Seroprevalence of Paratuberculosis in Rural Bovine Herds from Different Agro-Climatic Zones of Punjab

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ABSTRACT

Paratuberculosis (Johne’s disease) is an OIE listed notifiable economically important contagious mycobacterial disease of ruminants caused by Mycobacterium avium subspecies paratuberculosis. The present study reports the seroprevalence of paratuberculosis in bovines of the rural area from five different agro-climatic zone of Punjab. A total of 736 serum samples from both cattle and buffalo herds were evaluated for the presence of antibodies to MAP using a commercially available paratuberculosis screening enzyme-linked immunosorbent assay (ELISA) kit. Twenty three animals were positive and the seroprevalence was found as 3.125%. Highest prevalence was recorded in western plain zone (6.66%) followed by western zone (3.07%), central zone (2.68%), sub mountain undulating zone (2%) and nil in undulating plain zone

Keywords: Johne’s disease, seroprevalence, enzyme-linked immunosorbent assay, MAP

Paratuberculosis (Johne’s disease/JD) is an OIE listed notifiable economically important disease of ruminants (OIE, 2008). It is caused by Mycobacterium avium subspecies paratuberculosis (MAP). The disease has worldwide distribution with varying prevalence in different countries. Although MAP primarily infects ruminants but there are reports of the organism infecting the non-ruminants, wild animals as well as human (Feller et al., 2007). In humans, MAP is regarded as one of the causative agent of Crohn’s disease.

The disease is characterized by persistent diarrhoea, progressive weight loss and protein losing enteropathy followed by death. Johne’s disease is considered to be one of the most serious diseases affecting dairy animals as there is no effective treatment and disease can cause significant economic loss in affected herds. Losses are associated with reduced milk yield, lower reproductive efficiency, premature culling and decreased cull cow values. Diagnosis and control of JD is difficult since the disease persists for many years without showing any clinical signs (Manning and Collins, 2001). Thus the disease creates numerous difficulties and represents an important issue for both veterinarians and cattle growers. Evaluation of the regional distribution of infected herds is needed for a successful development of disease prevention and control program. Many diagnostic tests are available but they have their own limitations. As per World Organization for Animal Health (OIE, 2008), enzyme-linked immunosorbent assay (ELISA) is the most sensitive and specific test for serum antibodies to Mycobacterium avium subspecies paratuberculosis in cattle. Limited seroprevalence studies on JD from different districts of Punjab in small ruminants (Dixit et al., 2013) and dairy herds (Garg et al., 2015) have been reported. The objective of the present study was to determine the seroprevalence of paratuberculosis in cattle and buffaloes of the rural population in different agro climatic zones of Punjab.

Collections of samples: Blood samples were randomly collected by Animal Disease Research Centre, GADVASU, Ludhiana, Punjab. Sampling was carried out in apparently healthy animals from dairy farms located at the village level in different districts from five major agro-climatic zones viz., central plain zone, undulating plain zone, western zone, western plain zone, and sub-
mountain undulating zone of Punjab (India). Maximum of 25 samples were collected from cattle and buffaloes in one village. A total of 736 blood samples were collected from dairy animals (341 cattle, 395 buffalo). Hundred samples were collected from sub mountain undulating zone, 50 from undulating plain zone, 336 from central zone, 120 from western plain zone and 130 from western zone. Serum was separated and stored at -80°C until use. The age group of animals from which the samples were collected was between 3 years to 8 years.

Detection of antibodies against MAP: Serum samples were tested for specific antibodies against *Mycobacterium avium* subspecies *paratuberculosis* (MAP) using a commercially available indirect ELISA kit (IDEXX Paratuberculosis Screening Ab Test, IDEXX Laboratories, Inc., Westbrook, Maine, USA) as per the manufacturer’s protocol. Optical density (OD) was measured at 450 nm using ELISA Microplate Reader (Lab Systems, Multiskan). The results were categorized into positive and negative results, after the transformation of OD values into sample/positive ratios (S/P).

The data was analyzed using SPSS (Statistical Package for Social Sciences) for Window version 11.0.1 SPSS Inc. USA computer software program. The associations were evaluated between binary outcome variable and risk factors viz. age, species etc. in order to identify any risk factor associated with seroprevalence of disease.

The total of 736 blood samples from cattle and buffaloes were collected from five agro climatic zones of Punjab and 23 animals were positive. The overall prevalence was found as 3.125 %. Highest prevalence was recorded in western plain zone (6.66%) of which 5% prevalence was in cattle and 1.66% in buffaloes. This was followed by western zone (3.07%), central zone (2.68%), sub mountain undulating zone (2%) and nil in undulating plain zone (Table 1). The prevalence of disease in cattle was 4.3% while that of buffaloes was 2.02%. It was observed that animals only above 5 years were exhibiting the antibodies in the sera sample. Dieguez et al. (2007) have reported that increase in the antibody response depends directly on the disease progress. Statistical analysis revealed that seroprevalence was non-significantly higher in cattle as compared to buffaloes (Chi Square = 2.667 and P value = 0.1025) and in western plain zone as compared to other zones (Chi Square = 2.667 and P value = 0.1025).

Paratuberculosis is a chronic, incurable and often remains in a subclinical state for years. Thus, it is important to diagnose the disease as early as possible to reduce losses and also spread of infection to susceptible animals and even to humans. Many diagnostic tests are available for diagnosis of JD, but each has its own limitations. According to OIE (2008), ELISA technique is considered the main screening test for serological diagnostic of paratuberculosis and control of infectious contagious diseases in animals.

Further this technique is preferably used due to its low cost, rapid and potential to be used for massive testing. MAP has been detected serologically by many workers from different part of the world viz., 1.6% animal-level in small ruminants from Grenada and Carriacou, West Indies (Kumthekar et al., 2013); 3.48% animals in 38 herds of Zulawy, Poland (Sztyn et al., 2012); 1.9% animals of 202 herds of Belgium (Vangeel et al., 2012); 1.2% and 6.1% for beef cattle and dairy cattle, respectively, 3.3% animals of 27 herds in in Gyeongnam province, Korea (Lee and Jung, 2009); 7.3%. animals from South West England (Woodbine et al., 2009); 3.6 % cows from Canada (Tiwari et al., 2009); 2% cattle from Texas (Pearce et al., 2008). In India, few studies on seroprevalence on JD are reported. JD has been investigated in buffaloes and cattle by various techniques like Johnin test (Vanamaya et al., 1987); 48% by PCR (Yadav et al., 2008); 14.5% by PCR and bacterial culture (Sivakumar et al., 2005); molecular epidemiology (Kaur et al., 2011, Garg et al., 2015); 32.1% out of 115 lactating cow by milk ELISA with m-culture; m-PCR (Sharma et al., 2008) and 15.14% (53/300) animals by ELISA (Gupta et al., 2012).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Agroclimatic zone</th>
<th>Total animals tested</th>
<th>Positive animals</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sub-mountain undulating zone</td>
<td>100</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Undulating zone</td>
<td>50</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Central zone</td>
<td>336</td>
<td>9</td>
<td>2.68</td>
</tr>
<tr>
<td>4</td>
<td>Western Plain zone</td>
<td>120</td>
<td>8</td>
<td>6.66</td>
</tr>
<tr>
<td>5</td>
<td>Western zone</td>
<td>130</td>
<td>4</td>
<td>3.07</td>
</tr>
</tbody>
</table>

Table 1: Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* in different agro-climatic zones of Punjab
Seroprevalence of paratuberculosis in Punjab

However, in the present study, various districts of Punjab were covered and seroprevalence was evaluated using ELISA technique. Reports are available regarding the sero-prevalence of disease using indigenous ELISA (Sivakumar et al., 2005; 2007; Singh et al., 2008; Garg et al., 2015). Singh et al. (2008) had screened a total 1425 bovine (Buffaloes and cattle) serum samples showing sero-prevalence of Johne's disease 31.9% and 23.3% in Uttar Pradesh and Ludhiana district of Punjab, respectively. Garg et al (2015) reported a bio-load of MAP infection in dairy herds using serum ELISA and milk ELISA as 15.6 and 16.3 percent respectively. Tripathi et al. (2007) has studied the sero-prevalence of JD in selected population of ruminants from different parts of India. Out of 1822 sera screened, 22.5%, 20%, 11.6% and 4% of 414 indigenous cattle, 264 cross-bred cattle, 465 sheep and 359 goats, respectively, were found to be positive. As compared to the previous reports, seroprevalence of JD was low in the present study. Woodbine et al. (2009) stated that prevalence of paratuberculosis was lower in cattle herds with ≤100 animals. In the present study, the cattle herd size was small and cattle herd ≥100 animals were seropositive. Various studies reported that the possibility for being positive for paratuberculosis increases with herd size (Collins et al., 1994; Dieguez et al., 2007; Woodbine et al., 2009).

It is well established that Mycobacterium avium subspecies paratuberculosis infected animals start developing antibodies in advanced stage of disease (Stabel, 1998) therefore infected animals in early stages of the diseases will be missed when antibodies are targets for diagnosis. The differences in the sero-prevalence pattern of JD with other studies may be contributed due to the difference in methods/techniques employed, different MAP ELISA kit, different time point analysed and area wise difference in sampling designs and target populations.

CONCLUSION
The present study may contribute to the current knowledge on the prevalence of paratuberculosis in different agro climatic zone of Punjab. This study may help in prevention and control of disease in the herd and also in humans from this region. Further studies are required to detect the disease in large populations using multi-disciplinary approach.

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REFERENCES


