

## PROGRESSIVE PATHOLOGICAL CHANGES IN SUBCLINICAL EXPERIMENTAL PARATUBERCULOSIS IN GOATS

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### ABSTRACT

*An experiment was carried out to study the development of sequential histological lesions and efficacy of certain diagnostic tests in experimentally induced subclinical paratuberculosis infection in goats. Twelve goats of 8-12 weeks age were infected with  $4.23 \times 10^9$  *Mycobacterium avium* subsp. *Paratuberculosis* on 8 occasions. Eight goats were kept as in-contact controls and 4 as uninfected controls. Diagnostic tests, gross and histopathological studies were carried out at 3, 6, 9 and 12 months post-infection. Two goats were positive in the AGID test at 12MPI. Three goats, one at 9 MPI and two at 12 MPI were positive in faecal culture. Tissue PCR detected one goat positive each at 9 and 12 MPI. Six (50%) of 12 infected goats had gross and histological lesions. Marked enlargements of the mesenteric lymph nodes were observed in the early stages (3 and 6 months post infection) of infection. Mucosal thickening and corrugation of the jejunal and ileal mucosae, enlargement and oedema of the mesenteric lymph nodes, dilatation of lymphatics and gelatinisation of mesenteric fat were observed in goats euthanised at 9 and 12 MPI. Histological lesions consisting of flat and broad villi with mild infiltration of lymphocytes and macrophages in the intestinal villi and crypts were common at 3 MPI and 6 MPI. Focal infiltration of macrophages in Peyer's patches and giant cells were found in the mesenteric lymph nodes at 6 MPI. Broadened villi and increased infiltration of lymphocytes and macrophages with Langhan's giant cells were observed in the villi and crypts at 9 MPI and 12 MPI goats. Acid-fast bacilli were detected in 3 goats, one each at 6, 9 and 12 MPI.*

**Keywords:** paratuberculosis, goat, sub-clinical, pathology

### INTRODUCTION

Johne's disease is an economically important chronic bacterial disease of all domestic and wild ruminants. The disease, caused by *Mycobacterium avium* subsp. *paratuberculosis* is characterized by chronic granulomatous enterocolitis, lymphangitis and lymphadenitis. The clinical manifestations of the disease include incurable diarrhoea, progressive weight loss and decline in the productivity of animals (Williams *et al.*, 1979; Chiodini *et al.*, 1984; Benedictus *et al.*, 1987; Buergelt and Ginn, 2000; Godfroid *et al.*, 2000; Kramsky *et al.*, 2000). The bacterium of Johne's disease also has been implicated with an inflammatory bowel disease in humans known as Crohn's disease (Stabel, 1998; Selby, 2000).

In India, paratuberculosis in goat was first reported by Pande in 1942 from Assam. The disease now been reported from many parts of the country with prevalence varying from 2.5 to 18.9 % (Srivastava and More, 1987; Kumar *et al.*, 1988; Singh *et al.*, 1998; Tripathi and Parihar, 1999, Tripathi *et al.*, 2002). Various authors have described lesions in paratuberculosis, which occurs due to host's inflammatory immune response to the invading mycobacteria (Paliwal and Rajya, 1982; Rubin and Habekar, 1999; Hirsh and Zee, 1999).

The pathological changes in natural cases of paratuberculosis of goats were studied in detail by several workers (Nakamatsu *et al.*, 1968; Majeed, 1972; Paliwal and Rajya, 1982; Corpa *et al.*, 2000). Corpa *et al.*, (2000) described lesions in the natural cases of caprine paratuberculosis as focal, diffuse multibacillary, diffuse lymphocytic and diffuse mixed based on the severity and pattern of cellular infiltration. Sigurdardottir *et al.*, (1999) studied the pathological and immunological response in early subclinical stages of experimental paratuberculosis in goat kids. Similarly, Storset *et al.*, (2001) carried out immunological and microbiological study in subclinical paratuberculosis in goats following experimental infection. Valheim *et al.*, (2002) described lesions in subclinical paratuberculosis in goats, which were associated with persistent gut associated lymphoid tissues (jejunal and ileo-caecal valve Peyer's patches). The studies on sequential development of lesions and their relationships with immunological, serological and bacteriological findings in early stages of paratuberculosis are scarce in goats. Therefore, this study was aimed for comparative assessment of commonly used diagnostic tests during subclinical infection in experimental infection in goats.

## **MATERIALS AND METHODS**

### **Experimental design**

Twenty-four goats were randomly divided into three groups, namely, the infected (n=12), the in-contact control (n=8) and the uninfected control (n=4). The animals in the infected group were orally administered with 5 ml of bacterial suspension ( $4.23 \times 10^9$ /ml) on eight occasions at 3 days interval within a month. Similarly, the uninfected control animals received 5 ml sterile PBS. The animals of the in-contact group were administered similarly with PBS and kept along with the animals of the infected group. Five animals (3 infected, one in-contact and one uninfected control goats) were euthanised by intravenous injection of Thiopentone injection (Anesthal, Jagsonpal Pharmaceutical, Faridabad, India) at 3, 6, 9 and 12 months post-infection (MPI).

### **Preparation of inoculum**

Small intestine from mid jejunum to the terminal ileum of an advanced case of paratuberculosis goat (multibacillary goat, case no.12A/2003) was collected at necropsy and the bacterial suspension was prepared as described previously (Kurade *et al.*, 2004). The material was then filtered through the sterile gauze to remove tissue debris and to recover the bacterial suspension. The approximate number of acid-fast organisms in the suspension was found to be  $4.23 \times 10^9$  AFB/ml (Talwar, 1983). The inoculum was stored in screwcapped plastic containers in 50 ml aliquots at  $-20^\circ\text{C}$ .

The suspension was subjected to culture on the Herrold's egg yolk medium (HEYM) and mycobactin – J dependent *M. a. paratuberculosis* was isolated. The identity of the strain was confirmed by the demonstration of the IS900 sequence by polymerase chain reaction and confirmed by restriction enzyme analysis (Sivakumar *et al.*, 2005).

### **Clinical signs**

All the goats of the infected, in-contact and uninfected control groups were examined regularly for development of the clinical signs. The body weights of all the animals were recorded at monthly intervals.

### **Johnin testing**

The test was carried out by single intradermal inoculation of 0.1 ml of Johnin purified protein derivative (1 mg/ml, BP Division, IVRI, Izatnagar) at a shaven site on one side of the neck. The thickness of skin was measured before inoculation and at 72 hours after injection with the help of Vernier callipers. Animals showing an increase of 2 mm in thickness of skin were considered positive (OIE Manual, 2000; Perez *et al.*, 1996).

### **Faecal and tissue smear examination**

Faecal samples (2.0 g) collected directly from the rectum of each goat, was mixed well in 15 ml of distilled water and left overnight to settle. 5 ml of the supernatant was centrifuged at 1500 g for 20 min and smears were prepared from the sediment. Similarly scraping smears were prepared from the jejunum, ileum, ileocaecalcalve and mesenteric lymph nodes. After heat fixation, smears were stained by Ziehl Neelsen's (ZN) method and examined under microscope using oil-immersion objective. The presence of 2-3, strongly acid-fast bacilli in clumps were considered as positive (OIE Manual, 2000, Anonymous, 2004).

### **Bacterial culture of faeces and tissue samples**

Faeces (2.0 g) and pooled tissue samples (2.5 g) from small intestine and mesenteric lymph node were homogenized in sterile distilled water and decontaminated with 0.9% hexadecylpyridinium chloride (final concentration 0.75%) as described previously (Whipple *et al.*, 1991; Rajukumar, 1998; Tripathi *et al.*, 2000). The samples were cultured on Herrold's egg yolk medium (HEYM, pH 7.1-7.4), with and without Mycobactin - J (Allied Monitor Inc., Missouri, USA) at 37°C for 16 weeks. The culture was checked every 4 weeks. The growth of acid-fast colonies on Mycobactin J supplemented HEYM tubes were identified as positive, which were also subjected to the IS900 PCR confirmation.

### **Polymerase Chain Reaction (PCR)**

IS900PCR was carried out in faecal and tissue samples at 3, 6, 9 and 12 months post infection. The PCR mixture included 10x buffer(5µl), Mgcl<sub>2</sub>(5µl), dNTP(10µl), primers BN<sub>1</sub> and BN<sub>2</sub>(2.5µl each), Taq. Polymerase(0.5µl), DNA(0.5µl) and water(24µl). Oligonucleotide primersBN<sub>1</sub>: 5' GTT ATT AAC GAC GCC CAG C-3' and BN<sub>2</sub>: ACG ATG CTG TGT TGG GCG TTA G-3' flanking a region of 626 bp fragments, designed from published sequence of IS 900 of *M.a. paratuberculosis*(Sivakumar *et al.*, 2005 and Green *et al.*, 1989) was used. Initial denaturation (1 cycle) was carried out at 94C for 4

min, denaturation (30 cycles) at 94° C for 1 min., annealing (30 cycle) at 60°C, synthesis (30 cycles) at 72°C for 4 min, final elongation (1 cycle) at 74°C for 4 min and holding at 4°C for 30 min.

### Serological tests

**a) Agar gel immunodiffusion test (AGID):** AGID test was carried out in 1% agarose prepared in PBS (pH 7.4) as per the procedure already developed in the laboratory (Goat Lab Division of Pathology IVRI).

**b) Absorbed ELISA:** The capture antigen from MAP strain 316 and the adsorbing antigen from *Mycobacterium phlei* were prepared as described previously (Rajukumar *et al.*, 2001). The flat bottom 96-well plates (Maxisorp, Nunc, Denmark) were used. The ELISA procedures including optimum concentration of capture and adsorbing antigens, sera and conjugate (antigoat IgG-HRP, Sigma, St. Louis, MO, USA) were carried out at Goat Disease Lab, Division of Pathology, IVRI. The colour was developed by addition of hydrogen peroxide and O-phenylenediamine (Sigma). The optical density was measured at 492 nm in and ELISA reader (Multiscan Ex; Becton Dickinson, Franklin Lakes, NJ, USA). The serum samples were incubated with optimum concentration of adsorbing antigen (*M. phlei* antigen).

The positive ELISA results were determined by S/P value (sample to positive ratio) using the following formula:

$$S/P = \frac{\text{Sample mean} - \text{Negative control mean}}{\text{Positive control mean} - \text{Negative control mean}}$$

The samples showing S/P value of 0.35 (cut off value) or more were considered positive. The cut off value was determined based on sera collected from 100 goats from an organized farm that had no history of occurrence of paratuberculosis and for last 3 consecutive years, had been testing negative in faecal culture, PCR and AGID.

### Pathological studies

The experimental animals were euthanised at 3, 6, 9 and 12 months post infection. Detailed necropsy examination was conducted, and the gross pathological changes were recorded. The tissue collection for the microscopic examination included 1 section of duodenum, 4 sections of jejunum, 3 sections of ileum, 1 section each of the mesenteric lymph node, ileocaecal lymph node, caecum, colon and rectum. One section each from other organs such as liver, kidneys, spleen, adrenals, pancreas, heart, lungs and aorta were also collected. All tissue samples were fixed in 10% neutral buffered formalin and cut into pieces of 2-3 mm thickness before conventional processing. The embedded tissues were sectioned at 4µm thickness and stained with haematoxyline and eosin (Culling, 1968). Adjacent sections from all tissues were also stained by Ziehl Neelsen's (ZN) method.

## RESULTS

### Clinical signs

The body weight did not show significance difference between the infected, in-contact and the uninfected control animals. Three goats, one at 9 MPI and two at 12 MPI. showed unthriftiness and mild muscle wasting. Oedema of the intermandibular space in two goats (One at 9 MPI and one at 12 MPI) and intermittent soft faeces (two at 9 MPI and one at 12 MPI) which were observed during 7 months of infection and persisted for 3 weeks. The remaining animals of the infected and control groups had normal appetite and pelleted faeces during the observation period.

### Johnin testing

Three goats (Goat No 3, 4 and 28) tested positive at 6 MPI. Five goats (Goat No. 1, 3, 4, 5, and 6) tested positive at 9 MPI. Two goats (Goat No 3 and 4) tested positive both at 6 and 9 MPI. Two of 3 goats that were positives at 9 MPI became negative at 12 MPI (Table 1).

**Table 1:** Result of different diagnostic tests employed in 12 experimental infected animals

Goat No.	Sacrifice MPI	DTH	AGID	ELISA	Faecal smear	Faecal culture	Faecal PCR	Tissue smear	Tissue culture	Tissue PCR	H/p lesion	ZN stain
1	12	+	+	+	+	+	-	+	+	+	+	+
2	12	-	-	-	-	-	-	-	-	-	-	=
3	12	+	+	+	-	+	-	-	-	-	-	=
4	9	+	-	-	+	+	-	+	-	+	+	+
5	9	+	-	-	-	-	-	-	-	-	-	=
6	9	+	-	-	-	-	-	-	-	-	-	=
7	3	-	-	-	-	-	-	-	-	-	-	=
23	6	-	-	+	-	-	-	-	-	-	-	=
26	3	-	-	-	-	-	-	-	-	-	-	=
27	6	-	-	+	-	-	-	-	-	-	+	+
28	6	+	-	+	-	-	-	-	-	-	-	=
31	3	-	-	-	-	-	-	-	-	-	-	=

### Serological examination

#### Agar gelimmunodiffusion test (AGID)

In the infected group, 2 goats (Goat No.1 and 3) were positive at 12 MPI in the AGID test. All goats of the in contact and uninfected control groups were tested negative in the AGID test.

#### Absorbed ELISA

There were increased S/P values in both infected and in-contact groups as compared with the control group. However, the mean S/P values were negative at all time points. A total of 5 experimentally infected goats (Goat N. 1, 3, 23, 27 and 28) were found positive at various month post infection. Only one goat showed positive after 1 MPI, two were found positive after 5 and 6 MPI and other two goats were tested positive after 8 and 10 MPI.

However monthly mean S/P values of entire infected, in-contact and control group were found negative for ELISA for paratuberculosis infection (Table 1).

### **Bacterial culture**

*M.a. paratuberculosis* was isolated from faeces of 3 animals, one goat (Goat no.4) at 9 MPI and two goats (Goat No.1 and 3) at 12 MPI. Remaining animals of infected group and all the animals of the in contact and uninfected control groups were negative for bacterial culture at all time points. Tissues from one infected goat(Goat No 1) was positive for bacterial culture (Table 1).

### **Polymerase Chain Reaction (PCR)**

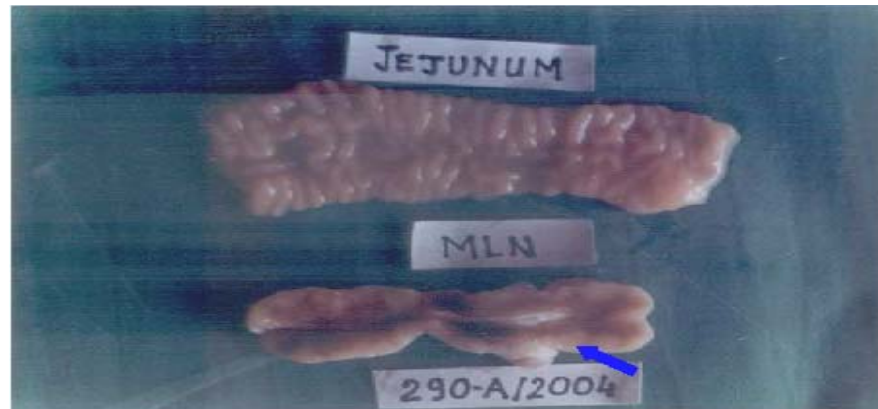
All the experimental animals were negative in the faecal PCR at all time points. IS900 PCR carried out on tissue samples revealed positive amplification in two goats, one each at 9 MPI (G4) and 12 MPI (G1) (Table 1).

### **Gross Pathology**

The important gross lesions consisted of thickening and corrugations of the intestinal mucosa and enlargement and oedema of the mesenteric lymph nodes. Enlargement of the mesenteric lymph nodes was marked in goats necropsied on 3 and 6 MPI. Pale discoloration of mucosa, thickening and corrugation of jejunum and ileum were more prominent in goats necropsied at 9 and 12 MPI. Gelatinisation of mesenteric fat was observed in two goats (G1 and G3) each at 9 and 12 MPI (Fig 1 and 2).



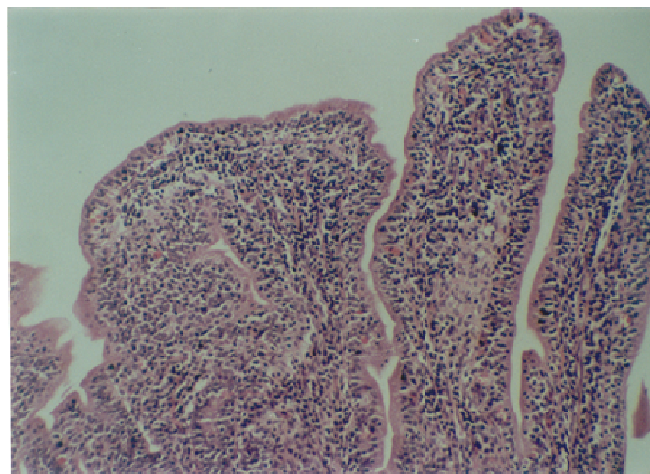
**Fig.1:** Intestinal tract (6 MPI): enlarged MLN (blue arrow) and thickened intestinal wall (white arrow).



**Fig.2:** Jejunum and MLN (12 MPI): Mucosal corrugation in the jejunum and note enlarged and oedematous jejunal MLN with distinct grayish white area (blue arrow) in the cortex.

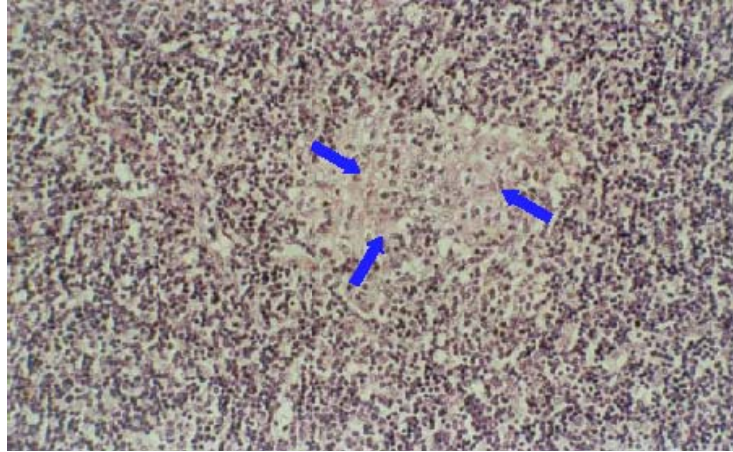
### **Histopathology**

In goats at 3 MPI, slightly flat and broad villi with increased infiltration of lymphocytes and macrophages were noticed. Focal infiltration of macrophages was observed in the ileal Peyer's patches of two goats (G26 and G31) and ileo-caecal valve Peyer's patches and mesenteric lymph node of one goat. Acid-fast bacilli were not detected in any of 3 infected goats. In the infected goats at 6 MPI, there were more infiltration of lymphocytes and macrophages in the ileum and jejunum. The Peyer's patches of jejunum and ileum showed focal infiltration with macrophages. Multinucleated giant cells of Langhans type were observed in the paracortical region of the mesenteric lymph node. Acid-fast bacilli were detected in ZN stained sections of the jejunum and ileocaecal valve of one goat (G27) (Fig 3 and 4).



**Fig.3:** Ileum (3 MPI): Broad villi showing infiltration of lymphocytes and macrophages. H&E X

160

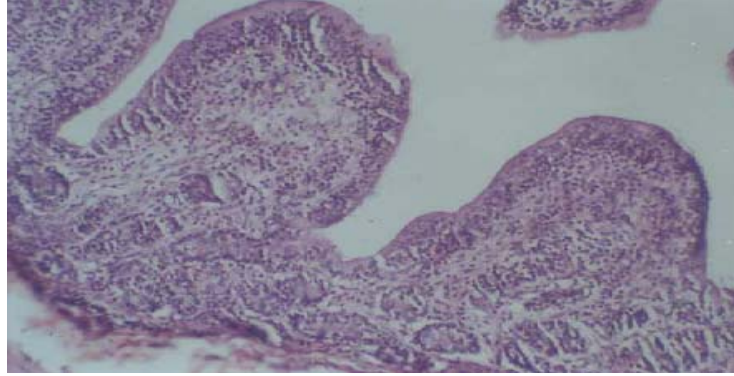


**Fig.4:** Mesenteric lymph node (3 MPI): Focal granuloma (blue arrow) consisting of macrophages in the paracortical area. H&E X160.

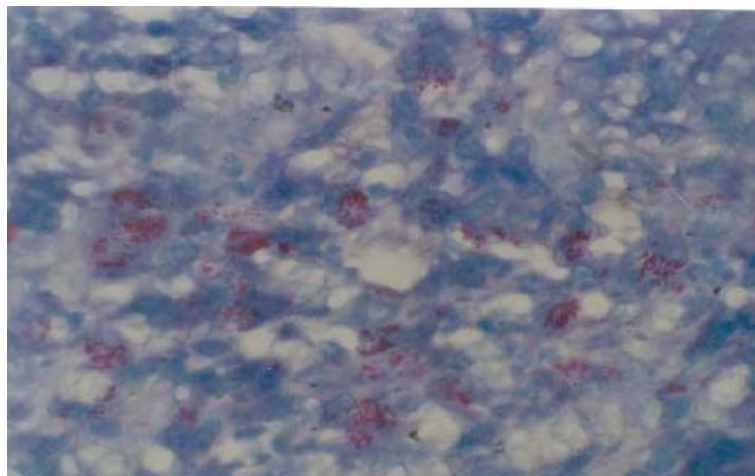
Microscopic lesions in infected goats at 9 months post infection were more severe as compared with 3 and 6 MPI groups. The intestinal villi were broad, and at places fused and showed diffuse infiltration with lymphocytes, macrophages and occasionally multinucleated giant cells in the mucosa of the jejunum and ileum. Focal granulomas were frequently detected in the jejunal and ileal villi in one goat (G4). In the same goat, ganglion cells of Meissner's plexus were surrounded by the lymphocytes and macrophages. Acid-fast bacilli were detected in the ZN stained sections of jejunum, ileum and ileocaecal valve of one goat (G4). In the mesenteric lymph nodes, microgranulomas along with multinucleated giant cells were observed in the cortical and paracortical regions without demonstrable acid-fast bacteria.

In infected goats at 12 MPI villi were broad, short and had shown diffuse infiltration with lymphocytes and macrophages. There were infiltrations of macrophages in the Peyer's patches of jejunum. Giant cells were present in the crypt region of ileum. Acid-fast bacilli were detected in ZN stained tissue section of ileo-caecal valve of one goat (G1). Interfollicular areas of cortex, and the paracortex showed infiltration with macrophages and formation of granuloma at the cortico-medullary junction. The in-contact control goat at 12 MPI revealed mild thickening but mild infiltrations of lymphocytes and macrophages in small intestine were common in most of the in-contact control goats ( Fig 5 and 6).





**Fig. 5:** Ileum (12 MPI): Shortening and broadening of villi with infiltration of macrophages. H&E X160.



**Fig. 6:** Ileocaecal valve(12 MPI) with clumps of acid fast Bacilli in crypts

## DISCUSSION

In this study few infected animals showed unthriftiness, mild muscle wasting, intermittent soft faeces and oedema of the inter mandibular space, which was in contrast to previous long term studies in which there were no symptoms of paratuberculosis (Sigurdardottir *et al.*, 1999; Munjal,2004).

Six goats from the infected and none from the in-contact groups yielded positive delayed type hypersensitivity (DTH) reaction using Johnin PPD at different MPI. Two animals, which were positive at 9 MPI in DTH failed to respond positively at 12 MPI. Fluctuation in CMI response has been reported frequently in paratuberculosis in goats (Munjal, 2004; Storset *et al.*, 2001) and other small ruminants (Kurade *et al.*, 2004). Out of 6 DTH positive goats, acid-fast bacilli were detected in 3 goats, which also had lesions compatible with paratuberculosis. In other 3 goats, although there were specific tissue reactions, acid-fast organisms could not be demonstrated. The negative DTH results in all the in-contact goats suggested that the excretion of bacilli from the infected animals was not sufficient to sensitise the in-contact goats.

In the present study, only 2 of 3 goats at 12 MPI were positive in the AGID test, suggesting poor sensitivity of the test in the subclinical paratuberculosis (Shulaw *et al.*, 1993; Clarke *et al.*, 1996). Out of 12 infected goats, 5 goats were found to be positive in the ELISA, 4 of these were positive at 5 MPI. In the experiment of Sigurdardottir *et al.*, (1999), only one goat of 8 infected goats was positive in the ELISA after 8 months of infection. Storset *et al.*, (2001) detected antibodies against *M. a. paratuberculosis* from 15-20 weeks in 4 of 7 animals and one additional animal became positive by 35th week. Munjal (2004) detected 4 goats positive in ELISA at 180 days post infection. Our results alongwith the previous studies suggested the utility of the ELISA in diagnosing subclinical infection in goats.

In the present study faecal or tissue culture and tissue PCR detected 2-3 goats positive after 9 months of infection (write culture results of other workers (Sigurdartottir *et al.*, 1999, Storset *et al.*, 2001, Vathiem *et al.*, 2002, Munjal, 2004).

Sensitivity of bacterial culture and PCR have been reported to be comparable (Sigurdardottir *et al.*, 1999; Collins *et al.*, 1993; Anonymous, 2004) and poor in the experimentally infected small ruminants because of variable incubation period (Colgrove, 1989; Collins *et al.*, 1993; OIE Manual 2000; Carrigan and Seaman, 1990; Sigurdardottir *et al.*, 1999).

The sensitivity of the bacterial culture and PCR was reported to be variable in the clinical sample (Collins, 1996) and depends on various factors such as number of bacteria, DNA extraction procedure, concentration of eukaryotic DNA and presence of PCR inhibitors (Van der Geison. *et al.*, 1993; Collins *et al.*, 1993; Collins, 1996). In the current study, low numbers of bacteria in subclinical infection appeared to be the major factor for poor sensitivity of the bacterial culture and PCR.

Initially (3 and 6 MPI) gross lesions were more prominent in the merenteric lymph node than in the small intestine where thickening and corrugations were more obvious after 9 months of infection. While these findings generally agree with those reported previously (Fodstad and Gunnarson, 1979; Paliwal and Rajya, 1982; Collins *et al.*, 1984; Rajukumar, 1998; Sigurdardottir *et al.*, 1999; Corpa *et al.*, 2000, Munjal, 2004), mesenteric lymph node lesions should be examined carefully in early cases. In the small intestine, comparatively severe lesions observed in the jejunum than ileum corroborated earlier observation correlating lesions with the Peyer's patches, which persist in adult life (Corpa *et al.*, 2000; Valheim *et al.*, 2002; Munjal, 2004).

At 3 MPI, except for the presence of increased proliferation of lymphocytes and macrophages in the mucosa and infiltration of macrophages in the peyer's patches specific granulomas with acid-fast bacteria were not observed. While these lesions cannot be considered specific for paratuberculosis, there was no means to absolutely rule out that such initial lesion would not be caused by *Mycobacterium avium paratuberculosis*. Sigurdardottir *et al.* (1999), failed to detect lesions in experimental goats at 11 weeks post infection (WPI) but at 17 WPI, inflammatory cell infiltrate and giant cells were detected in

the jejunum and ileum. Munjal (2004) reported granulomatous lesion in the ileo-caecal valve in one goat at 2 MPI. Experimental infection of goats with *M. a. paratuberculosis* (human isolate) produced granulomatous lesions consisting of clusters of epithelioid cells and giant cells in the interfollicular and basal regions of the ileal and jejunal Peyer's patches at 3 MPI (Van Kruiningen *et al.*, 1986; Clarke 1997). The mild lesions observed in the present study could be due to the dose and strain variation and age of the animals, which were comparatively younger in previous studies (Van Kruiningen *et al.*, 1986; Clarke 1997).

At 6 MPI histological lesions were more developed along with demonstration of AFB than those seen at 3 MPI and were similar to those reported by Sigurdardottir *et al.*, (1999) at 24-week post-infection, and Munjal (2004) at 30 weeks post-infection in the experimental goats. In the experimental goats sacrificed at 9 MPI had increased infiltration with lymphocytes and macrophages, and multinucleated Langhan's giant cells in the lamina propria of the jejunum and ileum of one goat. Proportions of macrophages were more in comparison to lymphocytes. Acid-fast organisms were detected in the jejunum, ileum and ileo-caecal valve of one goat. Sigurdardottir *et al.*, (1999) detected microscopic lesions of inflammatory infiltrates in the jejunum and ileum at 37 weeks post infection. Munjal, (2004) detected lesions in intestine consisting of few multinucleated giant cells and accumulation of AFB in the intestinal villi at 270 DPI.

In the present study, experimental goats sacrificed at 12 MPI also revealed broad and flattened villi with more infiltration of macrophages. Multinucleated Langhan's type of giant cells was also seen in the crypt region of ileum. Peyer's patches were infiltrated with macrophages. Granulomas were observed in the cortical and paracortical areas of the mesenteric lymph nodes. Acid-fast organisms were detected in the ileo-cecal valve of one goat. In comparison to other studies that were carried out for more than one year (Sigurdardottir *et al.*, 1999; Storset, 2001), typical histological lesions of paratuberculosis were observed in goats in the present study.

In spite of observation of corrugations of intestinal mucosa in 9 infected goats, granulomas and giant cells were seen in the small intestines of one infected goat each at 9 and 12 MPI. The presence of giant cells in the paracortical region of lymph node was observed in one goat at 6 MPI. These findings suggest that thickening and corrugation of intestinal mucosa in goats can be due to infiltration of lymphocytes and macrophages in subclinical paratuberculosis without presence of classical lesions. This is important from diagnosis point of view in naturally infected cases, wherein such lesions even without demonstration of acid-fast bacteria should be suspected for paratuberculosis.

In the present study, acid-fast organisms were mainly detected in the ileo-caecal valve and associated Peyer's patches indicating the major involvement of ileocaecal valve in the progression of subclinical infection in goats. A previous study on experimental paratuberculosis using young goats showed that ileal Peyer's patches can take up the bacteria and hence it could be a site of early histological lesions (Valheim *et al.*, 2002). In the present study, most of the infected goats had infiltration with macrophages in the Peyer's patches. Only one goat at 12 MPI revealed the presence of acid-fast bacilli in the

Peyer's patches of ileo-caecal valve confirming that these were the sites of bacterial invasion multiplication and further dissemination. Munjal (2004) demonstrated acid-fast bacilli in 2 experimentally infected goats, one at 60, and other at 210 days post infection with similar lesions as observed in the present study.

Van Kruiningen *et al.*, (1986) reported that *M. a. paratuberculosis* infection began in the ileo-caecal or jejunal Peyer's patches, the only intestinal lymphoid tissues persisting in the adult animals (Landverk *et al.*, 1991; Perez *et al.*, 1996). As seen in this study, macrophage infiltration in the jejunal Peyer's patches were also noticed by Corpa *et al.*, (2000) in natural cases of paratuberculosis suggesting that initial focus of histological changes starts around Peyer's patches.

In the present study, mononuclear cell infiltration around ganglion cells of Meissner's plexus in small intestine in one goat at 9 MPI was like those observed in cattle and sheep with histological evidence of paratuberculosis (Gwozdz *et al.*, 2001). Buergelt *et al.*, (1978) described the intestinal neural lesions, which were characterised by infiltration of myenteric ganglia with globular leucocytes. Chiodini *et al.*, (1984) highlighted the possibility of axonal degeneration of sciatic and brachial plexuses. Binford, (1985) noticed distinct aggregation of mononuclear cells around nerves in the ileal submucosa and muscular layer of 8 naturally and 5 experimentally infected sheep. It has been suggested that the neural lesions may represent an autoimmune reaction triggered by antigens expressed in nerves or nerve associated cells in affected parts of the intestine since nerve runs parallel to lymphatics (Gwozdz *et al.*, 2001). Though, in the present study, goat had only soft faeces, neuritis causing involuntary muscle relaxation in the small intestine during infection, may potentiate diarrhoea that has been correlated with type 1 or III hypersensitivity (Tizard, 1996).

Mild thickening of intestines in the in-contact control goats at 12 MPI and infiltrations mainly of lymphocytes in the small intestine and ileo-caecal valve in most of the in-contact goats could be the result of the sensitization of animals by exposure to *M. a. paratuberculosis* organisms but it might not have been enough to cause the detectable infection. None of the in-contact animals could be detected positive by any diagnostic tests employed. Moreover, it should be kept in the mind none of infected animals were heavy shedders as most of them were in subclinical stage of disease in the present study.

The results of the present and earlier studies (Sigurdardottir *et al.*, 1999; Valheim *et al.*, 2002; Storset *et al.*, 2001; Munjal, 2004), initially there were no distinct granulomas and clinical disease could not be induced even with higher dosage up to 2 years of age (Storset *et al.*, 2001). On the contrary, in sheep with similar experimental design (Kurade *et al.*, 2004) like the present study and in others (Begara-McGorum *et al.*, 1998) well delineated granulomas were reported as early as 4 weeks after infection and production of clinical disease within a year of infection. Though comparison of infection process in goat from sheep was not the aim of this study, evidences suggest that early pathogenetic mechanism differ between these two species. While goat could be a better experimental model for persistence of infection without developing immune granuloma, sheep for the clinical disease model that early pathogenetic mechanism differs between sheep and goat and

latter does not appear to be a better experimental model for clinical paratuberculosis. This conclusion may be true only for experimental paratuberculosis, but it raises other questions, as clinical disease is not produced in goats, if goat is resistant, it should develop immune granulomas to resist and clear the infection, which was not obvious in any experimental studies. This means that the mechanism of resistance to *Mycobacterium avium paratuberculosis* infection in goat differs from sheep, inferring that the mechanism to intracellular resistance could be characteristics of the species and hence may vary. While goats may not be a good experimental model for paratuberculosis, it could be a good subject to study the resistance of Map infection in goats for quite long time without showing any distinct granuloma.

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