Buffalo Bulletin (January-March 2023) Vol.42 No.1
https://doi.org/10.56825/bufbu.2023.4213817

SERODIAGNOSIS OF MAP INFECTION BY LATEX AGGLUTINATION TEST IN SLAUGTERED BUFFALOES OF MALWA REGION (M.P., INDIA)

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Received: 06 August 2020
Accepted: 26 March 2023

ABSTRACT

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of chronic enteritis which is commonly called as Johne's disease (JD) in animals which is associated with several incurable, auto-immune diseases like Crohn's disease in human beings. In the current study, 19 serum samples of buffaloes were collected irrespective of their age, sex and breed which were slaughtered at Cantonment board slaughterhouse, Mhow and Nagar Nigam, Indore. These animals were brought from different places of Malwa region of Madhya Pradesh. For detection of anti-MAP antibodies, the latex agglutination test was performed following the standard procedure (Cheong Koo *et al*., 2004). The present investigation recorded 52.63% MAP infection in slaughtered buffaloes.

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

INTRODUCTION

Worldwide, Mycobacterium avium subspecies paratuberculosis (MAP) is responsible for Johne's disease which is characterized by chronic granulomatous enteritis, is highly prevalent in domestic animals like sheep, goat, cattle, and buffalo. It is highly infectious disease having zoonotic importance causing heavy economic losses to livestock owners. Country-wide programmes for the control of Johne's disease in domestic livestock have been initiated as, infected animals shed MAP organisms through milk, faeces, saliva, semen, and vaginal discharges acting as a source of infection for human beings.

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The MAP is a causative agent of Crohn's disease (CD) in human beings responsible for chronic enteritis.

Currently, sensitive, and specific tests are not available in field condition to diagnose the chronic diseases like Johne's disease; hence, it is rapidly spread in the animal population. But on the other hand, early and accurate diagnosis is very important to stop spread of this infection in animals and man. Earlier the latex agglutination test has been successfully used for detection of MAP infection in milk samples of animals (Singh *et al*., 2016). Therefore, present investigation reports development of a reliable and sensitive test (latex agglutination test) for detection of MAP antibodies in serum samples of slaughtered buffaloes of Malwa region of Madhya Pradesh, India.

MATERIALS AND METHODS

In the present study, 19 serum samples of buffaloes (irrespective of sex) slaughtered at Cantonment board slaughterhouse, Mhow and Nagar Nigam, Indore were collected aseptically in centrifuge tube without anticoagulant for the separation of serum. After collection of blood tubes were kept in the ice box and were carried to the Department of Veterinary Pathology. After centrifugation, separated serum were collected in 2 ml sample vials and preserved at -20°C in the deep freeze for its use for detection of anti-MAP antibodies by the latex agglutination test.

Latex agglutination test (LAT) was standardized for serum samples as per method described by Cheong Koo *et al*. (2004). In this method, at first, 10 µl of latex beads were taken in a 1.5 ml tube and added 20 µl of glycine saline buffer. Subsequently, 20 µl of MAP antigen (0.4 µg/ml) was added to the latex bead and incubated at 37°C for 2 h in shaker incubator, mixture was spin down at 5000 rpm for 10 minutes and supernatant was carefully aspirated. Subsequently, pellet was resuspended in 200 µl of blocking buffer (3% skimmed milk) and incubated in shaker incubator at 37°C for 45 minutes. Consequently, pellet was spin down at 5000 rpm for 10 minutes and supernatant was aspirated carefully. Reaction mixture was washed with 250 µl of 1X PBST, 3 times. Finally, 2 µl of coated latex beads were poured on microscopic slide followed by mixing of 4 µl serum samples to each spot separately with the help of a tip by mixing in a circular motion. As a positive control, 4 µl of antiserum was mixed as antigen spot and as a negative control 4 µl of glycine saline buffer was mixed to the spot. Slides were incubated for two minutes, and agglutination was observed.

RESULTS AND DISCUSSIONS

In the present study, 19 serum samples of buffaloes irrespective of age, sex and breed which were slaughtered at Cantonment board slaughterhouse, Mhow and Nagar Nigam, Indore. These animals were brought from different places of Malwa region of Madhya Pradesh and were screened for anti-MAP antibodies by latex agglutination test as a serodiagnostic tool. Out of 19 serum samples, 10 (52.63%) were found positive for anti-MAP antibodies in the form of agglutination on the glass slides. The results of screening of anti-MAP antibodies by latex agglutination test are shown in Table 1, Figure 1, 2 and 3. The present investigation was in consonance with the findings of Singh *et al*. (2016) who observed 51.4% infection in bovine milk samples and with 50.9% infection in paneer samples (Stephen *et al*., 2016).

Table 1. Detection of MAP by latex agglutination test (LAT) (n=19).

S. No.	Status	No. of animals	Incidence (%)
1	Positive	10	52.63
2	Negative	09	41.37

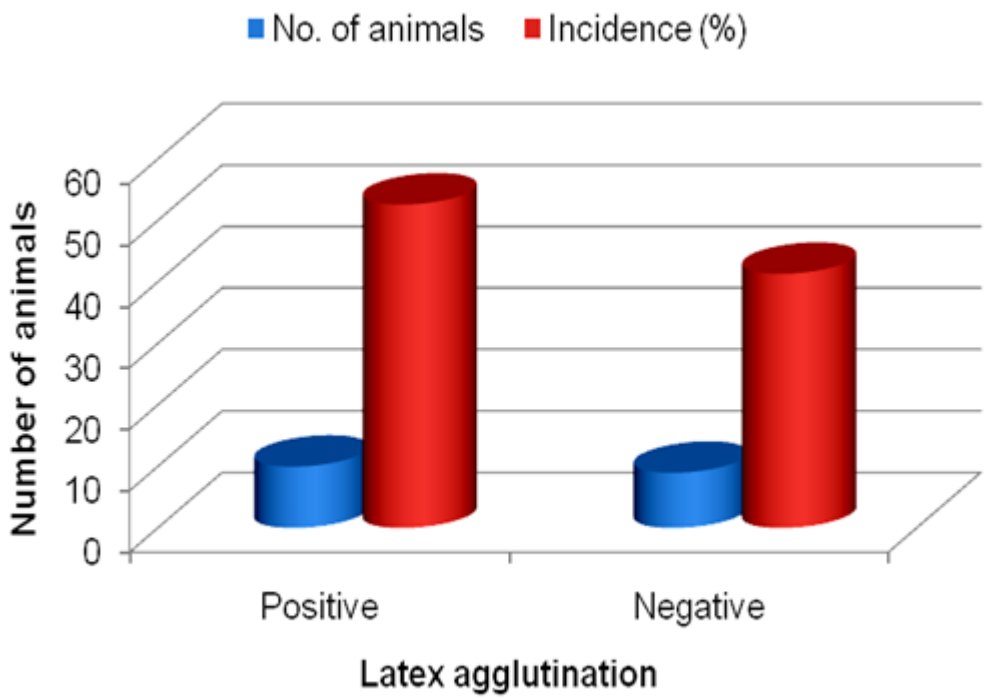

Figure 1. Detection of anti-MAP antibodies by latex agglutination test (LAT).

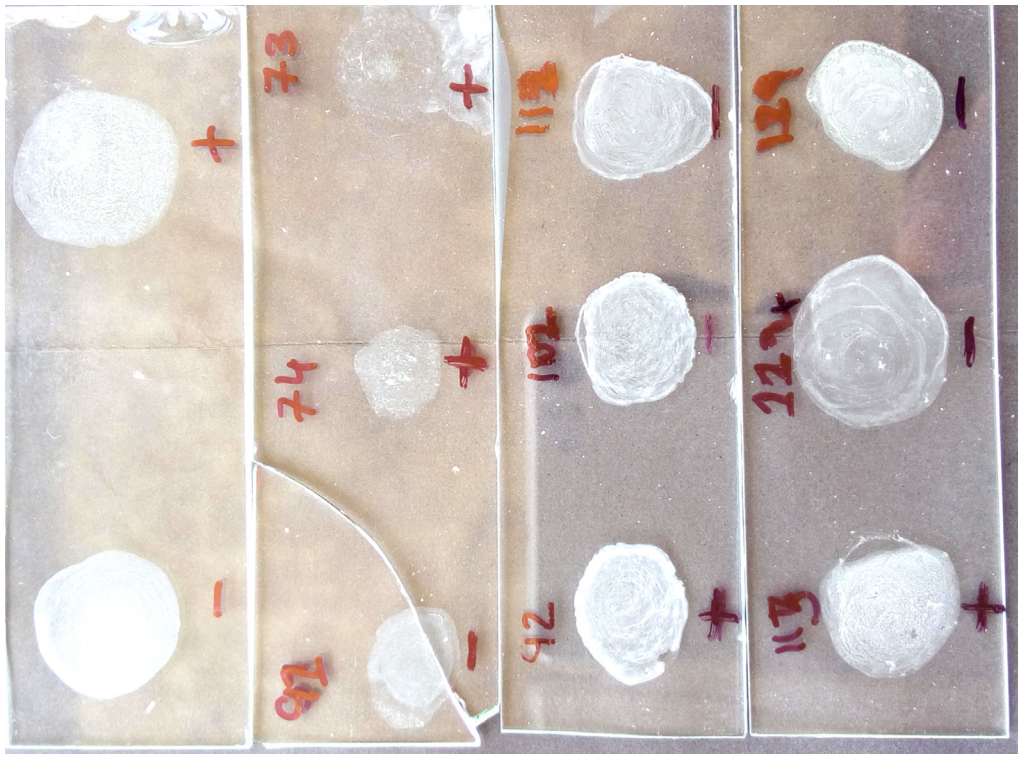

Figure 2. Photograph showing latex agglutination test on the slides (arrows) I.

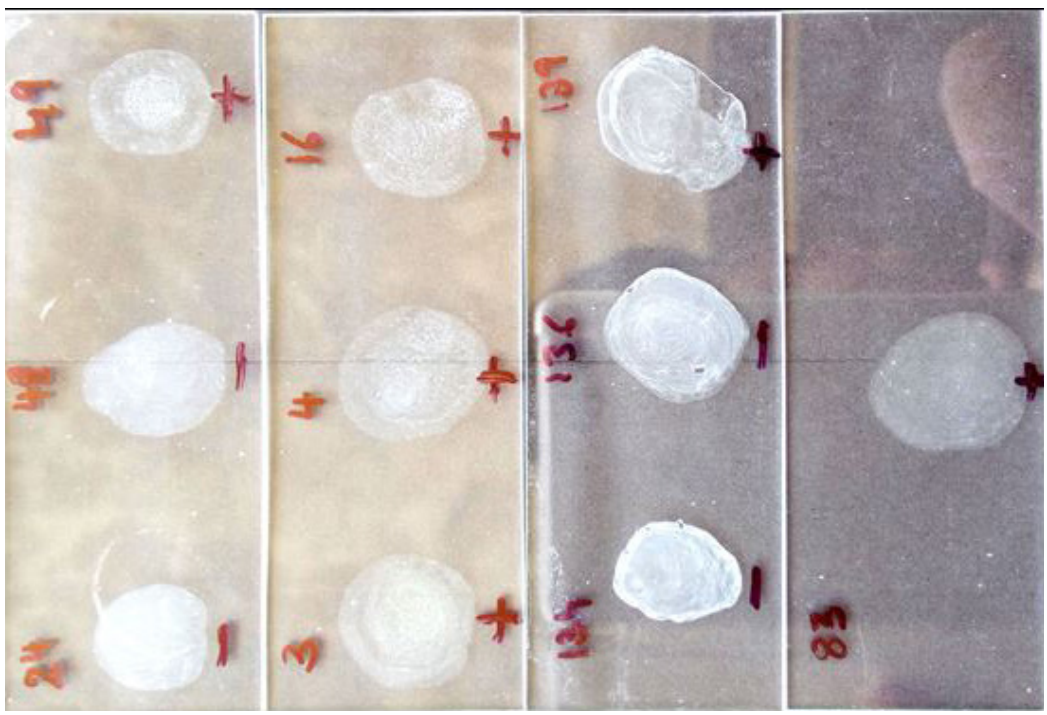

Figure 3. Photograph showing latex agglutination test on the slides (arrows) II.

ACKNOWLEDGEMENTS

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

REFERENCES

Cheong Koo, H., Y.H. Park, J. Ahn, W.R. Waters, M.J. Hemilton, G. Barrington, A.A. Mosaad, M.V. Palmer, S. Shin and W.C. Davis. 2004. New latex bead agglutination assay for differential diagnosis of cattle infected with *Mycobacterium bovis* and *Mycobacterium avium subsp. paratuberculosis*. *Clin. Diagn. Lab. Immun.*, **11**(6): 1070-1074. DOI: 10.1128/CDLI.11.6.1070-1074.2004

Singh, S.V., B.J. Stephen, M. Singh, S. Gupta, K. Chaubey, Sahzad, T.K. Sachan, J.S. Sohal, K. Dhama, S. Mukartal and Z. Hemati. 2016. Comparison of newly standardized 'Latex milk agglutination test', with 'Indigenous milk ELISA' for 'on spot' screening of domestic livestock against Mycobacterium avium subsp. paratuberculosis infection. *Indian J. Biotechnol.*, **15**(4): 511-517.

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