The present study was aimed to assess the seroprevalence of paratuberculosis among bovines using commercial enzyme-linked immunosorbent assay as a serodiagnostic aid. Four hundred sixty samples (60 indigenous cattle, 60 cross bred cattle and 340 buffaloes) were collected randomly from various villages during the months of September, 2014 to February, 2015 in Krishna district, Andhra Pradesh, India and tested with IDEXX® paratuberculosis antibody test kit. Results revealed an overall seroprevalence of 2.82% among large ruminants. Among cattle, 10.83% (18.33% from indigenous and 3.33% from cross bred cattle) were found positive. None among the 340 samples of buffaloes revealed any positives. Presence of antibodies against bovine paratuberculosis in the studied area indicates the need of continuous epidemiological studies among animals to periodically assess the prevalence and mode of transmission. There is an utmost need to put the animal legislations to proper use for control and eradication of diseases like JD, along with proper financial assistance to livestock farmers to cope with the loss of animal.

Key words: Buffaloes, Cattle, Johne’s disease, Prevalence

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the causative bacterium for Johne’s disease (JD) and is a major problem in domestic animals worldwide (Pahangchopi et al., 2014). In 1895 the bacteria was isolated for the first time by Dr. Heinrich Albert Johne that was unable to multiply outside the animals in nature, but in contaminated soil or water, it survived probably more than a year as it could withstand cold, heat and drying (Singh et al., 2014). Even though JD is primarily a disease of ruminants, it also infects equines, canines, felines, pigs, rabbits, deer and non-human primates characterised by granulomatous enteritis leading to progressive wasting, emaciation and death (Pahangchopi et al., 2014). In United States, Bovines Johne’s diseases accounts for an annual loss of $200-250 million(Ott et al., 1999). In India also many authors (Pahangchopi et al., 2014 and
Rawat et al., 2014) reported the endemicity of JD causing high morbidity and reduced productivity. Vinodhkumar et al. (2013) reported economic loss of Rs. 1840 per sheep/farmer/year. 85% of animals get infected in the first days, 5% during the first year of their life (Sing, 2014) by ingestion of food contaminated with faeces from other infected animals. As the incubation period varies from months to several years, the disease is not manifested clinically until the animals are adults (Collins, 2003). There is no effective treatment and the effected animals become increasing emaciated and ultimately die as a result of dehydration and cachexia (Whitlock and Buergelt, 1996).

Cattle and buffalo population accounts for 37.28% (190.9 million) and 21.23% (108.7 million) of total livestock population of India. Recent years have seen the rise in the number of cross bred cattle to 19.42 million with an increase of 34.78% over the last census (DAHD, 2012). In this regard, a study was conducted to ascertain the seroprevalence of Johne’s disease in Krishna district of Andhra Pradesh, India.

MATERIALS AND METHODS

Sample collection: Four hundred sixty samples (60 indigenous cattle, 60 cross bred cattle and 340 buffaloes) were collected randomly from adult animals of various villages in the months of September, 2014 to February, 2015 in Krishna district, Andhra Pradesh, India. Samples were collected aseptically from jugular vein using BD® vaccutainers. Serum was allowed to clot at room temperature and transferred to laboratory at the earliest possible. Serum was separated and stored at -20°C until further use.

ELISA procedure:

IDEXX® paratuberculosis antibody test kit is very similar to that described in the 5B/009 recommendations (OIE, 1991). The antigen coated in the bottom of the wells is a protoplasmic extract of Mycobacterium paratuberculosis. Sera samples were diluted at the rate of 1 in 20 of dilution buffer and incubated in an uncoated microplate for 45 minutes at 37°C. 100 µl from each of the preplate was transferred to appropriate wells of the coated plate and incubated for 45 minutes at 37°C. All the wells were washed thrice and 100 µL of conjugate was added to each well and incubated for 30 minutes (min) at 37°C. Washing step was repeated as before and TMB substrate was added and incubated for 10 min at 37°C. The reaction was stopped using designated stop solution. Optical densities of both tuberculosis and paratuberculosis samples were read at 450 nm using BioTek® microplate reader. The results were analysed with xChekPlus® software.

RESULTS

In the present study 60 indigenous, 60 cross bred cattle and 340 buffaloes were tested. 10.83% (13/120) of cattle, among which 11 (18.33%) from indigenous and 2 (3.33%) from cross bred cattle were positive for MAP antibodies. None among the 340 samples of buffaloes revealed any positives for MAP antibodies. Overall seroprevalence among large ruminants was 2.82% (13/460) (Table 1).
DISCUSSION

Limited reports available on the prevalence of Johne’s disease in various states of the India, ranging from 15.60 to 22.50% seropositivity in cattle and buffaloes (Tripathi et al., 2008 and Trangadia et al., 2012). Seroprevalence of 10.83% in cattle observed in this study are proximate to the reports of 7% (Trangadia et al., 2014) and 15.14% (Gupta et al., 2012). In contrast, higher infection rates of 21.47% (Tripathi et al., 2008), 27.1% (Kumar et al., 2014), 29.8% (Singh et al., 2008), 50.6% (Pahangchopi et al., 2014), 77.9% (Trangadia et al., 2014) and 86.9% (Singh et al., 2007) were also reported. Zero seroprevalence observed in buffaloes was in complete agreement with the reports of Tripathi et al. (2008). On contrary higher prevalence of 3.73% (Sikander et al., 2012) and 28.6% (Singh et al., 2008) was reported. Overall prevalence of 15.6% in Gujarat and 19.31% in Andhra Pradesh (Trangadia et al., 2012) was reported, whereas very low overall prevalence of 2.82% was observed in this study. Comparative low prevalence of JD compared to other reports in various areas in India may be due to better management practices, small scale animal holdings, farmer awareness, hygiene and proper veterinary aid in the studied area. Samples from stray cattle and old cows taking shelter in Goshala were also collected in the present study, which might have contributed for the sero prevalence of MAP antibodies in cattle.

*Mycobacterium avium* subspecies paratuberculosis has emerged as major and successful animal pathogen with public health and zoonotic concerns (Verma, 2013). After infection bacilli parasites various organs leading to malabsorption, diarrhoea, decrease in milk yield and loss of body condition (Delgado et al., 2009). Many workers (Chi et al., 2002; Dufour et al., 2004; Stott et al., 2005 and Tiwari et al., 2008) in western countries reported on economic losses due to MAP infection, mostly based on reduced milk yield and increased culling and mortality (Tiwari et al., 2008). Studies on the economic impact of MAP infection among Indian bovines are very meagre (Rawat et al., 2014).

Higher detection rate of MAP by PCR and culture in gut samples from Crohn’s patients suggest the emerging zoonotic potential of JD in causing inflammatory bowel disease (IBD)/Crohn’s disease (CD) (Naser et al., 2014). Heat resistant nature of MAP towards pasteurisation and Indian’s

### Table 1. Details of samples screened and prevalence of MAP antibodies in bovines

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Species</th>
<th>No. of samples collected</th>
<th>No. positive</th>
<th>Present Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cross bred cattle</td>
<td>60</td>
<td>2(55)</td>
<td>0(5)</td>
</tr>
<tr>
<td>2.</td>
<td>Indigenous</td>
<td>60</td>
<td>9(50)</td>
<td>2(10)</td>
</tr>
<tr>
<td>3.</td>
<td>Buffaloes (GMB)</td>
<td>240</td>
<td>0(320)</td>
<td>0(20)</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>460</td>
<td>11</td>
<td>2</td>
</tr>
</tbody>
</table>

*Mycobacterium avium* subspecies paratuberculosis among bovines: a serological survey

Table 1. Details of samples screened and prevalence of MAP antibodies in bovines
habit of consuming raw milk may lead to increased chances of transmission of organism from bovines to humans (Grant et al., 2005). Most of the available diagnostic tests lack the sensitivity and specificity in diagnosing JD. Culture remains the Gold Standard, but it takes lot of time (Verma, 2013). In this regard, ELISA serves as an easy, rapid and reliable method for diagnosing JD.

The most striking result observed in this study is seronegativity of Johne’s disease in buffaloes. Prevalence was observed in cattle in the same area and chance of contamination is quite high as both species are generally housed together. Hence, it may be pertinent to cross verify the results with other standard tests and if found same, it is advisable to undertake scientific research regarding ability of buffaloes to resist JD when compared to cattle. In India, cow is regarded as a holy animal. Normally open grazing is a common practice followed in India, which is leading to spread of infection from effected to healthy animal in a rapid manner. There is an utmost need to put the legislations of various animal acts to proper use for control and eradication of diseases like JD, along with proper financial assistance to livestock farmers to cope with the loss of animal.

From this study it is concluded that ELISA is a simple and reliable method for diagnosing JD. High seroprevalence of JD in cattle need further exhaustive epidemiological studies. Seroprevalence of Mycobacterium avium subspecies paratuberculosis among bovines stresses the stringent use of animal legislations along with proper financial assistance to cope with the loss of animals for effective control and eradication of the disease.

Conflict of Interest
Authors declare that they have no significant competing financial, professional or personal interests that might have influenced the performance or presentation of the work described in the manuscript.

ACKNOWLEDGEMENT
Facilities provided by Director of Animal Husbandry, Andhra Pradesh for conducting the research are greatly acknowledged.

REFERENCES


Delgado F, Etchechoury D, Gioffre A, Paolicchi F and Viera FB et al., 2009. Comparison between two in situ methods for Mycobacterium avium subspparatuberculosis detection in


Singh SV, Singh AV, Shukla N, Singh PK and Sohal JS et al., 2007. Sero-prevalence of Johne’s disease in prospective young bulls of Hariana breed by indigenous ELISA kit, using
protoplasmic antigen from ‘Bison type’
genotype of *Mycobacterium avium*


Tripathi BN, Sonawane GG, Munjal SK, Bind RB and Gradinaru D et al., 2008. Seroprevalence of paratuberculosis in selected population of ruminants in India. Proceedings of the 9th International Colloquium on Paratuberculosis, Tsukuba, Japan, 29 October-2 November 2007, pp 246-249


*Article received on 12.08.2015 and accepted for publication on 17.11.1215*