Indian J. Vet. Pathol., ***31****(1): 62-63, 2007* **SHORT COMMUNICATION**

Seroprevalence of paratuberculosis in Indian buffaloes

P. Sivakumar, Nem Singh, B.N. Tripathi†, P. Ezhil Praveena and D. Saravanan
Division of Pathology, Indian Veterinary Research Institute
Izatnagar-243122, Bareilly (UP) India

ABSTRACT

Sivakumar P, Singh Nem, Tripathi BN, Ezhil Praveena P, and Saravanan D (2007). Seroprevalence of paratuberculosis in Indian buffaloes. Indian J. Vet. Pathol. ***31****(1): 62-63.*

The prevalence of paratuberculosis in Indian buffaloes is largely unknown. The present study reports the seroprevalence of paratuberculosis in Indian buffaloes. Of 365 sera samples tested, absorbed-ELISA and agar-gel immunodiffusion test developed in the laboratory for the detection of antibodies specific to paratuberculosis identified 14.5% and 3.2% sera positive for the infection, respectively.

Key words: absorbed-ELISA, Johne's disease, water buffaloes

Paratuberculosis (Johne's disease) is considered to be the most serious debilitating disease in ruminants and is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease is prevalent worldwide and causes huge economic losses due to decline in lifetime productivity and death of animals in clinically advanced stage of disease. An estimate of prevalence of paratuberculosis is generally assessed by bacterial culture or by detection of antibodies to MAP in serum or milk[2,3]. Bacterial isolation, though considered most specific, is less sensitive, cumbersome and time-consuming. Alternatively, a number of serological assays including complement fixation test (CFT), agar-gel immunodiffusion (AGID) and absorbed-ELISA are mostly used for prevalence studies in most countries[4,7,8]. Among them, absorbed ELISA has been reported to be more sensitive and specific for paratuberculosis[2,3].

Indian buffalo population is the largest in the world, which significantly contributes to the total production of milk and meat in the country. Although the disease was reported to be present in buffaloes, based on microscopic examination of smears and johnin skin test, neither confirmation nor estimation of the prevalence by most specific tests was reported in India[1,5,6,10]. The present study reports the seroprevalence of paratuberculosis infection in buffaloes estimated by absorbed ELISA and AGID test.

A total of 365 serum samples including 50 sera (group A) from southern part of India, 105 sera (group B) from the institute farm and 200 sera (group C) from Bareilly slaughterhouse were used in serological assays. The capture-antigen was prepared from 316F laboratory strain of MAP grown in Middle-brook liquid medium and absorbing-antigen from *Mycobacterium phlei* grown in glycerin nutrient broth as described previously[8].

Sera including known-positive and negative controls, and unknown test samples were analysed for MAP-specific antibodies by an in-house developed absorbed-ELISA procedure described previously[8]. An ELISA ratio (ER) was used to determine the cut off values of the assay, in which absorbance of positive duplicates was divided by absorbance of negative duplicates. The sera with ER values of 2 and more were considered as positive for the assay. Sera were also subjected to AGID test as described previously [8]. The appearance of single clear precipitation line was recorded as positive for the test.

Of the 365 sera samples screened for MAP-specific antibodies, 54 (14.5%) samples were positive in absorbed-ELISA with a group wise distribution of seropositivity as 15, 20 and 21 in group A, B and C, respectively. Although, studies on seroprevalence of MAP infection in buffaloes were not available as such, the reports based on other tests have been used for the comparison. The prevalence reported in the present study is relatively higher than those reported in a number of previous studies including a study by Mukerjee and Lahiri[6], who reported 1% on the basis of tissue smear examination of slaughterhouse specimens in West Bengal, and Kulsrestha *et al*.[5], who reported 3.6% positive johnin reactors in the northern states of India. In two other different studies, it was reported that 0.5 and 7% of buffaloes, respectively, were johnin positive[1,10]. These estimates may not be as accurate as they were based on smear examination and johnin test, whose sensitivity and specificity were reported to be low and were hardly used for prevalence studies in other parts of world[3]. In a most recent study, 2% of buffaloes were reported to have histological lesions compatible with paratuberculosis, which was further confirmed by IS*900* tissue-PCR and bacterial culture[9]. Although, the observation of high prevalence of infection in the present study was different from the low prevalence reported previously, it was in agreement with a recent report describing up to 21% seroprevalence of paratuberculosis in cattle suggesting that the disease is prevalent in cattle and buffaloes in

†Corresponding author

larger proportion in the country. Absorbed ELISA is one of the most sensitive and specific serological methods available for the diagnosis of paratuberculosis in bovines[3]. The absorption of test sera with *M. phlei* has endowed greater specificity to the assay[3,8]. Keeping a panel of positive and negative sera, the efficacy of absorption of sera with *M.phlei* was monitored and it was found that absorption step had beneficial effect in removing cross-reacting antibodies[4].

The AGID test, the most specific for serological test for the diagnosis of paratuberculosis in cattle, identified 12 (3.2%) buffalo sera positive for the infection in the present study[7]. Since the sensitivity of AGID is several time lesser than the ELISA, the observation of low positivity in AGID was not surprising, but the specificity of AGID was reported to be more than ELISA and equal to bacterial culture[7]. AGID test-positive animals in the present study were also positive in absorbed-ELISA suggesting that AGID test could be considered a specific test for detection of antibodies to MAP infection[7].

Testing serum samples by ELISA and AGID has advantages over the bacterial culture and PCR. They are comparatively less expensive and the results are available in lesser than 48 hr. In addition, the positive serological tests suggest the most possibility for the presence of infection as seroconversion in paratuberculosis infected animals occurs during later stage of the infection and, therefore, ELISA positive animals cannot be declared free of infection in the absence of testing by bacterial isolation or other tests. It has also been reported that ELISA test is effective in detecting herds that have been infected over a prolonged period of time (Cocito *et al.,* 1994; Collins, 1996). However, it is desirable that some of the ELISA positive animals must be confirmed by the bacterial culture and PCR. Although the data on test sensitivity and specificity of ELISA was not determined in the present study, the specificity was expected to be more possibly due to pre-absorption of sera, which could reduce the cross-reacting antibodies (Cox *et al.,* 1991). Thus, ELISA could be used as preliminary screening test for detecting the infection in a large population with unknown status of the disease, before resorting to the costlier and time-consuming tests.

REFERENCES

1. Bapat JA and Bangi MI (1985). Tuberculosis and Johne's disease among cattle and buffaloes in maharastra state. *Indian J. Anim. Sci.,* **55**: 1022-1023.
2. Cocito C, Gilot P, Coene M, Mde Kesel, Poupart P, Vannuffel P (1994). Paratuberculosis. *Clin. Microbiol. Rev.* **7**: 328-345.
3. Collins M T (1996). Diagnosis of paratuberculosis. *Vet. Clin. North Am. Food Anim. Pract.,* **12**: 357-371.
4. Cox J C, Drane D P, Jones S L, Ridge S and Miller A R (1991). Development and evaluation of a rapid absorbed enzyme immunoassay test for the diagnosis of Johne's disease in cattle. *Aust. Vet. J.,* **68**: 157-160.
5. Kulsrestha RC, Singh J, and Chandirmani NK (1980). A survey on the prevalence of tuberculosis and Johne's disease in cattle and buffalo in Haryana state. *Haryana Veterinarian.,* **19**: 139-141.
6. Mukerjee A and Lahiri A (1960). Investigation of Johne's disease in buffaloes. *Indian Vet. J.,* **37**: 349-353.
7. Sherman D M, Markhan R J F and Bates F (1984). Agar-gel immunodiffusion test for diagnosis of clinical paratuberculosis in cattle. *J. Am. Vet. Med. Assoc.,* **185**: 179-182.
8. Sivakumar P, Singh N, Tripathi BN, Ezhil Praveena P, and Saravanan D (2005a). Seroprevalence of paratuberculosis in cattle. *Int. J. Cow Sci.* **1**: 65-67.
9. Sivakumar P, Tripathi BN, Singh N (2005b). Detection of *Mycobcateriun avium* subsp.*paratuberculosis* in intestinal and lymph node tissues of water buffaloes by PCR and culture. *Vet Microbiol*., **108**: 263-270.
10. Vanamayya PR, Sharma AK and Pari TP (1987). Evaluation of tuberculin and Johnin test with pathological lesions in buffalo. *Indian J. Anim. Sci.,* 57: 189-190.