

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

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### 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

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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 anti-bovine horseradish peroxidase conjugate (Sigma Aldrich, 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).

$$\text{S/P percent} = \frac{[(\text{Sample OD} - \text{Negative OD}) \times 100]}{(\text{Positive OD} - \text{Negative OD})}$$

## 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).

Tests	Indigenous - ELISA status n (%)				
	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% bio-load 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- $\gamma$  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%). Waqas *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, sero-prevalence 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.

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