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Incidence of *Mycobacterium avium* Subspecies *paratuberculosis* in Mehsani and Surti Goats of Indian Origin using Multiple Diagnostic Tests

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Abstract: *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the causative agent of chronic enteric granulomatous inflammation in animals and is known as Johne's Disease (JD) or Paratuberculosis. JD, being spectral in nature, presents variable bacteriological, immunological and pathological spectra leading to variable efficacy of diagnostic methods at different points of time during the course of infection. The present study aimed to estimate the incidence of MAP in two important breeds of goats (Mehsani and Surti) from South Gujarat region of India by applying conventional, molecular and serological methods. A total 219 goats were screened and categorized into Group-I (123 Mehsani goats), Group-II (76 Surti goats) and Group-III, (20 Non-descript goats). Percent positivity by faecal smear examination, delayed type hypersensitivity (DTH), agar gel immunodiffusion (AGID), IS900 polymerase chain reaction (PCR) and indigenous enzyme linked immunosorbent assay (ELISA) kit was 9.2 (7/76), 21.9 (27/123), 10.9 (24/219), 12.5 (5/40) and 43.3% (95/219), respectively. Of the 123 goats of Group-I, 27 (21.9%) were positive in DTH test. Of the 5 faecal positive goats which also showed clinical signs, 2 (3.5%) goats died during study were negative by Johnin test. Similar to these findings, sensitivity of Johnin test in goats ranged between 18-30% with least specificity in both preclinical and advanced stage of disease. Of 34 cases of caprine paratuberculosis, 73.5% goats were positive for Johnin test. In the present study, out of the 5 infected goats, 3 (60%) were positive in Johnin test. Rectal pinch smear examination was carried out in 27 DTH positive goats and all smears were negative for the presence of acid fast *bacilli*. Screening tests (Indigenous ELISA and DTH) showed very high incidence of MAP infection in the goat population. The utility of multiple diagnostic tests is suggested for confirmatory detection and epidemiological diseases investigations of MAP in animals.

Key words: Johne's disease, *Mycobacterium avium* subspecies *paratuberculosis*, johnin test, microscopy, ELISA, IS900PCR, AGID, Ziehl-Nielsen, artificial insemination, complement fixation test

INTRODUCTION

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of chronic enteric

granulomatous inflammation in animals known as Johne's disease (JD) or Paratuberculosis (Chiodini *et al.*, 1984; Tripathi *et al.*, 2002; Sweeney *et al.*, 2012). It is either a saprophytic or obligatory microbe which is an ancient

pathogen (Deb and Goswami, 2011). It causes enterocolitis, lymphadenitis and lymphangitis in domestic and wild ruminants (Perez *et al.*, 1996; Clarke, 1997; Collins *et al.*, 2001; Singh *et al.*, 2012). The disease has an endemic distribution globally reducing production of food in both developed as well as developing countries. The disease in most of the instances is restricted to sub-clinical infection. But there is rapid loss in condition leading to quick death because of nutritional deficiency resulting due to absorption loss from the gut (Collins, 2003). In India, limited studies (Pande, 1940; Singh *et al.*, 1996, 2007a) have reported the variable incidence (2-18%) in domestic livestock (Tripathi *et al.*, 2002). Animals may either receive infection from infected parents through semen, in-utero, milk or colostrums (Shankar *et al.*, 2010) or pick up infection from contaminated environment soon after birth. Then it follows a protracted incubation period which may last few months to years. In this century, MAP is considered as a major pathogen of animals as well as human beings (Manning and Collins, 2001; Tripathi *et al.*, 2002; Momotani, 2012; Sweeney *et al.*, 2012). The disease is primarily being responsible for low per animal productivity in the ruminant population (domestic) of the country. In the domestic livestock inspite of high prevalence and endemicity of the MAP the impact of the disease has neither been realized nor estimated at the National level. This is primarily due to the lack of indigenous diagnostic tests as well as kits. Sharing of MAP between species (inter-species transmission) has been frequently reported (Singh *et al.*, 2012). Clinical symptom being non-specific (weight loss and diarrhea) are not visible before the disease gets fully established in the body (clinical). Progressive weakness ultimately leads to either un-timely death or culling. Internally, granulomatous enteritis leads to thickening of intestinal mucosa and regional lymphangitis and lymphadenitis in small ruminants (Chiodini *et al.*, 1984). However, corrugations are rare as animals goes out much before from the production system. Recently, antibiotic resistance is an also an emerging problem to be tackled for effective treatment of the disease (Tiwari *et al.*, 2013a).

Johne's disease (JD) being spectral in nature presents variable bacteriological, immunological and pathological spectra leading to variable efficacy of diagnostic methods at different points of time during the course of infection (Chiodini *et al.*, 1984). Cultivation of MAP from faeces and tissues (ileo-caecal lymph nodes and small intestines) though specific but is time consuming and may require up to 12 weeks (Cocito *et al.*, 1994) and presents lesser sensitivity (Socket *et al.*, 1992). Polymerase Chain Reaction (PCR) is a rapid diagnostic

test for the detection of MAP from faeces, blood and tissues with sensitivity comparable with bacterial culture (Tripathi *et al.*, 2002; Singh *et al.*, 2010; Dhama *et al.*, 2011). Monoclonal antibody based diagnostics have also been reported to be useful for JD (Deb *et al.*, 2013). Infected animals elicit strong Cell-Mediated Immune (CMI) response in the early stages of infection and strong humoral immune response in the later stages (Clarke, 1997; Perez *et al.*, 1999). CMI based tests such as cutaneous testing with Johnin Purified Protein Derivative (PPD), gamma-interferon assay and lymphocyte stimulation test, although reported to be sensitive, have been less frequently evaluated for the detection of early stages of MAP infection in sheep and goats (Molina *et al.*, 1991; Storset *et al.*, 2001; Kurade *et al.*, 2004). Antibody assays for JD include Complement Fixation Test (CFT), Agar Gel Immunodiffusion (AGID) and Enzyme-linked Immunosorbent Assay (ELISA) tests (Kurade, 1999). The latter is sensitive in clinical infection but performs poorly in sub-clinical stages of infection (Stewart *et al.*, 2006). In view of these facts, this study aimed to evaluate the efficacy of commonly used diagnostic tests including of conventional, serological and recent molecular tools for the detection of MAP infection in goats.

MATERIALS AND METHODS

Faecal smear: About 2-3 g of faecal sample were collected from each of the goats directly from rectum, brought to the laboratory on the same day and processed. One gram of faecal sample was properly crushed with the help of sterilized pestle and mortar in sterilized distilled water. Mixture was then centrifuged at 2,600 rpm for 45 min. After centrifugation, supernatant was discarded and from the semisolid middle layer smear was prepared on a clean slide. Smear was air dried, heat fixed and stained with Ziehl-Nielsen (ZN) method and examined under microscope. Positive faecal samples were stored at -20°C for DNA extraction and culture.

Rectal pinch smear: Rectal pinch smear were examined in goats positive in Johnin test. Rectal pinch was collected with a sterilized artificial insemination sheath and smears were prepared. Smears were air dried, heat fixed, stained with Zeihl Nielson (ZN) method and observed under microscope. Smears exhibiting presence of short acid fast bacilli were considered positive for MAP.

Delayed type hypersensitivity (DTH)/Johnin test: Single Intra-dermal (DTH) test was performed on 123 goats of group-I by inoculation of 100 µg of Johnin PPD, obtained from Division of Biological Products, Indian Veterinary

Research Institute (IVRI), Izatnagar (India) in the form of Heat Concentrated Synthetic Medium (HCSM) of *Mycobacterium paratuberculosis*. Test was carried out as per the instructions of manufacturer on the side of neck. Skin thickness was measured with Vernier Calipers at pre, 24, 48 and 72 h post inoculation. Animals showing hot, edematous, painful skin thickness of more than 3 mm after 48 h were considered positive.

Post mortem and histopathology: Goats died of natural infection were subjected to post mortem examination. Tissues (Ileo-caecal junction of intestine, ileum and mesenteric lymph nodes) exhibiting gross lesions were collected and stored at -20°C without adding any preservative for DNA isolation and PCR. For histopathology, tissues were stored in 10% formalin at room temperature. Formalin fixed tissues were cut into thin (2-3 mm) pieces and washed thoroughly with water for several hours before putting in ascending grades of alcohol for dehydration. Dehydrated tissues were cleared in xylene, embedded in paraffin blocks and 5 micron thick sections were prepared (Luna, 1968). Sections were stained with haematoxylin and eosin (H and E) and Ziehl Nielsen method. Smears made from tissues (Ileocaecal junction, mesenteric lymph nodes) were heat fixed and stained with ZN method for the presence of acid fast *bacilli* indistinguishable to MAP.

Agar gel immunodiffusion (AGID) test: The protocol of AGID was followed as per Ferreira *et al.* (2002). Agarose was dissolved at 0.75 in 0.85% NaCl solution and buffered to pH 9.0 with 0.01 M tris (hydroxymethyl)-aminomethane and sodium azide 0.02%. Gel was poured in plates on a 4 mm thick layer and wells of 5 mm diameter and in a hexagonal pattern of six peripheral wells for serum samples and a central well for antigen were made. Protoplasmic Antigen (PPA) (Allied Monitor, USA) was used as antigen at the concentration of 10 mg mL⁻¹. Positive serum was placed into wells adjacent to test sera. Diameter between the centers of all the adjacent wells was 8 mm. After the wells were filled plates were incubated in covered, moistened glass dishes to avoid drying. Plates were examined after 24 and 48 h of incubation at room temperature. Appearance of one or more clearly definable precipitation lines before or at 48 h constituted a positive test result. Absence of any precipitation lines was recorded as a negative test result. Serum from clinically infected animals was used as positive control.

DNA ISOLATION

From tissues: After collection of tissues (mesenteric lymph nodes and intestines) these were grounded and treated with 0.9% HPC (Hexa decylpyridinium chloride) overnight. The sediment (0.5-1.0 mL) was taken in 2.0 mL

capacity eppendorf tubes and washed 3-4 times with Phosphate Buffered Saline (PBS) by spinning and vortexing. Pellet was subjected to DNA isolation as per Van Embden *et al.* (1993) method with some modification. Briefly, washed pellet was suspended in 1.0 mL 1X Tris EDTA (TE) buffer (pH 8.0) and centrifuged at 8,000 rpm for 15 min. Pellet was re-suspended in 450 µL 1X TE buffer and subjected to freezing and thawing (heating to boiling and snap cooling at -20°C) and the process repeated 3-4 times. Then, 40 µL of lysozyme was added and the tubes incubated at 37°C for 2 h. Proteinase K (6 µL) and 10% Sodium Dodecyl Sulphate (SDS) (56 µL) were added and the tube incubated at 65°C for 30 min. After that 64 µL of CTAB+80 µL 5M NaCl were added and incubated at 65°C for 30 min. Equal volume of Chloroform+Isoamyl alcohol (24:1) was added, mixed properly and centrifuged at 10,000 rpm for 10 min. DNA was precipitated by chilled absolute ethanol and washed with 70% chilled ethanol. DNA pellet was re-suspended in 30 µL 1X TE buffer and stored at -20°C until further use.

From faeces: Faecal samples were centrifuged at 2,600 rpm for 45 min and *bacilli* were concentrated in the interface of solid and watery layers. After discarding the top layer, semi-solid middle layer was collected by sterilized swab and stirred in tubes containing 40 mL 0.9% HPC for decontamination (18-24 h) at room temperature. For DNA isolation, 0.5-1.0 mL of sediment, after discarding the decontaminated layer was kept into a 2.0 mL capacity sterilized eppendorf tubes. Sediment was washed with PBS, 3-4 times by spinning and vortexing. Pellet was subjected to DNA isolation as per method described by Van Embden *et al.* (1993) with some modification.

Polymerase chain reaction (PCR): DNA was isolated from decontaminated fecal and tissue samples were subjected to specific IS900 PCR as per Green *et al.* (1989) and Vary *et al.* (1990). Briefly, PCR was set in volume of 25 µL, using 1.0-5.0 ng template DNA, 12.5 µL of 2X PCR Master Mix (Genei, Bangalore) and 1.0 µL of each primer (10 pmole). Total of 35 cycles were performed in a thermocycler (MJ Research) for complete amplification reaction (Table 1). Thermal cycling conditions for primer 1 were: Initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 10 sec, annealing at 50°C for 10 sec, extension at 72°C for 10 sec and final extension at 72°C for 3 min. Thermal cycling conditions for primer 2 were: Initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 64°C for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 5 min (Table 2, 3 and 4). Presence and yield of specific PCR product was analyzed by 1.5% agarose gel electrophoresis.

Table 1: Details of primers used for PCR

Sequence 5' to 3'	Amplicon size	Reference
Primer 1		
NT 05 (F) GAT CGC CTT GCT CAT CGC TGC CG	218 bp	Green <i>et al.</i> (1989)
NT 06 (R) GAT CGG AAC GTC GGC TGG TCA GG		
Primer 2		
(F) CCG CTA CTT GAG AGA TGC GAT TGG	229 bp	Vary <i>et al.</i> (1990)
(R) AAT CAA CTC CAG CAG CGC GGC CTC G		

Table 2: Faecal and rectal pinch smear examination

Group	Tested n			Positive n (%)		
	M	F	Total	M	F	Total
I	4	52	56	1 ^D (25)	2 ^D +2* ^D =4 (7.6)	5 (8.9)
III	2	18	20	1 (50)	1 (5.5)	2(10.0)
Total	6	70	76	2 (33.3)	5 (7.1)	7 (9.2)

D: Goats died during study, *Two goats found negative by PCR, n: No. of animals

Table 3: Delayed type hypersensitivity (DTH) /Johnin test

Age (year)	Tested n			Positive n (%)		
	M	F	Total	M	F	Total
0.5-1	2	23	25	0 (0.0)	2 (8.7)	2 (8.0)
1-3	5	64	69	1 (20)	17 (26.5)	18 (26.0)
> 3	3	26	29	1 (33.3)	6 (23.0)	7 (24.1)
Total	10	113	123	2 (20)	25 (22.1)	27 (21.9)

n: No. of animals

Table 4: Enzyme linked immuno sorbent assay

Group	Tested n			Positive n (%)		
	M	F	Total	M	F	Total
I	10	113	123	4 (40)	53 (46.9)	57 (46.3)
II	5	71	76	1 (20)	29 (38.1)	30 (39.4)
III	2	18	20	1 (50)	7 (38.8)	8 (40)
Total	17	202	219	6 (35.2)	89 (44.0)	95(43.3)

n: No. of animals

ELISA testing: Serum samples were screened by ‘indigenous ELISA kit’ as per the method of Singh *et al.* (2007b). Briefly, 10 µg plate⁻¹ of MAP ‘Indian Bison type’ protoplasmic antigen was taken in 10 mL of carbonate-bicarbonate buffer (pH 9.6) and coated in duplicate wells of flat bottom 96 well ELISA plates. Plates were incubated at 4°C overnight, washed thrice with washing buffer (PBS+0.05% Tween-20, pH 7.4- PBST), blocked (3% skimmed milk powder in PBS) and incubated at 37°C for 1 h. Plates were washed thrice with PBST and stored at 4°C till further use. *M. phlei* (Allied Monitor Inc., USA) was mixed in serum dilution buffer before the dilution of each test serum sample and plate used for serum dilution was incubated for one hr at 37°C. After incubation, 100 µL of each adsorbed test serum was transferred to duplicate wells of ELISA plate and plates incubated for 2 h at 37°C. Then the plates were washed thrice with PBST. After washing, 100 µL of optimally diluted (1:8000) rabbit anti-goat horse radish peroxidase conjugate was added to wells and incubated at 37°C for 1 h. Plates were washed thrice with PBST and 200 µL of freshly prepared substrate Orthophenyl diamine (OPD), 5 mg plate⁻¹ in substrate buffer (pH-5.0) was added to

each well and incubated in dark for 30 min at room temperature. Absorbance was read at 450 nm in an ELISA reader.

Data analysis and statistics: Data were managed in Tables created using Microsoft Office Excel; Microsoft Corp., Redmond, Washington, USA. Positive and strong positive samples were considered positive for MAP infection in ELISA as per Collins (2002) by converting the OD values into sample-to-positive (S/P) ratio (using formula; Sample OD-Negative OD/Positive OD-Negative OD in Excel cells). Relative sensitivity, specificity, kappa values and proportional agreement were calculated comparing all the five tests with each other (Table 1 and 2).

RESULTS AND DISCUSSION

In animals and human beings, infection of *Mycobacterium avium* subspecies *paratuberculosis* have been associated with chronic inflammation of the intestines and mesenteric lymph nodes in animals (JD) and Inflammatory Bowel Disease (IBD) or Crohn’s disease

Table 5: Agar gel immuno diffusion (AGID)

Group	Tested n			Positive n (%)			
	M	F	Total	M	F	Total	
I	10	113	123	1 (10)	14 (12.3)	15(12.2)	
II	5	71	76	0 (0.0)	5 (7.0)	5 (6.5)	
III	2	18	20	1 (50)	3 (16.6)	4 (20)	
Total	17	202	219	2 (11.7)	22 (10.8)	24(10.9)	

n: No.of animals

Table 6: IS 900 Polymerase chain reaction (PCR)

Group	Tested (n)			Positive n (%)		
	M	F	Total	M	F	Total
I	4	24	28	1 (25)	2 (8.3)	3 (10.7)
III	2	10	12	1 (50)	1 (10)	2 (16.6)
Total	6	34	40	2 (33.3)	3 (8.8)	5 (12.5)

n: no. of animals

in human beings (Sweeney *et al.*, 1998; Sechi *et al.*, 2001, 2005). For the diagnosis of MAP infection, the choice of the diagnostic test is essential for the success of the control programs but it depends on the purpose and should invariably involve multiple tests in case of chronic MAP infections (Singh *et al.*, 2009a). In the faecal smear examination, out of 56 goats of Group-I, 5 (8.9%) goats were positive, whereas, in Group-III, out of 20 goats, 2 (10.0%) goats were positive. Of a total of 76 goats screened, 7 (9.2%) were positive (Table 5) for MAP. Out of 5 faecal positive goats of Group-I, 3 died during the study. Clinical signs were observed in all the 7 faecal smear positive goats and these were also positive in ELISA and AGID. Tripathi *et al.* (2006) showed that out of 36 known cases of caprine paratuberculosis diagnosed by clinical and faecal smear examination, 72.2% goats were shedding MAP *bacilli*. Faecal smear examination with acid-fast staining detected more goats as positive as compared to bacterial culture and PCR (Munjal *et al.*, 2007). Screening of 71 animals (55 goats and 16 sheep) belonging to Central Institute for Research on Goat (CIRG) located at Makhdoom (India) revealed that 40% goats and 31.2% sheep were shedding MAP in faeces (Singh *et al.*, 2007a, b).

Rectal pinch smear examination was carried out in 27 DTH/Johnin positive goats. All the rectal pinch smears were negative for the presence of MAP. In accordance to this, OIE (2000) has described that the smears of rectal mucosa biopsies are challenging to get and may not be very helpful because the disease process is less likely to involve the rectum in sheep and goats (<http://ohioline.osu.edu/vme-fact/0003.html>). Of the 123 goats of Group-I, 27 (21.9%) were positive in DTH test. Out of 5 faecal positive goats which also showed clinical signs, 2 (3.5%) goats died during the study were negative by Johnin test. Similar to these findings, Paliwal and Rajya (1982) and Rajukumar (1998) stated that sensitivity of



Fig. 1: Mehsani goat breed from south Gujarat

Johnin test in goats ranged between 18-30% with least specificity in both preclinical and advanced stage of disease. Tripathi *et al.* (2006) reported that out of 34 cases of caprine paratuberculosis, 73.5% goats were positive for Johnin test. In the present study, of 5 infected goats, 3 (60%) were positive in Johnin test. For screening of MAP antibodies, ELISA is a better choice as it improves the rate of detection or better estimation of MAP infection. There is moreover significant increase in the rate of detection (Braun *et al.*, 1990). Of the 219 goats tested, 95 (43.3%) were positive by ELISA. Of 123 Mehsani goat breed (Group-I) (Fig. 1), positive reactors were higher 57 (46.3%) and had history of recurrent diarrhea (Table 6). Sweeney *et al.* (1995) reported that sensitivity of ELISA was only 15.0% in animals excreting low quantities of MAP *bacilli* in their faeces as compared to 87.0% in animals with clinical signs of Johnes' disease. In this study, similar results were obtained i.e., all the 7 goats showing clinical signs as well as faecal smear positivity were also positive by ELISA. Paolicchi *et al.* (2003) and Singh *et al.* (2009b) observed that absorbed ELISA was useful to detect positive animals and those goats shedding MAP in faeces. Similarly, in the

present study all the animals shedding MAP in feces were positive in the ELISA test also. Indigenous ELISA kit developed by CIRG, Makhdoom was most sensitive and specific test for the detection of MAP infection (OIE, 2000).

As far as the AGID test is concerned it is well proven that animals either in the early stage or advanced stage of the disease are often found negative and there is every possible of chance of obtaining either false positive or false negative result (Stabel, 1998). Of 219 goats tested in the present study, 24 (10.9%) were positive by AGID. Group-III consisting of 20 non-descript goats showed higher number (4 or 20%) of goats as positive reactors. All the 7 goats showed clinical signs and were positive in smear examination and AGID. Similarly, Sherman *et al.* (1984) recorded that out of 33 AGID positive cattle, 32 (96.9%) were confirmed for MAP infection by culture or necropsy. Similarly, Ferreira *et al.* (2002) found AGID specificity was 92.5% and sensitivity was 57%. Mainar-Jaime *et al.* (1998) recorded 11.7% (64 of 546) sero-prevalence of MAP in sheep and goat flocks using AGID test.

PCR was carried out on faecal samples of suspected animals of Group-I and III with history of recurrent diarrhoea or pasty faeces and tissue samples of three goats died in Group-I. Of 40 goats, 7 showed clinical signs and were also positive by faecal smear examination (Fig. 2). Among these 7 goats, faecal PCR detected 5 goats as positive while 2 were negative. Munjal *et al.* (2007) observed that faecal smear examination with acid fast staining detected more goats to be positive than bacterial culture and PCR. Microscopic view of acid fast bacilli has been shown in Fig. 2. Previous studies also reported the higher prevalence of MAP in farm herds as compared to farmer's herds (Kumar *et al.*, 2007). Tissue samples of all the 3 goats died during study were positive by PCR (Fig. 3). In the last two decades with the advent of molecular assays like IS900 progress has been made in order to differentiate MAP strains as well as isolates for classifying them into various types and subtypes (Sohal *et al.*, 2009). None can deny the fact that IS900 sequence is the most commonly targeted sequence for confirmation of MAP at molecular level. There are however certain concerns that the sequence may not be

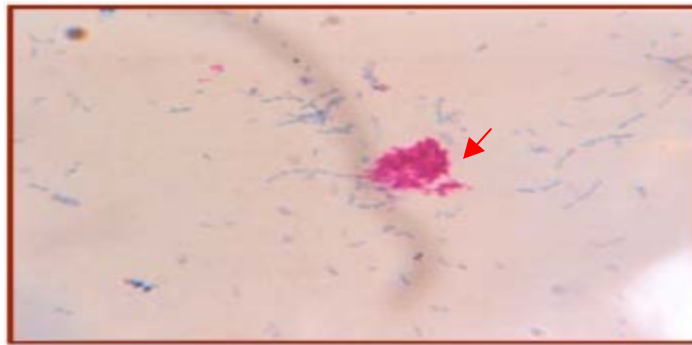


Fig. 2: Microscopic view of acid fast *bacilli* (indistinguishable to MAP)

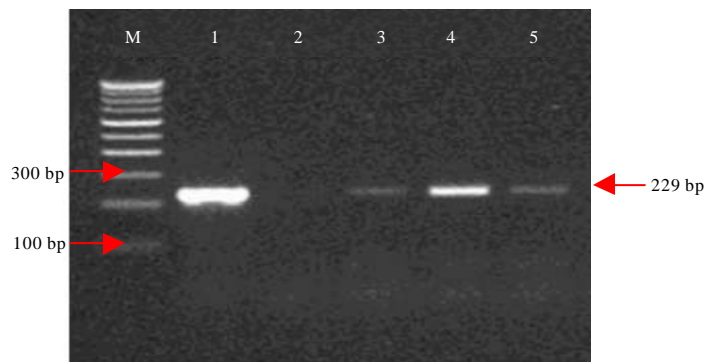


Fig. 3: MAP specific amplicons (229 bp) using IS900 PCR, Lane M: 10 bp DNA marker, Lane 1: Positive control (MAP DNA), Lane 2: Negative control (miliQ water), Lane 3-5: Tested DNA samples



Fig. 4: Gross changes showing enlargement of mesenteric and ileocecal lymph nodes



Fig. 6: Gross changes showing affected rectum



Fig. 5: Gross changes showing corrugation and thickening of intestinal mucosa

100% unique to MAP. Due to homologous IS900-like elements in related mycobacterial species false positive results can occur due to cross reaction (Deb *et al.*, 2011). Stabel (1998) reported that IS900 based PCR could be more sensitive in tissue samples in confirming diagnosis at necropsy (Singh *et al.*, 2013a, b).

All the three goats died during the present study in Group-I were subjected to post mortem examination which revealed enlargement of mesenteric and ileo-cecal lymph nodes (Fig. 4) and thickening of ileum and ileo-cecal junction wall. In one goat slight corrugation of ileal mucosa was observed. Carcass showed depletion of fat depots. Rajukumar (1998) observed similar lesions with enlarged, edematous and congested mesenteric and ileocecal lymph nodes (Clarke, 1997; Collins *et al.*, 2001). Corrugation of ileal mucosa (Fig. 5) observed in only one goat as described into Ohio State University Fact Sheet for Johne's disease, that sheep and goats may exhibit some gross and microscopic lesions not typical of those seen in cattle. Ridges and thickening of the small intestine (Fig. 5) and caecum are not always seen as is common in cattle. Gross changes present in rectum are indicated in Fig. 6 (Stabel, 1998; Sechi *et al.*, 2005).

Storset *et al.* (2001) observed more number of goats with lesions in jejunum as compared to ileum while the lesions in ileum were detected in only one goat. In contrast to this lesions in jejunum were not observed in the present study. Histopathological changes in goats died of JD revealed slightly congested intestinal mucosa with infiltration of mononuclear cells consisting of lymphocytes and macrophages in lamina propria. Villi were thickened and blunt at some places. Mesenteric lymph nodes exhibited infiltration of lymphocytes and macrophages. Buergelt and Ginn (2000) as well as Munjal *et al.* (2005) observed similar lesions such as thickening of the intestinal villi with flat and wide tips, infiltration of lymphocytes, macrophages and epitheloid cells in lamina propria of intestine and inter-follicular area of mesenteric lymph nodes. Similar histopathological changes were also observed by Paliwal and Rajya (1982) and Rajukumar (1998).

In conclusion, the utility of multiple diagnostic tests including of conventional, serological and recent molecular tools is suggested for reaching to a confirmatory diagnosis of MAP infection in animals and studying the prevalence and epidemiology this important disease (JD). The advances in molecular diagnostic tools (Deb *et al.*, 2011, 2013; Dhama *et al.*, 2013a, b), inventing effective prophylactics (Dhama *et al.*, 2008, 2013c), novel therapeutic regimens (Mahima *et al.*, 2012; Dhama *et al.*, 2013d, e; Tiwari *et al.*, 2013b) along with suitable preventive and control measures (Tripathi *et al.*, 2002; Momotani, 2012; Sweeney *et al.*, 2012) have to be adapted and explored fully for alleviating huge economic impacts due to this significant pathogen of animals having public health concerns, particularly in the Era of One Health, One Medicine (Grant, 2005; Dhama *et al.*, 2013e).

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