# DIAGNOSIS OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* BY DOT- ELISA IN BUFFALOES OF MALWA REGION (MADHYA PRADESH, INDIA)

**Gaya Prasad Jatav[1,*], Ravi Sikrodiya[2], Anantrao Kashiram Jayraw[3], Shyamsagar Keshavrao Karmore[4], Shoorvir Singh[5], Daljeet Chabbra[2], Nidhi Shrivastava[1], Vivek Agrawal[3] and Supriya Shukla[1]**

## ABSTRACT

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) is an etiological agent of paratuberculosis (JD), causing chronic persistent diarrhoea in small and large ruminants. Now-a-days, it is increasingly considered as a problem which is adversely affecting animal health leading to significant economic losses to the livestock industry. Recent emerging evidences demonstrated a relationship between MAP and Inflammatory bowel disease (IBD), Crohn's disease (CD) confirming its zoonotic significance hence the present study was carried out to record the MAP infection in buffaloes of Malwa region (Madhya Pradesh) by Dot ELISA. A total of 112 serum samples of buffaloes of either sex were examined for diagnosis of MAP infection which were slaughtered at Cantonment Board slaughter house, Mhow and Nagar Nigam Indore, belonging to different places of Malwa region aged between 1 to 10 years. Out of 112 serum samples, 92 (82.14%) samples were recorded positive for Anti-MAP antibodies or MAP infection in the serum samples of buffaloes by Dot-ELISA as a serological diagnostic test.

**Keywords**: *Bubalus bubalis*, buffaloes, Anti-MAP antibodies, Crohn's disease, Dot-ELISA

## INTRODUCTION

*Mycobacterium avium* subsp. *paratuberculosis* causes a chronic infection of ruminants which is called as Johne's disease (JD) which occurs worldwide in domesticated ruminants, including cattle, sheep, goat and deer. This is a deadly intestinal disease which causes weight loss, diarrhoea (intermittent or continuous) and emaciation in animals. Economic losses are caused due to reduced productivity in

[1]Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Madhya Pradesh, India, *E-mail: drgpjatavpath@gmail.com

[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Madhya Pradesh, India

[3]Department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Madhya Pradesh, India

[4]Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, Madhya Pradesh, India

[5]Department of Biotechnology, Institute of applied Science and Humanities, GLA University, Uttar Pradesh, India

terms of milk yield (quality and quantity), shorter life expectancy, reduced fertility, longer calving interval, premature culling, reduced salvage value at slaughter, increased treatment cost and risk of contracting and culling due to other diseases (McNab *et al*., 1991), hence it has a significant impact on the global economy (Sweeney, 1996). Information regarding paratuberculosis in small and large ruminants and buffaloes in particular from Malwa region of Madhya Pradesh is limited due to high cost of imported diagnostic kits. Paratuberculosis is most effectively identified by culture of MAP from faeces (Singh *et al*., 2009) but being an expensive, less sensitive and time consuming test, its use is limited. On the other hand, serology provides rapid and cost effective diagnosis, thus the current investigation was undertaken to diagnose anti-MAP antibodies by Dot-ELISA in buffaloes of Malwa region (Madhya Pradesh, India).

## MATERIALS AND METHODS

The current investigation was carried out in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Mhow, Indore (M.P.). Blood samples from buffaloes before slaughter were collected aseptically from the jugular vein in 15 ml calibrated centrifuge tube without anticoagulant for the separation of serum from the blood. After collection of 5 to 6 ml blood in the centrifuge tube these tubes were kept in the ice box for easy clotting of blood and were carried to the Department of Veterinary Pathology for further separation of serum by centrifuging the blood samples in the centrifuge tubes in centrifuge machine at 3000 rpm for 2 to 5 minutes. After centrifugation, separated serum were collected in 2 ml sample vials and preserved them at -20°C in the deep freeze for further serological diagnosis. All the samples were properly labeled and documented.

In the present study, dot-ELISA was standardized and performed with 112 serum samples. Nitrocellulose combs were coated with 0.8 µl (4 µl/ml) of antigen solution and incubated at 37°C for 2 h. Combs were stored at 4°C in the fridge for further use. Subsequently, 250 µl of blocking solution was added to the used and cleaned ELISA plate wells and the combs were incubated at 37°C for 1 h. Furher, combs were washed thrice in PBST solution and dipped in 250 µl of serum samples, incubated at 37°C for 1 h followed by washing in PBST (3 times). The rabbit anti-species HRP conjugate (250 µl) was added to each well and combs were incubated at room temperature for 30 minutes. Finally, 250 µl of substrate solution (DAB) was added in new wells of used and cleaned ELISA plates, wherein combs were dipped till development of colour. Reaction was stopped by dipping the combs in water, dried and then observed for appearance of dots on comb fingers.

## RESULT AND DISCUSSION

In the present study, 112 buffaloes irrespective of sex screened for anti MAP antibodies in the serum samples by using dot-ELISA test. Out of 112 buffalo serum samples, 92 (82.14%) samples were found positive for anti-MAP antibodies. The results of screening of anti-MAP antibodies by using dot- ELISA are shown in Table 1, Figure 1, 2 and 3.

In spite of the advances made in diagnosis of bacterial, viral, and protozoal disease diagnostic methods have to be renewed to be more rapid,

Table 1. Detection of anti- MAP antibodies by using dot- ELISA (n=112).

| S. No. | Status | No. of animals | Incidence (%) |
|---|---|---|---|
| 1 | Positive | 92 | 82.14 |
| 2 | Negative | 20 | 17.86 |

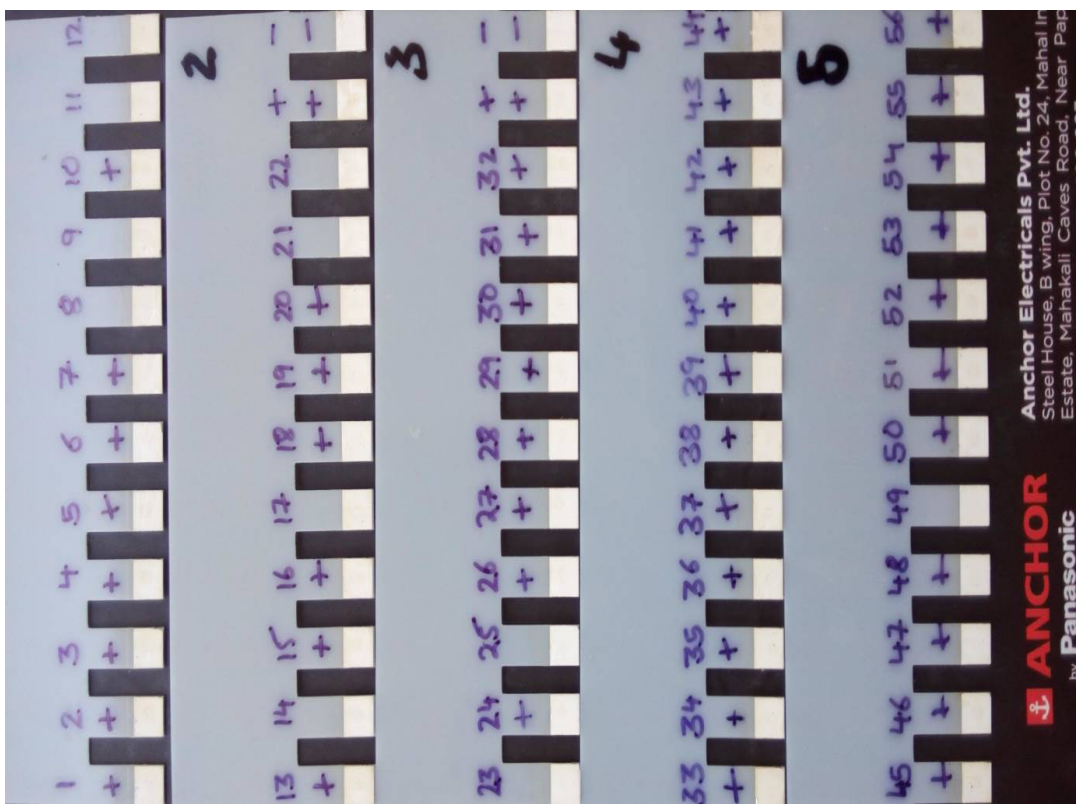


Figure 1. Nitro-cellulose combs showing dots in positive samples for anti-MAP antibodies (arrows) (I).

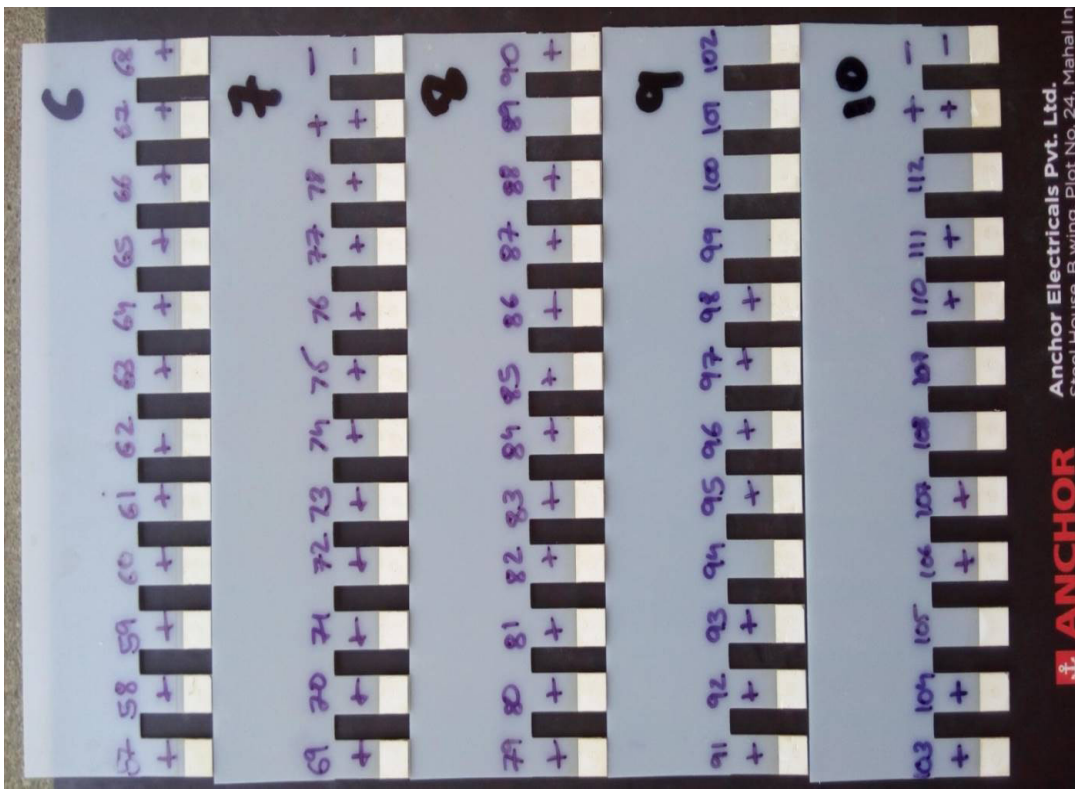


Figure 2. Nitro-cellulose combs showing dots in positive samples for anti-MAP antibodies (arrows) (II).

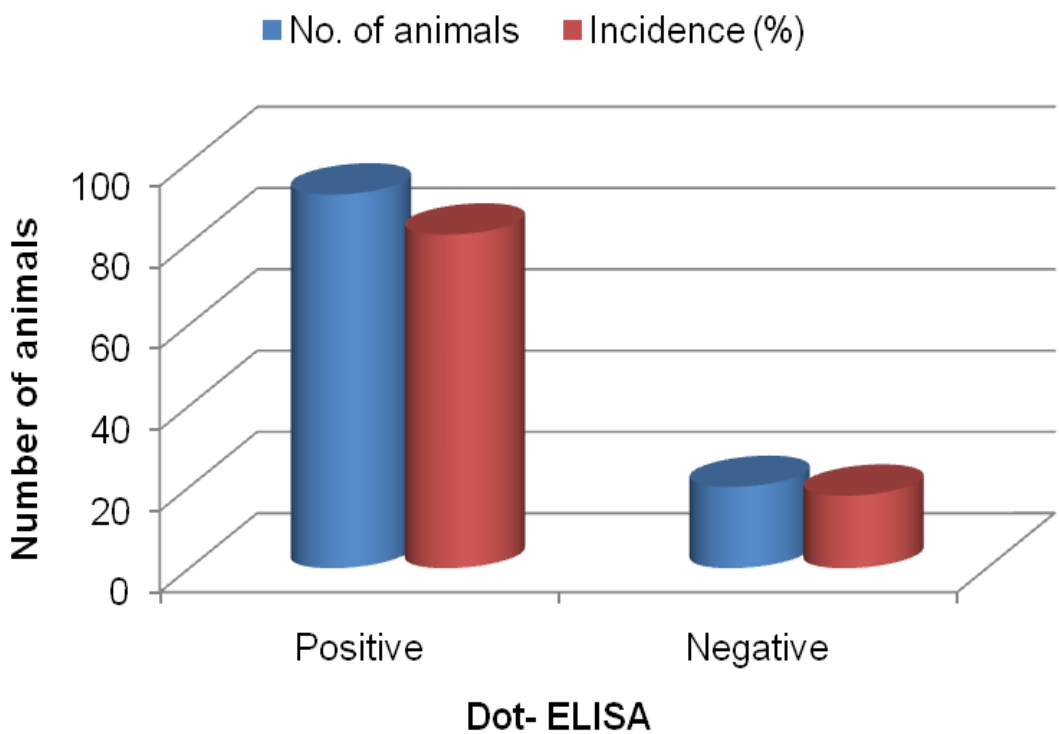


Figure 3. Detection of anti- MAP antibodies by Dot-ELISA.

sensitive, and specific. During the past few years there has been an increased interest in disease diagnosis using different techniques which are rapid, simple and inexpensive. Tests such as IHA, CFT, CIA and Indirect fluoroscopy are very tedious, difficult to standardize, conduct and interprete. The most used assay iFAT requires expensive fluorescent microscopy and trained technicians. Dot-ELISA which is simple to perform and do not require expensive equipments to detect the antibodies. Hence the present research work was carried out to diagnose anti-MAP antibodies by dot-ELISA in serum samples of buffaloes slaughtered at the Cantonment board slaughter house, Mhow. Out of 112 serum samples of buffaloes irrespective of age, breed and sex, 82.14% (92/112) were found positive for anti-MAP antibodies by using dot-ELISA as a diagnostic method for paratuberculosis (JD).

Findings of the current study are supported by observations of Stephen *et al.* (2016) who screened 55 paneer samples (fat and sediment layers) for MAP infection and reocorded 41 (74.5%) samples positive for MAP infection. Similarly, Nielsen *et al.* (1993) also noted 65.9 and 91.4% infection in cattle with faecal culture positive and faecal culture negative samples, respectively.

## ACKNOWLEDGEMENTS

Authors are highly thankful to the Dean, College of Veterinary Science and Animal Husbadry, Mhow and the Director, Central Institute for Research on Goats, Makhdoom, Mathura (Uttar Pradesh.) for providing the necessary facilities to carry out this research work.

## REFERENCES

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.

Nielsen, S.B., W.P. Shulam, P.L. Frandsen, J.B. Jorgensen, P. Ahrens and N.C. Feld. 1993. Use of dot-ELISA assay on absorbed sera for the diagnosis of bovine paratuberculosis. *Revue Scientifique et Technique (OIE)*, **12**(2): 617-627. DOI: 10.20506/rst.12.2.697

Singh, S.V., J.S. Sohal, P.K. Singh and A.V. Singh. 2009. Genotype profiles of *mycobacterium avium* subspecies *Paratuberculosis* isolates recovered from animal, commercial milk, and human beings in north India. *Int. J. Infect. Dis.*, **78**(5): e221-e227. DOI: 10.1016/j.ijid.2008.11.022

Stephen, B.J., M. Singh, S.V. Singh, S. Gupta, 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. *Veterinary Clinics of North America: Food Animal Practice*, **12**(2): 305-312. DOI: 10.1016/S0749-0720(15)30408-4