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DIAGNOSIS OF THE *Mycobacterium avium* subspecies *paratuberculosis* IN TISSUES OF SLAUGHTERED BUFFALOES USING FLUORESCENT ANTIBODY TEST

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ABSTRACT

Paratuberculosis (pTB) caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), infects domestic livestock leading to chronic diarrhoea (persistent, intermittent or no diarrhoea) and loss in body condition and weights. It is a major disease that adversely affects health and productivity resulting in colossal monetary losses to the livestock industry throughout the world. Association of MAP with inflammatory bowel disease / Crohn's disease (CD) in humans, established its zoonotic potential and public health significance. Tissues (corrugated mucosal folds) of 20 buffaloes (both the sexes, aged 1 to 10 years) from Malwa division of Madhya Pradesh were anlyzed by fluorescent antibody test (FAT). Out of 20 tissue smears screened, 11 (55.0%) were positive for MAP by FAT.

Keywords: *Bubalus bubalis*, buffaloes, fluorescent antibody test, MAP, Malwa region

INTRODUCTION

The MAP, the causative agent of paratuberculosis (pTB) is a chronic, persistent and incurable infection of domestic ruminants (buffaloes, cattle, sheep, goats) world-wide. Bacilli infects primarily large intestines in buffaloes causing transverse corrugations and granulomatous lesions in mucosa leading to diarrhoea (continuous or intermittent), weight loss and emaciation causing huge economic losses in livestock industry globally (Sweeney, 1996). These losses occur due to reduced productivity, low fertility, increased calving interval, premature culling, decreased milk and meat yield, increased treatment cost and culling besides zoonotic importance (McNab *et al*., 1991). Using modified Zeihl-Neelsen (ZN) staining of faecal samples, Jatav *et al*. (2018a) reported 79.33% prevalence of MAP infection faecal samples of buffaloes. MAP infection was also reported on the basis of gross pathological lesions and impression smear examination (Jatav *et al*., 2017) and by indigenous ELISA kit (Jatav *et al*.,

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2018b). Information was lacking on the prevalence of paratuberculosis in buffaloes of Malwa division of Madhya Pradesh. This was mainly due to non-availability of indigenous diagnostic kits and high cost of imported kits. Therefore, in the present investigation, help of referral laboratory for paraTB located at ICAR-CIRG, Mathura was taken, and FAT was conducted for diagnosis of paratuberculosis in tissue smears of buffaloes of Malwa region using fluorescent antibody test.

MATERIALS AND METHODS

Intestinal tissues were collected from slaughtered buffaloes in the sterile polythene bags and taken to the laboratory and were kept at -20°C in for further processing. Diagnosis of JD was made on the basis of detection of acid-fast bacteria (MAP) in the target tissues using fluorescent antibody test (FAT). Samples were properly labelled and documented. The FAT was carried out in tissue smears as per the method of Gilmour and Angus (1976) and modified by Singh *et al.* (2016). Tissue smears were prepared from aseptically triturated tissues (intestines and mesenteric lymph nodes) in sterilized pestle and mortar. After trituration, triturated material was centrifuged at 3000 rpm and smears were prepared from the sediment which was air dried followed by heat fixing. Slides were dipped in solution of 30% H_2O_2 in 90% methanol (3:7 ratio) and were incubated for 10 minutes at 37°C and were subsequently dipped in phosphate-citrate buffer (2.1% citric acid and 3.56% disodium hydrogen phosphate in 100 ml triple distilled water, pH -5) and were heated till boiling in microwave for 30 seconds (15 cycles) and a rest of 20 seconds was given after each heating cycle and this entire procedure was completed in 10 minutes. Air drying of slides was done at room temperature. Then primary antibody (whey as control in ratio of 1:4 and serum in ratio of 1:50) in serum dilution buffer (1% BSA in PBST) was poured on the slides. Further, the slides were incubated for 1 h at 37 °C in BOD incubator and were washed thrice in 1X PBS. Anti-species secondary antibody (FITC conjugate) was added in the ratio of 1:750 in 1X PBS (pH -7.6). Incubation of slides in dark was done for a period of one hour at 37 °C and 5 times washing of slides was done in 1X PBS in dark followed by air drying of slides was done in dark at room temperature. At last, slides were mounted with glycerine, covered with cover slip and examined immediately under fluorescent microscope. For positive control, smear prepared from the heat killed MAP culture was used for comparing the results. Positive slides for MAP infection showed green coloured fluorescence under fluorescent microscope (Figure 2).

RESULTS AND DISCUSSIONS

In the present study, 20 tissue samples of intestine and mesenteric lymph nodes were

Table 1. Detection of MAP organisms using fluorescent antibody test (n=20).

S. No.	Status	No. of animals	Incidence (%)
1.	Positive	11	55
2.	Negative	09	45

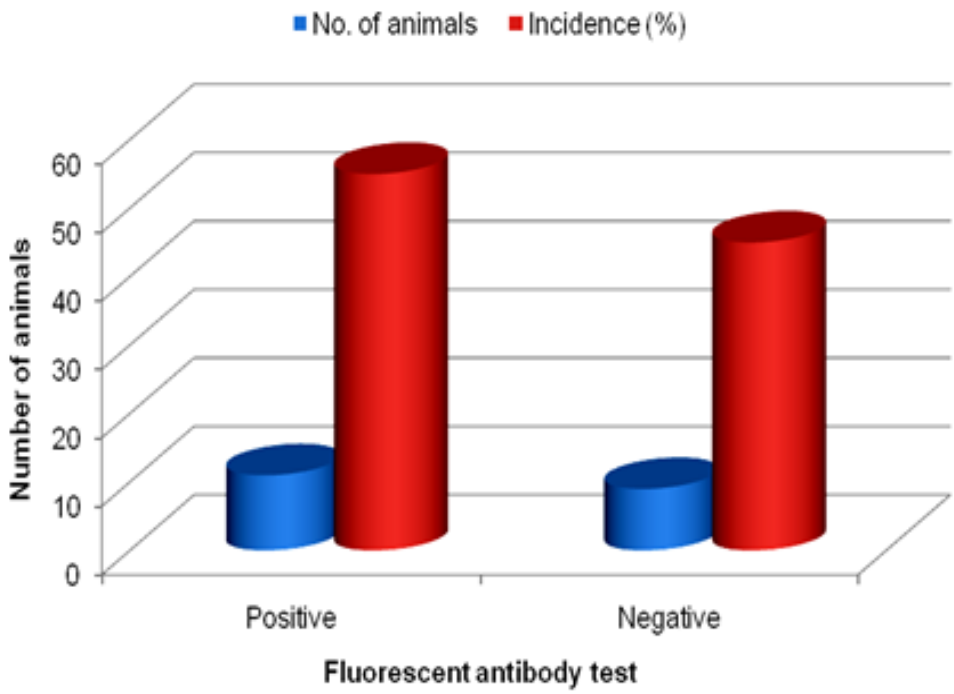

Figure 1. Detection of MAP using fluorescent antibody test.

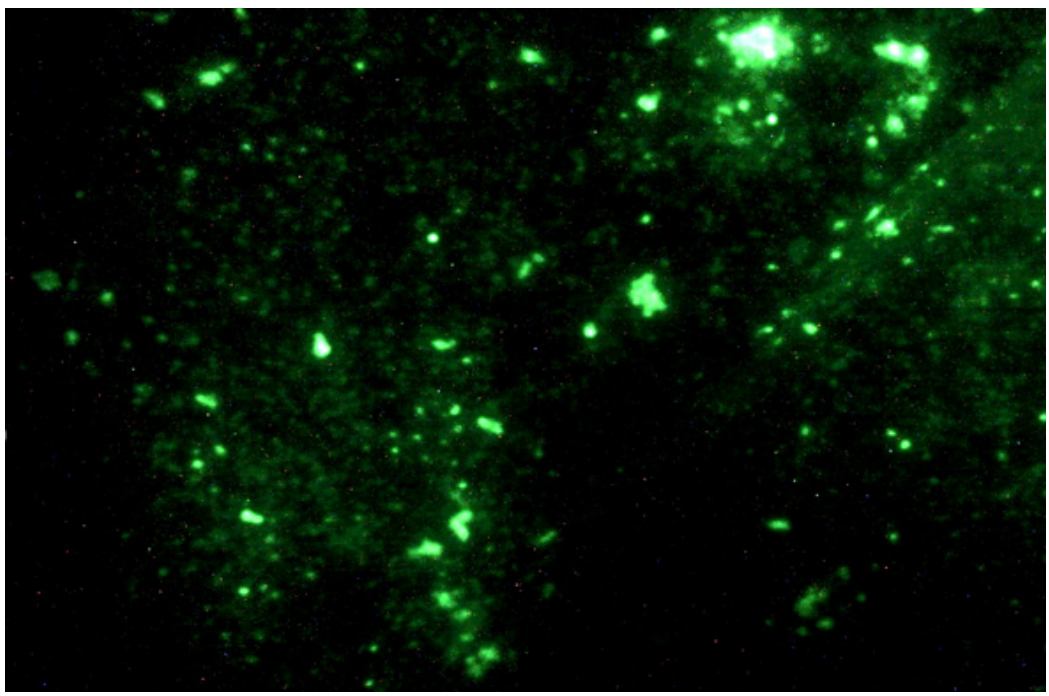

Figure 2. Photomicrograph showing clumps of fluorescent green colour rods of MAP organisms (FAT, 1000X) (arrows).

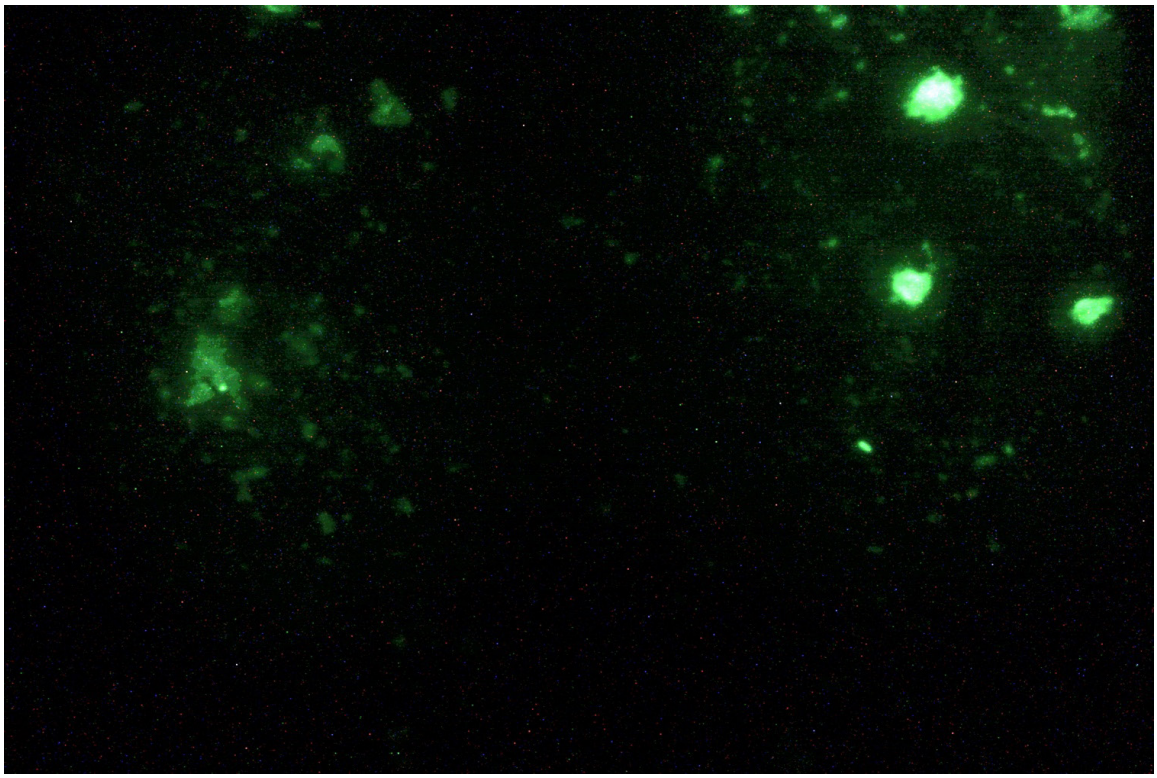

Figure 3. Photomicrograph showing fluorescent green coloured rod of MAP organism (FAT, 1000X) (arrow).

screened for confirmation of MAP bacilli using fluorescent antibody test. Of 20 intestinal tissues, specially corrugated mucosal fold tissue smears, 11 (55.0%) were positive for MAP infection. The MAP bacilli were seen as green fluorescence in the smears under fluorescent microscope (Table 1 and Figure 1, 2 and 3). The present study demonstrated the presence of MAP bacilli by FAT in tissue smears prepared by trituration of mucosal folds of intestinal tissue. Findings of the present investigation are in line with D'Haese *et al.* (2005) as this method detected very a smaller number of MAP organisms in pasteurized milk with 73.0% sensitivity. According to them, results of FAT may help to detect MAP organisms in tissues. Present findings are also in accordance with the results of Stephen *et al.* (2016) reporting 63.6% MAP positive cases in the paneer samples of cow milk using FAT for anti-MAP antibodies. Further, Singh *et al.* (2016) screened 372 milk samples (207 goats and 165 bovines) for the diagnosis of anti-MAP antibodies by indirect FAT and recorded 174 (46.7%) samples positive for MAP antibodies, which supported the present investigation in case of buffaloes of Malwa region (Madhya Pradesh), where the rate of MAP infection was also high.

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REFERENCES

D'Haese, E., I. Dumon, H. Werbrouck, V. D'Jonghe and L. Herman. 2005. Improved detection of mycobacterium paratuberculosis in milk. *J. Dairy Res*., **72**(S1): 125-128. DOI: 10.1017/s0022029905001226

Gilmour, N.J. and K.W. Angus. 1976. The specificity and sensitivity of the fluorescent antibody test in cattle experimentally infected with *Mycobacterium avium* and *Mycobacterium johnei*. *Res. Vet. Sci*., **20**(1): 6-9. DOI: 10.1016/S0034-5288(18)33470-2

Goudswaard, J., R.H. Van Dam and A.P.M.G. Bertens. 1977. Comparison of fluorescent antibody tests for tuberculosis and paratuberculosis with antigens coupled to insoluble spheres or taken up by macrophages. *Vet. Microbiol*., **2**(1): 39-47. DOI: 10.1016/0378-1135(77)90030-X

Jatav, G.P., U.K. Garg, S. Shukla, D. Chhabra, A.K. Jayraw, N. Shrivastava, S.K. Karmore and V. Agrawal. 2017. Incidence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection in slaughtered buffaloes (*Bubalus bubalis*) of Malwa region of Madhya Pradesh. *Indian Journal of Veterinary Sciences and Biotechnology*, **13**(2): 16-20.

Jatav, G.P., A.K. Jayraw, N. Shrivastava, R. Sikrodia, V. Agrawal and S.K. Karmore. 2018a. Detection of *Mycobacterium avium* subsp. *Paratuberculosis* (MAP) in faeces of buffaloes (*Bubalus bubalis*) from Malwa region of Madhya Pradesh. *International Journal of Livestock Research*, **8**: 73-78. DOI: 10.5455/ijlr.20170830105736

Jatav, G.P., S. Shukla, A.K. Jayraw, D. Chhabra, V. Agrawal, S.V. Singh and S.K. Karmore. 2018b. Detection of anti-MAP antibodies in serum samples of slaughtered buffaloes (*Bubalus bubalis*) by indigenous ELISA kit in Malwa region of Madhya Pradesh. *J. Anim. Res*., **8**(2): 1-3. Available on: https://ndpublisher.in/admin/issues/JARv8n2x.pdf

McNab, W.B., A.H. Meek, S.W. Martin and J.R. Duncan. 1991. Associations between dairy production indices and lipoarabinomannan enzyme-immunoassay results for paratuberculosis. *Can. J. Vet. Res*., **55**(4): 356-361.

Singh, S.V., B.J. Stephen, M. Singh, S. Gupta, K.K. Chaubey, Sahzad, S. Jayaraman, T.K. Sachan, G.K. Aseri, M. Dutta, J.S. Sohal, K. Dhama, S.Y. Mukartal and D. Rathnamma. 2016. Evalution of "indirect fluorescent antibody test" as potential screening test for *Mycobacterium avium* subspecies paratuberculosis using milk of lactating domestic livestock. *Journal of Experimental Biology and Agricultural Sciences*, **4**(5): 533-540. Available on: https://www.cabidigitallibrary.org/doi/pdf/10.5555/20163325717

Stephen, B.J., M. Singh, S.V. Singh, S. Gupta, K.K. Choubey, Sahzad, S. Jayaraman, M. Jain, J.S. Sohal, S.Y. Mukartal and K. Dhama. 2016. Bio-contamination estimation of MAP in fresh cottage cheese (Paneer) sold in rural, semi urban and peri urban regions of south U.P. using multiple diagnostic tests. *Advances in Animals and Veterinary Sciences*, **4**(8): 441-449. DOI: 10.14737/journal.aavs/2016/4.8.441.448

Sweeney, R.W. 1996. Transmission of paratuberculosis. *Vet. Clin. N. Am. Food-A*., **12**(2): 305-312. DOI: 10.1016/s0749-0720(15)30408-4