

# Detection of *Mycobacterium avium* subspecies *Paratuberculosis* (MAP) from goats of Jabalpur Region in India

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## ABSTRACT

Paratuberculosis or John's disease (JD) is one of the major economically important diseases of small ruminants worldwide. The disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The present study was carried out to detect *Mycobacterium avium* subspecies *paratuberculosis* from goats of the Jabalpur region. The study was conducted for eight months, from July 2017 to February 2018. Goats of either sex, above 6 months of age, belonging to different breeds were selected for this study. Total 230 samples (160 faecal and 70 tissues) were stained by Z-N staining method. The study revealed that 5% fecal and 7.14 % tissue samples were found positive for MAP.

**Keywords:** Acid-fast bacilli Goats, Jabalpur, Paratuberculosis, Ziehl-Neelsen.

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## INTRODUCTION

Paratuberculosis is recognized worldwide as one of the most economically important diseases caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a slow-growing acid-fast bacterium. Paratuberculosis has been frequently reported from farm herds and sacrificed/ slaughtered goats. Most losses occur due to the subclinical stage of diseases, increased susceptibility to other diseases, and eventually death (Kumar *et al.*, 2007). Therefore, the bacterium has also been implicated in Crohn's disease and is now considered a public health concern. The disease is regarded as incurable (Behr and Kapur, 2008).

In the absence of clear clinical symptoms, diagnosis of paratuberculosis is a challenge due to prolonged incubation, complex biology and pathogenesis and intracellular nature of bacilli, and poor sensitivity and specificity of diagnostic tests for the detection of subclinical infection (Chiodini *et al.*, 1984). Identification of subclinically infected animals is difficult as there is variation in the shedding of the MAP with the stage of infection (Chaturvedi *et al.*, 2017).

A major obstacle in the control of this disease is the difficulty of identifying infected animals, especially those in the subclinical stage or early clinical phase of infection (Sigurdardottir *et al.*, 1999). The caprine paratuberculosis is endemic in goat herds. Therefore the quick and accurate diagnosis is the need of the hour (Olsen *et al.*, 2002).

Early detection of MAP may be useful in monitoring the progression of paratuberculosis in ruminants. Fecal microscopy will be beneficial in early accurate diagnosis of the infection and provide links in establishing epidemiology of MAP infection in goats. The objective of the present study was to detect *Mycobacterium avium* subspecies *paratuberculosis* (MAP) from goats of the Jabalpur region using Z-N staining.

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## MATERIALS AND METHODS

The present study was carried out in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Jabalpur (MP). For a period of eight months from July 2017 to February 2018. Total 160 live and 70 dead/slaughtered goats were included in the present study. Goats above 6 months of age were selected randomly irrespective of sex and breed from the Goat Farm Amanala, goats registered at Teaching Veterinary Clinical Complex (TVCC), CoVSc and AH, Jabalpur, and goats from surrounding areas of Jabalpur region and from different slaughterhouses. Complete history *viz.* age, sex, breed, and health status of individual goat was recorded, and detailed clinical examination was carried out. For detection of MAP in live animals, 160 fecal samples were collected directly from the rectum of goats aseptically using sterile gloves. About 2-5 gm of fecal samples were collected from each goat and placed in sterile zipped polythene. For detection of MAP in dead/slaughtered animals, a total of 70 tissue samples

(intestine, mesenteric and ileocaecal lymph nodes ) were collected from different slaughterhouses of the Jabalpur region and animals bought for post-mortem to TVCC college of veterinary Sciences and AH Jabalpur. All the samples were transported to the laboratory under refrigerated condition (4°C) and were processed within 4-6 h of collection. From each goat, representative tissue samples were collected and transported to the laboratory under refrigerated conditions (-4°C). An individual tissue sample was processed for smear preparation.

### Ziehl Neelsen (Z-N) Staining

Collected fecal samples were processed to prepare fecal smears as per the method described by Barad *et al.* (2014) with slight modifications. Tissue smears were prepared from scraping of the intestinal mucosal surface, and impressions from mesenteric and ileocaecal lymph nodes on clean microscopic slides were allowed to be air-dried and heat-fixed.

Heat-fixed fecal smears, intestinal scarring smears and lymph node impression smear were stained with Ziehl-Nelson staining by using Z-N staining kit manufactured by Hi-media. Each slide was examined using oil immersion. Smear exhibiting the presence of clumps of short acid-fast bacilli was considered positive for MAP and in dispersed

form as suspected for MAP and negative if neither of the two forms was observed.

## RESULT AND DISCUSSION

On examination, the 160 goat fecal smears stained by Ziehl-Neelsen staining revealed that 08 (5.00 %) (Fig. 1) fecal smears were positive for the presence of typical clumps or individual pink colored, rod-shaped acid-fast bacilli (AFB) in the blue background under X 1000 magnification. In contrast, 7.14% (5/70) Intestine and lymph nodes smears showed typical acid-fast bacilli either individually or in clumps (Fig. 2). The intestine smears revealed acid-fast bacilli in clumps of 10 to 20 organisms in the cytoplasm of macrophages. The lymph node smears showed, individually or clump of three or more acid-fast bacilli in the cytoplasm of macrophages or outside of the cells.

The low (5%) prevalence of MAP in fecal samples was in corroboration with the earlier workers. Sulficar *et al.* (2009) reported a 2% prevalence of paratuberculosis in fecal samples of healthy goats in Kerala. In the present study low prevalence rate might be due to samples collected from healthy goats or maybe because of intermittent shedding of MAP in feces. On the contrary, various workers reported higher prevalence, from 21.6% to 77.5 % in goats (Singh *et al.*, 2010; Shah *et al.*, 2012; Singh *et al.*, 2013 and Bhat *et al.*, 2018) from various places of the country. In the present investigation, 7.14 percent of the intestine and lymph nodes smear showed typical acid-fast bacilli inside the cytoplasm of macrophages or outside the cells. The low prevalence rate observed in this study are very similar to that reported by earlier workers as 3.07% by Beygi *et al.* (2003) and 2% by Hajikolaei *et al.* (2006) in the direct smear of tissues for the presence of acid-fast bacilli by ZN staining. However, the higher prevalence (11 %) was also reported by Hailat *et al.* (2010), 12.76% by Sikander *et al.* (2013), 31.08% by Hajra *et al.* (2014), and 25% by Thakur *et al.* (2017). Moreover, Chiodini *et al.* (1984) and Harris and Berletta, (2001) reported that clumps of bacteria are shed only in the clinical stage of infection, and the animal in the subclinical stage eliminates only a few or no bacilli were observed.

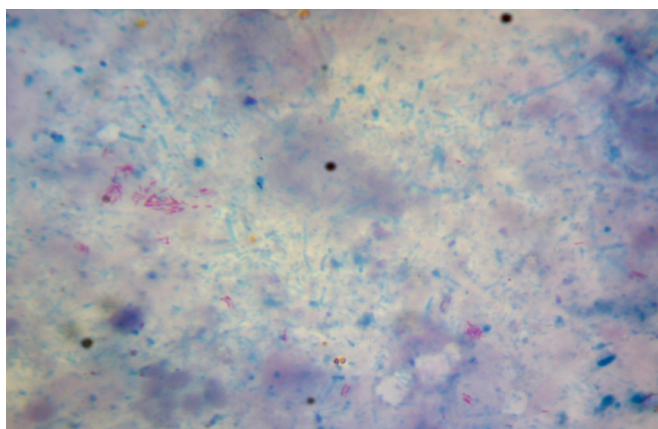


Fig. 1: Faecal smear showing acid-fast bacilli (arrow).

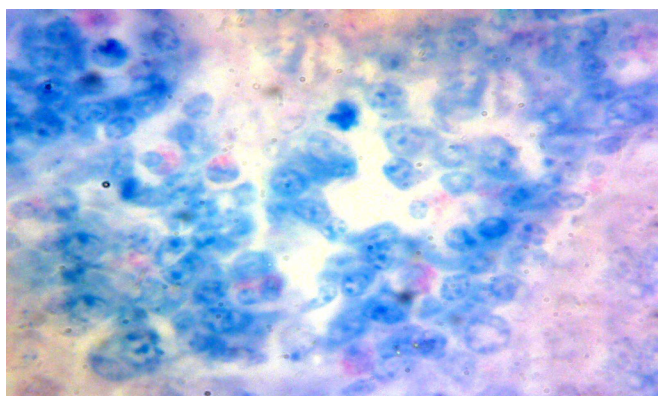


Fig. 2: Tissue scraping showing pink coloured, rod-shaped acid-fast bacilli (arrow).

## CONCLUSION

In the present investigation, a low prevalence rate in the fecal samples compared to the tissue samples by Ziehl-Neelsen staining was observed, which might be due to the early presence of bacteria in the tissue samples compared to fecal samples.

## REFERENCES

- Barad, D.B., Chandel, B.S., Dadawala, A.I., Chaouhan, H.C., Kher, H.S., Shroff, S., Bhagat, A.G., Singh, S.V., Singh, P.K., Singh, A.V., Soheli, J.S., Gupta, S., Chaubey, K.K., Chakarborty, S., Tiwari, R., Deb, R. and Dhama, K. (2014). Incidence of *Mycobacterium avium* subspecies *paratuberculosis* in Mehsana and Surti goats

- of Indian origin using multiple diagnostics tests. *Journal of Biological Sciences*, **14**(2): 124-133.
- Behr, M.A. and Kapur, V. (2008). The evidence for *Mycobacterium paratuberculosis* in Crohn's disease. *Current Opinion Gastroenterology Journal*, **24**: 17-21.
- Beygi, Y., Ramin, G.A.G., Faraji, V. and R. A. (2003). Study on the prevalence of subclinical cattle Johne's disease in the Urmia Abattoir. *Archives of Razi Institute*, **55**: 63-70.
- Bhat, A.M., Malik, H.U., Singh, S.V., Hussain, T., Chaubhey, K.K., Mir, M.S., Qureshi, S., Kashoo, Z.A., Nabi, S., Rehman, M., Yousuf, R.W. and Qadri, SI (2018). Bio-prevalence and molecular diagnosis of *Mycobacterium avium* subspecies *paratuberculosis* infection in small ruminant population of Ganderbal district of Kashmir valley. *Journal of Entomology and Zoology Studies*, **6**(1): 01-04.
- Chaturvedi, S., Singh, S.V., Srivastava, A.K., Ganagwar, N.K., Kumar, N., Rawat, K.D., Gupta, S., Chaubey, K.K., Singh, R., Singh, R. and Dhama, K. (2017). Comparative evaluation of FAT, IS900 PCR and microscopy vis a vis histopathology for the detection of *Mycobacterium avium* subsp *paratuberculosis* infection in tissues of goats naturally died in herds endemic for Johne's disease. *Indian Journal of Animal Sciences*, **87**(6): 18.
- Chiodini, R.J., Van Kruiningen, H.J. and Merkal, R.S. (1984). Ruminant paratuberculosis (Johne's disease): the current status and future prospects. *Cornell Veterinarian*, **74**: 218-62.
- Hailat, N.Q., Hananeh, W., Metekia, A.S., Stabel, J.R., Al-Majali, A. and Lafi, S. (2010). Pathology of subclinical paratuberculosis (Johne's disease) in Awassi sheep with reference to its occurrences in Jordan. *Veterinari Medicina*, **55**(12): 590-602.
- Hajikolaie, M.R., Ghorbanpoor, M. and Solaymani, M. (2006). The prevalence of *Mycobacterium paratuberculosis* infection in ileocecal valve of cattle slaughtered in Ahvaz abattoir, Southern Iran. *Iranian Journal of Veterinary Research*, **7**(2): 77-80.
- Hajra, S., Singh, S.V., Srivastava, A.K., Chakraborty, S. and Dhama, K. (2014). Pathobiology of spontaneous and experimental paratuberculosis (S-5 strain) in goats with special references to early lesions. *Asian Journal of Animal and Veterinary Advances*, **9**(8): 467-478.
- Harris, N.B. and Berletta, R.A. (2001). *Mycobacterium Avium* subsp. *paratuberculosis* in veterinary medicine. *Archive of Clinical Microbiology Reviews*, **14**(3): 489-512.
- Kumar, P., Singh, S.V., Bhatiya, A.K., Sevila, I., Singh, A.V., Whittington, R.J., Juste, R.A., Gupta, V.K., Singh, P.K., Sohal, JS and Vihan, V.S. (2007). Juvenile Capri-paratuberculosis (JCP) in India: incidence and characterization by six diagnostic tests. *Small Ruminant Research*, **73**: 45-53.
- Olsen, I., Sigurdardottir, O.G., Djonne, B. (2002). Paratuberculosis with special reference to cattle- a review. *Veterinary Quarterly*, **24**: 12-28.
- Shah, I.H., Darzi, M.M. and Mir, M.S. (2012). Comparative efficacy of rectal pinch, faecal smear and faecal polymerase chain reaction tests for surveillance of paratuberculosis in goats (*Capra hircus*). *Sher-e-Kashmir University of Agriculture Sciences and Technology of Kashmir Journal of Research*, **14**: 17-23.
- Sigurdardottir, O.G., Press, C.M., Sexgard, F. and Evensen, O. (1999). Bacterial isolation immunological response phase of experimental infection of goat kids with *Mycobacterium avium* subsp. *paratuberculosis* , *Veterinary Pathology*, **36**: 542-550.
- Sikandar, A., Cheema, A.H., Adil, M., Younus, M., Zaneb, H., Zaman, M.A., Tipu, Y. and Masood, S. (2013). Ovine paratuberculosis: a histopathological study from Pakistan. *The Journal of Animal and Plant Sciences*, **23**(3): 749-753.
- Singh, P.K., Singh, S.V., Kumar, H., Sohal, JS and Singh, A.V. (2010). Diagnostic Application of IS900 PCR Using Blood as a Source Sample for the Detection of *Mycobacterium avium* Subspecies *Paratuberculosis* in early and subclinical cases of caprine paratuberculosis. *Veterinary Medicine International*, **10**: 1-8.
- Singh, S.V., Singh, P.K., Gupta, S., Chaubey, K.K., Singh, B., Kumar, A., Singh, A.V. and Kumar, N. (2013). Comparison of microscopy and blood PCR for the diagnosis of clinical Johne's disease in domestic ruminants. *Indian Journal of Veterinary Research*, **14**(4): 354-349.
- Sulficar, S., Saseendranath, M.R., Krishnan, N.G., Tresamol, P.V. and Pillai, U.N. (2009). Comparative efficacy of various diagnostic tests for caprine paratuberculosis: a field study. *Journal of Veterinary Animal Sciences*, **40**: 35-36.
- Thakur, M., Maity, M. and Gupta, V.K. (2017). Pathology of naturally occurring paratuberculosis in Gaddi goats of Himachal Pradesh. *Indian Journal of Veterinary Pathology*, **41**(1): 25-30.

