in multibacillary than in the paucibacillary form of the disease. Other mycobacterial diseases and infections, including mammalian and avian tuberculosis, cause DTH and emerging of serum antibodies. It follows therefore that these diseases need to be differentiated fromparatuberculosis, both clinically and by the use of specific diagnostic tests. Exposure to environmental saprophytic mycobacteria may also sensitise livestock, resulting in nonspecific DTH reactions.Animals vaccinated against paratuberculosis develop both DTH and serum antibodies. Vaccination is an aid to theprevention of clinical disease, but does not necessarily prevent infection. It also interferes with programmes for thediagnosis and control of bovine tuberculosis. Thus, if it is necessary to attempt a diagnosis of infection invaccinates, only tests to detect *M. paratuberculosis* in the faeces can be used (Sung and Collins, 1998). In individual animals, especially from a farm in which the disease has not previously been diagnosed, a tentativeclinical diagnosis must be confirmed by laboratory tests. However, a definitive diagnosis may be warranted on clinical grounds alone if the clinical signs are typical and the disease is known to be present in the herd. Confirmation of paratuberculosis depends on the finding of either gross lesions with the demonstration of typicalacid-fast organisms in impression smears or microscopic pathognomonic lesions and the isolation in culture of *M.paratuberculosis.* The aim of this study was to detect intestinal epithelial cell atrophy in bovine Johne's disease.

2. Material and Methods

2.1. Analyzed Samples

Samples of ileum or ileocaecal tissue from 10 cows were used. Tissues were fixed in 10% formalin solution and embedded in paraffin following the standard histological procedures. Compatible lesions and acid fast bacilli were previously confirmed by hematoxylin and eosin and ZN staining performed following routine techniques.

3. Results

3.1. Slides Interpretation

After obtaining microscopic section from ileum tissue of Johne's disease infected cases, they were examined in the regard of atrophic cell existence. For this purpose, the numbers of atrophic cells were counted in 5 microscopic fields with ×40 magnification rates. Also, statistical analysis is provided in table and diagram with regard to Mean ± SD which have been obtained by T-test for treatment and control groups, respectively. These data demonstrate that always there is significant difference ($P<0.005$) in atrophic cells numbers between treatment and control groups.

Table 1: number of atrophic cells of ileum in healthy and ill animals

Animals	Mean±SD	P-value
Disease	18 ± 1.79	<0.005
Normal	1 ± 0.56	

3.2. Histopathologic findings

The histological changes in bovine ileum tissue found in Johne's disease are often indistinguishable from those caused by *M avium* and often similar to those caused by *M bovis.* Cases 1, 2, 3 and 4 corresponded to animals with severe granulomatous enteritis that showed marked lesions consisting of necrotic and atrophic epithelial cells many macrophages and giant cells spread throughout the mucosa, submucosa, muscle tunic and serosa. Macrophages, with foamy cytoplasm and also epithelioid cells, formed a diffuse infiltrate in the intestinal wall, producing severe thickening of the mucosa, with glands widely separated due to the infiltration. Often, fused granulomas were seen mainly in the villi bodies. Lymphocytes and Langhans giant cells were commonly seen in the epithelioid infiltrate. In most of the sections, intestinal glands were dilated and filled with necrotic debris. The submucosa was severely affected; an infiltrate formed almost exclusively of macrophages with some giant cells was present with edema and thrombus formation. Multifocal granulomas with lymphoid follicles were located in the interfollicular zone. Mononuclear cells infiltrated the muscular layer. The serosa was also affected by the presence of multifocal

granulomatous infiltrates. Lesions were found in the ileocaecal valve in all cases (figs 1 and 2).

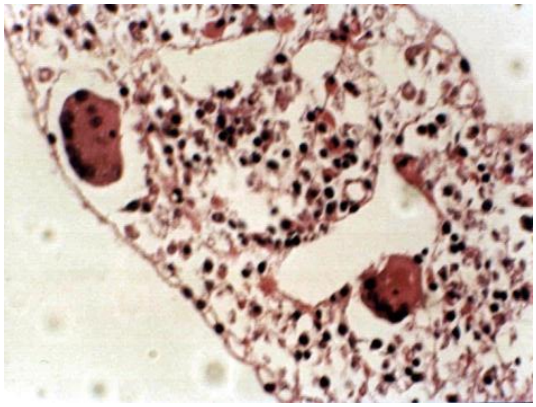

Fig 1: photomicrograph of bovine intestine tissue, showing granulomatous inflammation and intensive atrophic changes on epithelial cells. H&E, 20X.

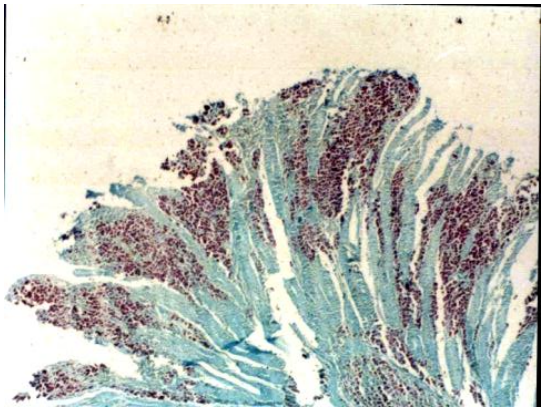

Fig 2: photomicrograph of bovine intestine tissue, showing leprematous inflammation and intensive atrophic changes on villi epithelial cells. ZN, 10X.

4. Discussion

The primary site targeted by Johne's disease is the lower part of the intestine known as the ileum. The wall of the ileum contains a large number of pockets of lymphoid tissue known as Peyer's patches that lie just beneath the interior surface of the intestine. Peyer's patches are clusters of macrophages and lymphocytes that are organized much like lymph nodes. Covering Peyer's patches are a layer of cells called M cells. These cells function to sample the content of the lumen of the intestines and pass antigens (bacteria) through to the underlying cells of the Peyer's patch to "show" these antigens to the macrophages and lymphocytes (Allen et al., 1967).
Unfortunately, when M cells bring *M. paratuberculosis* to the Peyer's patch, the bacteria find an ideal place for growth. Macrophages in Peyer's patches engulf *M. paratuberculosis* for the purpose of destroying the foreign invader, but for reasons that are unclear, these macrophages fail to do this. Inside a macrophage, *M. paratuberculosis* multiplies until it eventually kills the cell, spreads and infects other nearby cells. In time, other parts of the ileum and other regions of the body are teaming with millions of the mycobacteria. How *M. paratuberculosis* neutralizes or evades the normally efficient bacterial killing mechanisms of the macrophages is unknown, although the unusually resistant cell wall of mycobacteria likely plays an important role (Allen et al., 1967; Gilmour and Gardiner, 1969; Golde, 1968).

The animal's immune system reacts to the *M. paratuberculosis* invasion by recruiting more macrophages and lymphocytes to the site of the infection. The lymphocytes release a variety of chemicals signals, called cytokines, in an attempt to increase the bacterial killing power of the macrophages. Macrophages fuse together, forming large cells, called multinucleated giant cells, in an apparent attempt to kill the mycobacteria (Patterson et al., 1967). Infiltration of infected tissues with millions of lymphocytes and macrophages leads to visible thickening of the intestines. This prevents nutrient absorption, and diarrhea results. Late in the infection, antibody production by the animal occurs to *M.paratuberculosis* in serum of animals, and is an indicator that clinical signs of disease and death from the infection will soon follow (Chiodini et al., 1984).
For goats infected with this disease, the most apparent sign of having it is their body wasting away, even with a sufficient diet. If a goat develops Johne's and it has diarrhea, it is most likely going to die. When it has diarrhea, the goat is at the last stages of the disease. Herds should be tested once or twice a year to maintain the health and keep out the disease (Molloy et al., 1994).

MAP bacteria enter the intestinal wall through the small intestinal mucosa primarily in the region of the ileum via M cells (specialized absorptive mucosal cells) residing in the Peyer's patches (Allen et al., 1967). Where they are resistant to intracellular degradation, they are eventually phagocytosed by sub-epithelial macrophages (Gilmour and Gardiner, 1969). While the bacteria are in the mucosal tissue and submucosal macrophages, there is little or no detectable reaction to the infection. This delayed detectable humoral immune response is one reason for the poor sensitivity (Se) of serological diagnostic tests for MAP, as explained in detail later. Eventually, the infected macrophages migrate into local lymphatics (Patterson et al., 1968), spreading

the infection to regional lymph nodes. In the regional lymph nodes, the organisms are capable of stimulating inflammatory and immunological responses (Golde, 1968). The immune response towards MAP resembles that of other mycobacterial infections. Most animals mount a cellular immune response involving a variety of cells, most importantly T lymphocytes (Larsen et al., 1965). Cytokines produced by T helper cells also contribute to the protective response against mycobacterial infections, especially the cytokine gamma interferon (IFN-g). Production of IFN-g has been recognized as a key step in resistance against mycobacterial diseases in general, and it may provide a means to help monitor early infection in some animals (Chiodini et al., 1984). In some cows, the cellular immune response has been shown to be able to control the infection, with the cow's never developing clinical signs but remaining subclinically infected for life (Lambrecht et al., 1988). In those animals in which the cellular immunity is unable to control the disease, a detectable humoral immune response will develop, along with increased shedding of bacteria (2. Typically, the organism proliferates slowly in the ileal mucosa and regional lymph nodes. However, poor nutrition, stress related to transport, lactation, parturition, and immunosuppression by agents like bovine viral diarrhea virus have been proposed as accelerating or precipitating the onset of the clinical phase of infection (Laochumroonvorapong et al., 1996). The physiological mechanism for development of diarrhea in clinically affected animals is thought to be related to antigen-antibody reactions in infected tissue, with subsequent release of histamine (Patterson et al., 1968; Patterson et al., 1968; Patterson and Berrett, 1969; Patterson et al., 1969; Patterson and Sweasey, 1966). Macroscopic lesions, if present, are seen primarily in the intestine and it's draining mesenteric lymph nodes, more specifically in the region of the ileum, although they can occur throughout the whole length of the intestinal tract. The intestinal wall is thickened and edematous, and the mucosa has exaggerated transverse folds, mimicking the appearance of corrugated cardboard with atrophic villi cells. The serosal and mesenteric lymphatic vessels are dilated and thickened. Subsequent muscle atrophy, emaciation, alopecia, renal infarcts, anemia, andleukopenia are thought to be mediated by cytokines (Whitehead and Alleyne, 1972; Patterson et al., 1969).

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