



SHORT COMMUNICATION

Detection of Anti-Map Antibodies in Serum Samples of Slaughtered Buffaloes (*Bubalus bubalis*) by Indigenous ELISA Kit in Malwa Region of Madhya Pradesh

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ABSTRACT

Recent emerging evidences have established a link between *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and inflammatory bowel disease (IBD), suggesting its zoonotic importance hence the present study was designed to find out seroprevalence of MAP in buffaloes of Malwa region of Madhya Pradesh by using indigenous ELISA kit. The study was conducted in one to ten-years-old 150 buffaloes (120 females and 30 males) belonging to different places of Malwa region slaughtered at Cantonment Board slaughter house, Mhow and Nagar Nigam Indore. The overall seroprevalence was found to be 89.33% (134/150) whereas sex-wise, it was recorded as 91.56 and 80% in case of females and males, respectively.

Keywords: Buffaloes, ELISA, JD, Incidence and MAP

Johne's disease (JD) is a chronic infection of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis*. The disease occurs worldwide and is primarily a disease of domesticated ruminants, including cattle (both beef and dairy), sheep, goats and farmed deer. This is a deadly intestinal ailment causing weight loss, diarrhoea (intermittent or continuous) and emaciation and has significant impact on the global economy (Sweeney, 1996). Economic losses are due to reduced productivity in 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). Information on the prevalence of paratuberculosis in small and large

ruminants particularly in buffaloes in 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 expensive, low sensitivity and time consuming, hence its use is limited. Serology provides rapid and cost effective alternative diagnostic tool, thus the present study was designed to detect anti-MAP antibodies by using indigenous ELISA kit in buffaloes of Malwa region of Madhya Pradesh.

Blood samples of one to ten-years-old 150 buffaloes (120 females and 30 males) belonging to different places of Malwa region slaughtered at Cantonment Board slaughter house, Mhow and Nagar Nigam Indore were collected from September, 2015 to April, 2016, aseptically from the

jugular vein in 15 ml calibrated centrifuge tube without anticoagulant. The centrifuge tubes containing 5-6 ml blood were kept in the ice box for easy clotting of blood and were transported to the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Mhow, for further separation of serum by centrifuging in centrifuge machine at 3000 rpm for 2-5 minutes. After centrifugation, separated sera samples were collected in 2 ml sample vials and kept at -20°C in the deep freeze for further serological diagnosis. All the samples were properly labeled and documented.

Indigenous ELISA kit initially developed for goats (Singh *et al.*, 2007) has been previously standardized for screening of cattle in India (Sharma *et al.*, 2008) was used for serodiagnosis in the present study. Soluble PPA (purified protein antigen) was prepared from the native 'Indian Bison Type' genotype of MAP was isolated from a terminal case of Johne's disease in a goat (Sevilla *et al.*, 2005). Antigen from Indian bison type was standardized at 0.1 µg per well of the microtitre plate. Serum samples were used in 1:50 dilution and anti-cattle horseradish peroxidase conjugate (Sigma Aldrich, USA) in 1:3000 dilution. Serum samples from culture positive and negative samples of cattle were used as positive and negative controls, respectively. Optical densities (OD) were transformed and expressed as sample-to-positive (S/P) ratios as per the method of Collins (2002).

In order to declare tested animals as positive or negative for Johne's disease (by Indigenous ELISA test), animals in the categories of positive and strong positive (according to S/P ratio) were considered as 'Positive' for MAP infection, whereas other were considered as 'Negative'.

Out of 150 samples, 134 (89.33%) samples were found positive for anti-MAP antibodies. The sex-wise incidence was found to be 91.56 and 80% in case of females and males, respectively. Out of 150 serum samples, status of MAP was recorded as negative, suspected, low positive, positive and strong positive, in 6.66, 0.00, 4.00, 87.33, and 2.00% cases, respectively. The findings pertaining to status of anti-MAP antibodies by using indigenous ELISA kit are shown in Fig. 1.

The ELISA is a simple, rapid, cost effective and most frequently employed assay to determine the status of infection (Maroudam *et al.*, 2015). The ELISA is efficient for detecting small amounts of antibodies and therefore

has highest sensitivity among the serological tests used for detection of MAP (Harris and Barletta, 2001).

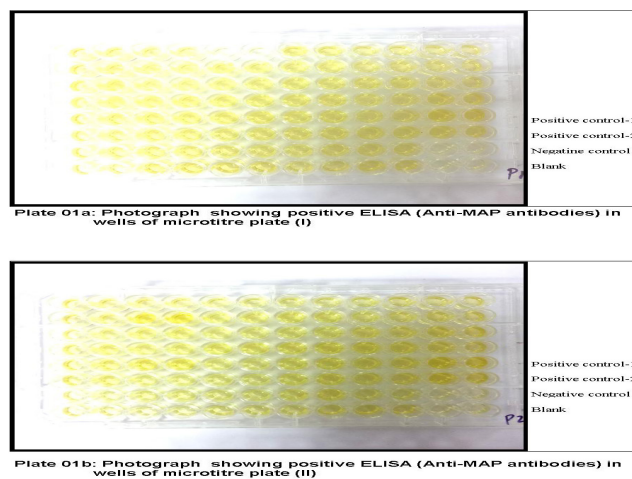


Fig. 1: Photograph showing positive ELISA (Anti-MAP antibodies) in wells of microtitre plate (I)

Hence, the present study was designed to detect anti-MAP antibodies in 150 serum samples of buffaloes by using indigenous ELISA kit. Out of 150, 134 (89.33%) buffaloes were found positive for anti-MAP antibodies and status of MAP was recorded as negative, suspected, low positive, positive and strong positive, in 6.66, 0.00, 4.00, 87.33 and 2.00% cases, respectively.

The high level of incidence (89.33%) of anti-MAP antibodies in the serum samples of buffaloes observed in the present study is in close proximity with the findings of Singh *et al.* (2007) who reported 73.30% seroprevalence in breeding bulls of Ludhiana (Punjab) on the basis of the type-II seroreactors, Singh *et al.* (2010) with 98.1% seroprevalence and Rawat *et al.* (2014) with 92.3% infection in HF cows.

When compared with the present study, earlier workers recorded low infection rate, viz. Mishra *et al.* (2009) with 23.6% infection in dairy herds of Mathura, Shisodiya *et al.* (2009) with 12.9% infection in animal keepers, Sikander *et al.* (2012) with 6 to 7% infection in cattle and buffaloes, Kumar *et al.* (2014) with 27.1% and Garg *et al.* (2015) with 15.6% infection in dairy cows in Punjab state of India.

According to Kumar *et al.* (2016) detection of MAP infection by ELISA technique appears to be dependent

on the disease stage of the tested animal and reported 85% sensitivity of ELISA for clinical cases, while about 15% for subclinical cases. The observations of Kumar *et al.* (2016) supports the present study for higher clinical incidence by Indigenous ELISA kit and also supports for overall incidence of JD recorded in the present study.

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