

ASSOCIATION BETWEEN MILK AND SERUM ANTIBODIES IN NATURALLY INFECTED BUFFALO (*Bubalus bubalis*) PARATUBERCULOSISO.R. Vinodh Kumar¹, L. Gunaseelan², M. Sekar², B.S.M. Ronald² and S.M. Sakthivelan²

ABSTRACT

From two organized farms with previous histories of paratuberculosis, 93 female buffaloes aged between 2 and 5 years were selected for screening for paratuberculosis using a cattle type ELISA kit. The milk and serum ELISA were performed and the association between ELISA S/P ratios was correlated. The correlation found was highly significant (0.816**, P=0.00). From this study it is concluded that milk ELISA can be a better alternative than serum ELISA since collection of blood from buffaloes is a very tedious while collection of milk is very simple.

Keywords: buffalo, paratuberculosis, serum ELISA, milk ELISA

INTRODUCTION

Riverine buffaloes (*Bubalus bubalis*), valued for milk, meat, draught power and efficient converters of low-quality feeds, are the preferred livestock in India. The world buffalo population (166.4 million) is spread over 129 countries; India with 96.9 million buffaloes is ranked first in the

world (FAO, 2003).

Paratuberculosis, a chronic granulomatous bowel disease of ruminants caused by *Mycobacterium avium* subsp. paratuberculosis (MAP), is a cause of severe economic losses all around the world. The disease affects both domestic and wild ruminants. Animals become infected early in life but often do not develop clinical disease until 3 years of age (Chiodini *et al.*, 1984). Infected animals with clinical disease and subclinical infections shed MAP bacteria in both milk and feces. Contamination of milk by MAP is widely reported in cattle. Milk is a potential source of disease transmission in young animals, therefore, important for diagnosis and control of Johne's disease in a population (Kaur *et al.*, 2010). Antibodies to MAP in serum can be detected by different methods such as complement fixation (Morris and Stevens, 1977), agar gel immune diffusion (AGID) and ELISA (Reichel *et al.*, 1999). ELISA is capable of detecting small amounts of antibodies and therefore shows the highest sensitivity of the serological tests for MAP (Harris and Barletta, 2001). Salgado *et al.* (2007) compared serum, milk ELISA and fecal culture in Chilean dairy goats and noted that paratuberculosis milk ELISA for goats is fast and inexpensive. There is very little work on comparison of milk and

¹Animal Health Division, Central Sheep and Wool Research Institute (CSWRI), Avikanagar, Rajasthan, India, E-mail: vinodhkumar.rajendran@gmail.com

²Department of Veterinary Epidemiology and Preventive Medicine, Madras Veterinary College, Chennai, India

serum ELISA in buffalo paratuberculosis infection. The aim of the present study is to find out the relationship between serum and milk antibodies in naturally infected buffaloes.

MATERIALS AND METHODS

The animals were selected from two organized farms with previous histories of paratuberculosis, which was confirmed by culture and PCR. Blood and milk samples were collected from 93 she buffaloes aged between 3 and 5 years.

Five milliliters of blood was collected from animals with a clot activator tube and the serum was separated. Milk and serum samples were kept in ice and transported to the laboratory for further analysis. Milk (15 ml) was collected aseptically from all four teats after discarding first few streaks. Milk samples were defatted by centrifugation for 20 minutes at 3000 g and 4°C. The defatted test milk samples and serum were stored at -20°C. To assess the humoral immune response, a cattle type absorbed ELISA kit ((Labor Diagnostik, Leipzig, Germany) was used in both milk and serum samples. The test serum sample was diluted 1:70 in sample dilution buffer containing an extract of *Mycobacterium phlei* and incubated for 120 minutes at room temperature. The defatted test milk samples were diluted with sample dilution buffer in the ratio of 1:2. Then 100µl of each of ready to use negative and positive control (in duplicates) and 100 µl of pre incubated serum / milk samples were transferred into the test plate wells. The absorbed Cattle type ELISA was performed as per manufacturer's protocol and the final optical density was read in ELISA reader at 450 nm wave length with reference wavelength at 620-650 nm.

Statistical analysis

The serum and milk ELISA S/P ratio were correlated using SPSS ver 18 and correlation was found out.

RESULTS AND DISCUSSION

Out of 93 milk samples from buffaloes subjected to ELISA for IgG antibodies, 29 were positive. Sera from the same 93 animals subjected to ELISA showed 29 positives. Two animals which were negative by serum ELISA were found positive in milk ELISA, and two animals which were negative by milk ELISA showed positive in serum ELISA. Pearson correlation of these results on 93 sera and milk samples showed a high degree of correlation between sera and milk antibodies (0.816**, P=0.00). From this study it was found that the relationship between milk and serum antibodies to MAP was significant and it is also inferred that milk is a better sample for detection of antibodies to MAP since it does not require handling the animals for blood collection. One drawback of using milk ELISA is that it can be used only in milking animals.

Humoral antibodies developed against the infection are primarily detected by ELISA (Vannuffel *et al.*, 1994). Immune response is observed in a rather late stage of the infection but still before appearance of clinical signs (Collins *et al.*, 1993). Lombard *et al.* (2006) used the serum ELISA as a reference and the reported relative sensitivity and specificity of the milk ELISA were 60.9 and 94.6%, respectively, and also found that the relationship between milk and serum MAP antibodies were significant. Sweeney *et al.*, (1995) used different ELISAs and the accuracies of different ELISAs were similar, irrespective

of whether they were used with milk or serum samples. All the above findings were in agreement with our study on buffaloes. Buergelt and Williams (2004) showed a positive correlation between high MAP ELISA readings in blood and increased probability of detection of MAP DNA in milk of clinical cows and also found ELISA is an effective tool for identification of MAP in milk as a first step of protecting the food chain. Salgado *et al.* (2007) compared serum and milk ELISA and fecal culture in Chilean dairy goats and found the sensitivity of ELISA on serum and milk relative to fecal culture was 74.3% and 60%, respectively, and also noted that paratuberculosis milk ELISA for goats was fast and inexpensive. Although the milk ELISA sensitivity is lower than that of serum ELISA, milk ELISAs can be an effective method for detecting heavy fecal shedders. In our study we found a good degree of agreement between serum and milk ELISA in buffaloes. Vinodhkumar *et al.* (2010) compared serum ELISA and gamma interferon

assay in cattle and buffaloes and found that ELISA was a better indicator of late stage of infection and gamma interferon assay can be used in the initial phase of infection.

The milk ELISA is similar to the serum ELISA in terms of testing time and cost. Since milk samples from individual cows are routinely collected on dairy farms. The milk ELISA may prove to be a less labor-intensive method for testing dairy buffaloes for MAP infection compared with the serum ELISA. In addition, the routine collection and testing of milk samples would allow producers to more consistently screen their herds for infection without the additional scheduling of serum or feces collection.

From our study we conclude that serum and milk ELISA gave similar results in buffalo paratuberculosis diagnosis; however, while collection blood from buffalo is tedious and requires great expertise, milk collection is a routine procedure in dairy operation and can effectively

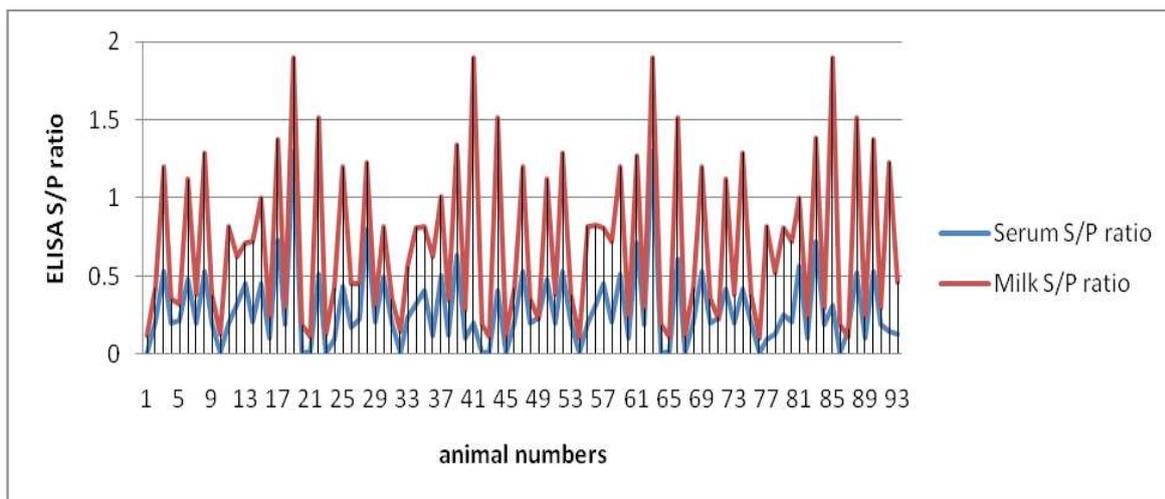


Figure 1. Association between serum and milk ELISA S/P ratio.

used for buffalo paratuberculosis screening.

REFERENCES

- Buergelt, C.D and J.E. Williams. 2004. Nested PCR on blood and milk for the detection of *Mycobacterium avium* subsp *paratuberculosis* DNA in clinical and subclinical paratuberculosis in Johne's disease. *J. Clin. Microbiol.*, **28**: 933-937.
- Chiodini, R.J., H.J. Van Kruiningen and R.S. Merkal. 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. *Cornell. Vet.*, **74**: 218-262.
- Collins, D.M., D.M. Stephens and G.W. de Lisle. 1993. Comparison of polymerase chain reaction tests and faecal culture for detecting *Mycobacterium paratuberculosis* in bovine faeces. *Vet. Microbiol.*, **36** : 289-299.
- FAO. 2003. FAOSTAT. Rome: Food and Agriculture Organization.
- Harris, N.B. and R.G. Barletta. 2001. *Mycobacterium avium* subsp *paratuberculosis* in Veterinary Medicine. *Clin. Microbiol. Rev.*, **14**: 489-512.
- Kaur, P., G. Folia, S.V. Singh, P.K. Patil and K.S. Sandhu. 2010. Molecular detection and typing of *Mycobacterium avium* subspecies paratuberculosis from milk samples of dairy animals. *Trop. Anim. Health Pro.*, **42**: 1031-1035.
- Lombard, J.E., T.M. Byrem, B.A. Wagner and B.J. McCluskey. 2006. Comparison of milk and serum enzyme-linked immunosorbent assays for diagnosis of *Mycobacterium avium* subspecies paratuberculosis infection in dairy cattle. *J. Vet. Diagn. Invest.*, **18**: 448-458.
- Morris, J.A. and A.E. Stevens. 1977. An improved antigen for the paratuberculosis complement fixation test. *J. Biol. Stand.*, **5**: 315-319.
- Reichel, M.P., R. Kittelberger, M.E. Penrose, R.M. Meynell, D. Cousins, T. Ellis, L.M. Mutharia, E.A. Sugden, A.H. Joens and G.W. de Lisle. 1999. Comparison of serological tests and faecal culture for the detection of *Mycobacterium avium* subsp. paratuberculosis infection in cattle and analysis of the antigens involved. *Vet. Microbiol.*, **66**: 135-150.
- Salgado, M., J. Kruze and M.T. Collins. 2007. Diagnosis of paratuberculosis by fecal culture and ELISA on milk and serum samples in two types of Chilean dairy goat herds. *J. Vet. Diagn. Invest.*, **19**: 99-102.
- Sweeney, R.W., R.H. Whitlock, C. Buckley and P.A. Spencer. 1995. Evaluation of a commercial enzyme-linked immunosorbent assay for the diagnosis of paratuberculosis in dairy cattle. *J. Vet. Diagn. Invest.*, **7**: 488-493.
- Vannuffel, P., B. Gilot, B. Limbourg, B. Naerhuyzen, C. Dieterich, M. Coene, M., L. Machtelinckx and C. Cocito. 1994. Development of a species-specific enzyme-linked immunosorbent assay for diagnosis of Johne's disease in cattle. *J. Clin. Microbiol.*, **32**: 1211-1216.
- Vinodh Kumar, O.R., L. Gunaseelan, K. Vijaya Rani and B.S.M. Ronold. 2010. A preliminary study on the usefulness of ELISA and Gamma interferon assay in buffalo and white cattle paratuberculosis diagnosis. *Buffalo Bull.*, **29**: 299-303.