STATUS OF JOHNE'S DISEASE IN BUFFALO POPULATION OF MALWA REGION USING GOAT BASED INDIGENOUS ELISA KIT

Sachin Digambar Audarya², Manju Singh¹, Bjorn John Stephen¹, Sahzad¹, Kundan Kumar Chaubey^{1,4}, Saurabh Gupta^{1,4}, Anjali Pachoori¹, Sujata Jayaraman³, Gajendra Kumar Aseri³, Jagdip Singh Sohal³, Ashok Kumar Bhatia⁴, Shoor Vir Singh¹,*

ABSTRACT

Present study estimated status of Johne's disease in the buffalo population of Malwa region using goat based Indigenous plate ELISA test. Of 156 serum samples screened 41.0% (64) and 85.8% (134) were positive for MAP infection by Indigenous plate ELISA kit; condition (A), condition (B), respectively. Study showed that despite high slaughter rate, prevalence of Johne's disease was high in native population of riverine buffaloes (Bubalus bubalis) and call for immediate control of disease. The present findings indicated that higher number of animals infected with JD may be responsible for reduced productivity or unproductivity and weight loss in buffaloes. Therefore systematic screening of buffaloes with indigenous sensitive and specific test for detection of Johne's disease at certain time interval is required. So that spread of infection to other animals and herds can be reduced or avoided. The goat based 'Indigenous ELISA kit' was good herd screening test with high sensitivity and is recommended for the large

scale screening of Johne's disease in the domestic livestock population.

Keywords: *Bubalus bubalis*, buffaloes, *Mycobacterium avium* subspecies *paratuberculosis*, plate-ELISA, Johne's disease, herd screening test

INTRODUCTION

Buffalo rearing is important source of livelihood to millions of farmers and is primarily raised for milk production and has emerged as important meat animal, since it has good salvage value. India at 127.8 million tonnes in 2011 to 2012 (DADF, 2013) is leading milk producer in the world and buffaloes contribution at 62.35 million tonnes was, 51.17% of the total milk produced in the country. Buffalo is also important source of meat and produced 0.80 million tonnes of meat, which was 16.53% of the total meat produced (BAHS, 2012). Buffalo population of the country has seen consistent increase from 43.40 million in 1951 to

¹Microbiology Lab, Animal Health Division, Central Institute for Research on Goats, Mathura, India, *E-mail: shoorvir.singh@gmail.com, shoorvir singh@rediffmail.com

²Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Madhya Pradesh, India

³Amity Institute of Microbial Technology, Amity University Rajasthan, Jaipur (Rajasthan), India ⁴Department of Microbiology and Immunology, GLA University, Uttar Pradesh, India

Department of Microbiology and Immunology, GLA University, Uttar Pradesn, Indi

105 million in 2007. Despite high slaughter rate of low or unproductive animals to meet the ever growing demand of meat for internal consumption and export, per animal productivity is low. The rates of morbidity are very high and dispersed over a time thus production losses are unnoticed and did not estimated in India despite low per animal productivity (Barbaruah and Joshep, 2008). This is mainly due presence of chronic infections, like Johne's disease. In absence of 'Indigenous diagnostic kits' the status of Johne's disease is not known in the native population of riverine buffaloes. Present pilot study was undertaken to estimate status of Johne's disease in native buffalo population of Malwa region using highly sensitive goat based 'Indigenous p ELISA kit'.

MATERIALS AND METHODS

Animals

The Madhya Pradesh livestock board (Kirtapur, Itarsi) is maintaining large number of buffaloes of Murrah, Jaffrabadi and Bhadawari breeds. Buffaloes were provided optimum to intensive feeding (green fodder, dry bhusa along with high quality of mineral mixture). Despite all these conditions a good number of buffaloes were identified as suspected for Johne's disease (body weight loss, ribs visible from distance, loss in production on regular interval). These identified animals along with apparently healthy were sampled for the screening of Johne's disease.

Serum samples

The period of sample collection was from March, 2015 to September, 2015. During the period, a total of 156 serum samples were collected from Murrah, Jaffrabadi and Bhadawari breeds of buffaloes. Samples collected from suspected and apparently healthy animals were stored at -20°C before processing.

Indigenous p_ELISA kit using goat based antigen

Semi-purified Protoplasmic antigen: Native MAP strain (S 5) characterized as 'Indian Bison Type' of goat origin (Jamunapari goat died due to Johne's disease) was used as antigen source (Singh et al., 2007a; Sohal et al., 2009). Indigenous ELISA, soluble PPA prepared from Indian Bison Type genotype of MAP isolated from terminal case of Johne's disease in a goat (Sevilla et al., 2005), was used for the screening of animals for sero-positivity against MAP (Singh et al., 2007b). Antigen (Indian Bison Type) was standardized at 0.1 microgram per well of the microtiter plate. Serum samples were used in 1:50 dilution and antibovine horseradish peroxidase conjugate (Sigma Aldirch, USA) in 1:2500 dilution. Serum samples from culture positive and negative samples of cattle were used as positive and negative controls, respectively. Strong positive and positive samples were considered as positive for MAP infection.

Interpretation

The optical density was measured at 450 nm. The OD values (optical densities) were transformed and expressed as sample-to-positive (S/P) ratios (Collins, 2002) (Table 1).

S/P percent = [(Sample OD - Negative OD) X 100 (Positive OD - Negative OD)]

RESULTS

Results were analyzed considering serum

samples in strong positive and positive categories of S/P ratio, as positives in p_ELISA. Out of 156 buffaloes screened, 41.0 (64) and 58.9% (92) were found positive and negative in p_ELISA, respectively in condition A while in condition B, 85.8 (134) and 14.1 (22) samples were found positive and negative in p_ELISA, respectively in condition B (Table 2). In p_ELISA, on the basis of S/P ratio, 0.6, 40.3, 44.8, 11.5 and 2.5% buffaloes were in the strong positive, positive, low positive, suspected and negative categories with respect to the status of Johne's disease infection. Considering low positives with strong positives and positives, as positives, 85.8% (134) buffaloes were found positive in p_ELISA kit.

DISCUSSION

Though India is highest milk producer in the world per capita milk availability has reached to 290 g / day (2011 to 2012) was higher than world average of 284 gm / day. Value of 14, 75, 526.01 MT of buffalo meat exported in 2014 to 2015 was Rs. 29,282.60 crores (APEDA, 2015). It is also provides 10.0% of the total draught power of the work animals. It also produces 0.52 million MT of skin and hides (CIRG, 2013). There are several routes by which MAP reach to animal and infect others animal. From these routes, oral and fecal route is regarded as the main horizontal transmission. The fecal and oral is the main route of transmission by which MAP spread from one animal to other animals and to other species (Sweeney, 1992; Greig et al., 1999; Ayele et al.,

Table 1. Sample to positive ratios and status of Johne's disease on the basis of likelihood ratio.

Sn	S/P ratios	Johne's disease status	
1	0.00 - 0.09	Negative	
2	0.10 - 0.24	Suspected or borderline	
3	0.25 - 0.39	Low positive	
4	0.4 - 0.99	Positive	
5	1.0 - 10.0	Strong positive	

Table 2. Incidence of Johne's disease in buffaloes of Malwa region using goat based indigenous-ELISA (n=156 samples).

	Indigenous - ELISA status n (%)				
Tests	Negative (N)	Suspected (S)	Low positive (LP)	Positive (P)	Strong positive (SP)
	4 (2.5)	18 (11.5)	70 (44.8)	63 (40.3)	1 (0.6)
Condition A	92 (58.9)			64 (41.0)	
Condition B	22 (14.1)		134 (85.8)		

2002). Transmission of MAP was also reported from semen, in-utero and fetus (Buergelt *et al.*, 2006).

Although Johne's disease was first reported in India in 1913 and is still a major health problem in domestic and wild ruminants (Singh *et al.*, 2014a) and still Nation-wide estimates on prevalence of MAP infection are not available. In a major study by Singh *et al.* (2014a), 28.3% bioload of MAP infection was reported in buffaloes from North India in last 28 years (1985 to 2013). The present study also showed that at least 41.0 can be considered as positive for MAP infection; using indigenous plate ELISA kit, since these buffaloes were detected positive in two tests combinations (Table 2).

Since, Johne's disease is endemic in domestic livestock (Singh et al., 2014a), low positive (44.8% in p ELISA) can be considered as positive. In a study from Agra region, Yadav et al. (2008) detected MAP from 48.0% tissues of slaughtered unproductive buffaloes for meat production. Using this 'Indigenous ELISA kit', the sero-prevalence of MAP infection in slaughtered buffaloes was 46.7%. Another study by Singh et al. (2008), wherein large scale sero-survey was conducted using this p ELISA kit and 28.6% in buffaloes from Northern India. Sero-prevalence was low (8.6 to 10.5%) in Murrah breed of bulls from Uttar Pradesh and Punjab using this kit. However, large number of bulls was on borderline (78.0 to 84.2%).

In a comprehensive study of 28 years (1985 to 2013), bio-load of MAP was moderately high (28.6%) behind cattle (39.3%), sheep (32.7%) and goats (20.1%). Sivakumar *et al.* (2005) reported 70.0 and 30.0% incidence in Buffaloes from India using PCR and culture. Lillini *et al.* (2002) reported 13.3% prevalence of MAP in the Latium region of

Italy using PCR in fecal samples of water buffalo herds. Molecular epidemiology studies by Kaur et al. (2011), revealed that as compared to 'Cattle type' biotype 'Indian Bison type' was the predominant (82.0%) biotype in domestic livestock including buffaloes. George's et al. (2011) reported 13.1% of water buffaloes were serologically positive for MAP in ELISA and 13.2% were positive in IFN-y test. They found significant association between age and sero-positive test results, (P=0.007, chi square 1 df, 95% confidence). Sezzi et al. (2010) studied on 1400 buffaloes belonging to 71 herds in the Latium region of Italy using two different commercial ELISA kits (Pourquier, ID.vet). In Pourquier kit none of the buffaloes was positive, whereas in ID.vet kit 3 buffaloes were positives (0.2% prevalence). Desio et al. (2013) carried out a study on 1350 buffaloes belonging to 56 herds in the Caserta province, of Campania region, Italy.

The prevalence of infected buffalo dairy herds was estimated by a commercial ELISA kit of individual blood samples of animals over 24 months of age. On the basis of performance (sensitivity 43%, specificity 99.3%) of ELISA test on serum, the resulting true prevalence at animal level and at herd level was 4% (95% CI 3% to 5%) and 74,1% (95% CI 71.8% to 76%). Wagas et al. (2015) reported 4.5% prevalence of MAP infection on the basis of suspected lesions in buffaloes with non-significant difference between age groups. However, prevalence was relatively higher in buffaloes of more than 10 years (6.1%)than buffaloes of less than 5 years (3.63%) of age and between 5 to 10 years (3.75%) years of age. Gamberale et al. (2014) reported bio-load of MAP in buffaloes over 12 months were subjected to yearly serological examination by ELISA and positive animals were culled. The overall yearly raw prevalence obtained was very low (1.0,

2.0 to 0%, 0%) between 2009 to 2012. Mohan et al. (2007) reported 5.8% sero-prevalence of paratuberculosis in buffaloes from unorganized dairy farms in Gujarat. Incidence of MAP was lower in rural dairy population than organized dairy farms. Tripathi et al. (2007) screened 320 buffaloes (central west India: 80, northern India: 240) sera for MAP infection by Pourquier ELISA kit, did not show antibody prevalence. Yadav et al. (2008) reported 48.0% buffaloes were positive by culture from tissues (MLN and large intestine) and 40% were positive by IS900 PCR for MAP infection. In another study, sero-prevalence of Johne's disease was 28.6% in buffalo in Northern India. Of 601 randomly sampled buffaloes, seroprevalence of MAP infection by indigenous ELISA kit was 40.3% (16.6% in young and 40.9% adults) and 25.5% (10.5% in young and 26.3% adults) in South and west Uttar Pradesh, respectively.

In another study, 699 serum samples screened from Ludhiana, Punjab, Sero-prevalence was 23.3% (12.1% in young and 24.4% in adults) in buffaloes. Here, Indigenous ELISA kit was used as a rapid, economic and sensitive test for large-scale screening of buffaloes and cattle population against incurable BJD (Singh et al., 2008). Sivakumar et al. (2005) was estimated sero-prevalence of MAP in buffalo to be 21.3% in Chennai. In another study, The Ziehl-Neelsen's stained tissue sections revealed acid-fast bacilli in grade-3 and grade-2 buffaloes and acid-fast granular debris were present in grade-1 buffalo. Of 20 buffaloes, 14 (70%) were positive by IS900 PCR and 6 (30%) were positive by MAP culture (Sivakumar et al., 2006). Singh et al. (2014b) screened 25 young Murrah bulls, 14 (56.0%) were positive for BJD. Sero-incidence of BJD was higher in young bulls of Murrah breed in their native tract.

Present study indicated that a routine

screening of farm for detection of Johne's disease at certain time interval is needed. So that, the infection can be stop to spread other animals and infected animals can be surrogate. The goat based Indigenous ELISA was good herd/farms screening test has higher sensitivity and recommended for screening of Johne's disease in domestic livestock population.

Conflict of interest

There is no conflict of interest to declare.

ACKNOWLEDGEMENTS

Authors are thankful to Semi government organized farm, Kiratpur and Madhya Pradesh State Livestock and Poultry development corporation, Bhopal for providing the samples and director, CIRG, Makhdoom for providing the facilities to screen the samples.

REFERENCES

- Ayele, W.Y, O. Fischer, P. Svastova, M. Alexa, M. Machachova and I. Pavlik. 2002. Dairy and beef cattle paratuberculosis survey in intensive and extensive farming conditions. *In* Juste, R.A., M.V. Geijo and J.M. Garrido. (eds). p. 340-344. *In Proceedings of the 7th ICP*, Spain.
- Bardhan, D. and M.L. Sharma. 2013. Technical efficiency in milk production in underdeveloped production environment of India. SpringerPlus, 2(65).
- Barbaruah, M.I. and A.K. Joshep. 2008. India: Dairy giant walking barefoot. *In Proceedings of the FAO-RAP Technical Meeting on Designing*

Effective Country-Specific Dairy Strategies, FAO, Rome, Italy. http://www.aphca.org/ workshop/Dairy_Workshop/India.doc.

- Buergelt, C.D., B.S. Williams, G.R.G. Monif,
 P. Pinedo and J.H. Decker. 2006. Nested polymerase chain reaction and prenatal detection of *Mycobacterium avium* subspecies *paratuberculosis* (Map) in bovine allantoic fluid and fetuses. *Int. J. Appl. Res. Vet. M.*, 4: 232-238.
- Central Institute for Research on Goats, (CIRG). 2013. Annual Report. Indian Council of Agriculture Research, Makhdoom, Farah, Mathura, Uttar Pradesh, India.
- Collins, M.T. 2002. Interpretation of a commercial bovine paratuberculosis enzyme-linked immunosorbent assay by using likelihood ratios. *Clin. Diagn. Lab. Immun.*, **9**: 1367-1371.
- Department of Animal Husbandry, Dairying and Fisheries, (DADF). 2013. *State of Indian Agriculture, 2012-2013*. Government of India Ministry of Agriculture, Department of Agriculture and Cooperation, Directorate of Economics and Statistics, New Delhi, India.
- Desio, G., S. Nizza, S. Montagnaro, S. Sasso,
 L. DeMartino, V. Iovane, R. Ciarcia,
 F. Casalinuovo and U. Pagnini. 2013.
 Estimated prevalence of Johne's disease in herds of Water buffaloes (*Bubalus bubalis*) in the province of Caserta. *Ital. J. Anim. Sci.*, 12: 1-8.
- Gamberale, F., G. Barlozzari, G. DeSantis, P. Scaramella, G. Pietrella, M. Sala and G. Macri, 2014. Paratuberculosis in Italian Mediterranean buffalo: Serological screening and prevalence study in a breeding farm in province of Rome (2009-2012).

In Proceeding of the 12th International Colloquium on Paratuberculosis, Parma, Italy.

- Georges, K., C.D. Ezeokoli, N. Seepersadsingh, N. Lambie, L. Rastogi, J. Ryan, M. Diptee and A.A. Adesiyun. 2011. Serological and subclinical evidence of Johne's disease (Mycobacterium paratuberculosis) in Water buffalo (Bubalus bubalis) on two farms in Trinidad, west Indies. West Indian Veterinary Journal, 11: 1-6.
- Greig, A., K. Stevenson, D. Henderson, V. Perez and I. Pavilik. 1999. Epidemiological study of Paratuberculosis in wild rabbits in Scotland. J. Clin. Microbiol., 37: 1746-1751.
- Kaur, P., G. Filia, S.V. Singh, P.K. Patil, G.V.P.P.S.
 Ravi Kumar and K.S. Sandhu. 2011.
 Molecular epidemiology of *Mycobacterium* avium subspecies paratuberculosis: IS900 PCR identification and IS1311 polymorphism analysis from ruminants in the Punjab region of India. Comp. Immunol. Microbiol. Infect. Dis., 34: 163-169.
- Lillini, E., F. Gamberale, L. DeGrossi, A. Cersini, B.
 Scherm and A. Fagiolo. 2002. Comparative study on detection of *Mycobacterium avium* subspecies *paratuberculosis* by PCR diagnosis and conventional culture on feces of Water buffalo herds in Latium region (Italy). *In* Juste, R.A., M.V. Geijo and J.M. Garrido. (eds). *In Proceedings of the 7th Intenational Colloquium on Paratuberculosis*. Bilbao, Spain.
- Ministry of Agriculture, Department of Animal Husbandry, Dairying and Fisheries. 2012. *Basic Animal Husbandry and Fisheries Statistics*. Krishi Bhawan, New Delhi, India. Ministry of Commerce and Industry, Government

of India. 2015. Agricultural and Processed Food Products Export Development Authority, (APEDA). http://www.apeda. gov.in/apedawebsite/SubHead_Products/ Buffalo_Meat.htm.

- Mohan, M.S., P. Duraisamy, P. Ezhil, P. Sivakumar, B.N. Tripathi and N. Singh. 2009. Prevalence of Paratuberculosis in cattle and buffaloes. *Indian Vet. J.*, 86: 4-6.
- Sevilla, I., S.V. Singh, J.M. Garrido, G. Aduriz, S. Rodriguez, R.J. Whittington, V. Saunders, R.H. Whitlock and R.A. Juste. 2005.
 Molecular typing of *Mycobacterium avium* subspecies *paratuberculosis* strains from different hosts and regions. *Rev. Sci. Techn.*, 24: 1061-1066.
- Sezzi, E., A. Gelli, G. Saralli, T. Zottola and L. DeGrossi. Extremely low prevalence of Paratuberculosis in Italian buffaloes: Biological reality or technical ineffectiveness? *Revista Veterinaria*, 21: 460-462.
- Singh, S.V., A.V. Singh, P.K. Singh, V.K. Gupta, S. Kumar and J. Vohra. 2007a. Seroprevalence of Paratuberculosis in young kids using 'Bison type', *Mycobacterium avium* subspecies *paratuberculosis* antigen in plate ELISA. *Small Ruminant Res.*, **70**: 89-92.
- Singh, S.V., A.V. Singh, P.K. Singh, J.S. Sohal and N.P. Singh. 2007b. Evaluation of an indigenous ELISA for diagnosis of Johne's disease and its comparison with commercial kits. *Indian J. Microbiol.*, 47: 251-258.
- Singh, S.V., A.V. Singh, R. Singh, S. Sharma, N. Shukla, S. Misra, P.K. Singh, J.S. Sohal, H. Kumar, P.K. Patil, P. Misra and K.S. Sandhu. 2008. Sero-prevalence of Bovine Johne's disease in buffaloes and cattle population

of north India using indigenous ELISA kit based on native *Mycobacterium avium* subspecies *paratuberculosis* 'Bison type' genotype of goat origin. *Comp. Immunol. Microb.*, **31**: 419-433.

- Singh, S.V., P.K. Singh, A.V. Singh, J.S. Sohal, N. Kumar, K.K. Chaubey, S. Gupta, K.D. Rawat, A. Kumar, A.K. Bhatia, A.K. Srivastav and K. Dhama. 2014a. Bio-load and bio-type profiles of *Mycobacterium avium* subspecies *paratuberculosis* infection in the domestic livestock population endemic for Johne's disease: A survey of 28 years (1985-2013) in India. *Transbound. Emerg. Dis.*, **61**: 1-13.
- Singh, S.V., R.K. Yadav, V.K. Gupta, S. Gupta, K.K. Chaubey, N.D. Agarwal and N. Kumar 2014b. Co-incidence of Bovine Johne's disease and *Bovine brucellosis* in young bulls of Murrah breed in their native tract (Rohtak, Haryana, India). *Adv. Anim. Vet. Sci.*, 2(15): 23-25.
- Sivakumar, P., B.N. Tripathi and N. Singh. 2005. Detection of *Mycobacterium avium* subspecies *paratuberculosis* in intestinal and lymph node tissues of Water buffaloes (*Bubalus bubalis*) by PCR and bacterial culture. *Vet Microbiol.*, **108**: 263-270.
- Sivakumar, P., B.N. Tripathi, N. Singh and A.K. Sharma. 2006. Pathology of naturally occurring Paratuberculosis in Water buffaloes (*Bubalus bubalis*). Vet. Pathol., 43: 455-462.
- Sohal, J.S., N. Sheoran, K. Narayanasamy, V. Brahmachari and S.V. Singh. 2009. Genomic analysis of local isolates of *Mycobacterium avium* subspecies *paratuberculosis*. *Vet Microbiol.*, **134**: 375-382.

Sweeney, R.W., R.H. Whitlock and A.E.

Rosenberger. 1992. *Mycobacterium paratuberculosis* isolated from foetuses of infected cows not manifesting signs of the disease. *Am. J. Vet. Res.*, **53**(4): 477-480.

- Tripathi, B.N., G.G. Sonawane, S.K. Munjal, R.B.
 Bind, D. Gradinaru, S.C. Dubey, S. Tripathi,
 D. Mondal, O.P. Paliwal and N. Singh.
 2007. Seroprevalence of Paratuberculosis in selected population of ruminants in India.
 p. 246-249. *In Proceedings of 9th ICP*, India.
- Waqas, A., M.T. Javed, K. Ashfaque and Q. Mehwish. 2015. An abattoir based study on Brucellosis, Bovine Tuberculosis and Paratuberculosis in buffaloes and cattle at Faisalabad, Pakistan. *International Journal of Veterinary Health Science and Research*, 3: 34-38.
- Yadav, D., S.V. Singh, A.V. Singh, I. Sevilla, R.A. Juste, P.K. Singh and J.S. Sohal. 2008.
 Pathogenic 'bison type' *Mycobacterium avium* subspecies *paratuberculosis* genotype characterized from riverine buffalo (*Bubalus bubalis*) in north India. *Comp. Immunol. Microb.*, **31**: 373-387.