Journal of Animal Research: v.6 n.4, p. 751-754. August 2016

DOI: 10.5958/2277-940X.2016.00094.2

SHORT COMMUNICATION

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

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Received: 27 February, 2016 **Accepted:** 05 July, 2016

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 -80ºC 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).

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

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

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 their 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 Zuławy, Poland (Szteyn *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 provine, 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).

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 16.3percent 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.

ACKNOWLEDGEMENTS

The authors acknowledged ICAR-Project Directorate on Animal Disease Monitoring and Surveillance for providing fund for the present study.

REFERENCES

Collins, M.T., Socket, D.C., Goodger, W.J., Conrad, T.A., Thomas, C.B. and Carr, D.J. 1994. Herd prevalence and geographic distrubition of, and risk factors for, bovine paratuberculosis in Wisconsin. *JAVMA*, **204:** 636-641.

Dieguez, F.J., Arnaiz, I., Sanjuan, M.L., Vilar, M.J., Lopez, M. and Yus, E. 2007: Prevalence of serum antibodies to Mycobacterium avium subsp. paratuberculosis in cattle in Galcia (northwest Spain). *Prev. Vet. Med.* **82:** 321-326.

Dixit, M., Filia, G., Singh, S.V. and Gupta, M.P. 2013. Prevalence of paratuberculosis in small ruminants in Punjab. *Indian Vet. J.*, **90(4):** 25-26

Feller, M., Huwiler, K., Stephan, R., Altpeter, E., Shang, A., Furrer, H., Pfyffer, G.E., Jemmi, T., Baumgartner, A. and Egger, M. 2007. *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease: a systematic review and meta-analysis. *Lancet Infect. Dis.*, **7(9):** 607-13.

Garg, R., Patil, P.K., Singh, S.V., Sharma, S., Gandham, R.K., Singh, A.V., Filia G, Singh P. K., Jayaraman, S., Gupta, S., Chaubey, K.K., Tiwari, R., Saminathan, M., Dhama, K. and Sohal, J.S. 2015. Comparative evaluation of different test combinations for diagnosis of *Mycobacterium avium* subspecies paratuberculosis infecting dairy herds in India. *BioMed Res. Int.*, doi:10.1155/2015/983978.

Gupta, A., Rani, S., Pushpa, A. and Gupta, P.K. 2012. Sero-Prevalence of Paratuberculosis (Johne's Disease) in Cattle Population of South-Western Bangalore Using ELISA kit. *Open J. Vet. Med.*, **2:** 196-200.

Kaur, P., Filia, G., Singh, S.V., Patil, P.K., Ravi, G.V., Kumar and Sandhu. K.S. 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.

Kumthekar, S., Elizabeth, J., Manning, B., Ghosh, P., Tiwari. K., Sharma, R.N. and Hariharan, H. 2013. *Mycobacterium avium* subspecies *paratuberculosis* confirmed following serological surveillance of small ruminants in Grenada, West Indies. *J. Vet. Diagn. Invest.*, **25(4):** 527–530.

Lee, K.W. and Jung, B.Y. 2009. Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* in cattle in Korea. *Vet. Rec.*, **165:** 661-662.

Manning, E.J., and Collins, M.T. 2001. *Mycobacterium avium subsp. paratuberculosis*: pathogen, pathogenesis and diagnosis. *Rev. Sci. Tech.*, **20:** 133-150.

OIE. 2008. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

Pearce, B.H., Fosgate, G.T., Ward, M.P., Roussel, A.J., Norby, B., Tavornpanich, S. and Ellis, D. 2008. Comparison of three methods of surveillance with application to the detection of Johne's disease seropositivity in Texas cattle. *Prev. Vet. Med*., **86:** 1–7.

Sharma, G., Singh, S.V., Sevilla, I., Singh, A.V., Whittington, R.J., Juste, R.A., Kumar, S., Gupta, V.K., Singh, P.K., Sohal, J.S. and Vihan, V.S. 2008. Evaluation of indigenous milk ELISA with m-culture and m-PCR for the diagnosis of Bovine Johne's disease (BJD) in lactating Indian dairy cattle. *Res. Vet. Sci*., **84:** 30-37.

Singh, S.V, Singh, A.V., Singh, R., Sharma, S., Shukla, N., Misra, S., Singh, P.K., Johal J.S., Kumar, H., Patil, P.K., Misra, P. and Sandhu, K.S. 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. Microbiol. Infect. Dis*., **31:** 419-433

Sivakumar, P., Singh, N., Tripathi, B.N. and Saravanan, D. 2007. Seroprevalence of paratuberculosis in Indian Buffaloes. *Indian J. Vet. Pathol.*, **31:** 62-63.

Sivakumar, P., Singh, N., Tripathi, B.N., Praveena, P.E. and Saravanan, D. 2005. Seroprevalence of paratuberculosis infection in cattle. *Int. J. Cow Sci.*, **2**: 65-67.

Stabel, J.R. 1998. Johne's disease: A hidden threat. *J. Dairy Res*., **81:** 1283-1288.

Szteyn, J. and Wiszniewska-Laszczych, A. 2012. Seroprevalence of *Mycobacterium avium subsp. paratuberculosis* infection in dairy herds in Zuławy, Poland. *Berl. Munch. Tierarztl. Wochenschr*., **125:** 397–400.

Tiwari, A., VanLeeuwen, J.A., Dohoo, I.R., Keefe, G.P., Haddad, J.P., Scott, H.M. and Whiting, T. 2009. Risk factors associated with Mycobacterium avium subspecies paratuberculosis seropositivity in Canadian dairy cows and herds. *Prev. Vet. Med.*, **88:** 32- 41.

Tripathi, B.N., Sonawane, G.G., Munjal, S.K., Bind, R.B., Gradinaru, D., Dubey, S.C., Tripathi, S., Mondal, D., Paliwal, O.P. and Singh, N. 2007. Seroprevalence of paratuberculosis in selected population of ruminants in India. In: *Proceedings of 9ICP – 2007*. Pp. 246-249.

Vanamaya P., Sharma A.K., Parai, T.P., Paliwal, O.P. and Parihar N.S. 1987. Evaluation of tuberculin and johnin tests with pathological lesions in buffaloes. *Indian J. Anim. Sci.*, **3:** 189-90.

Vangeel, I., Méroc, E., Roelandt, S., Welby, S., Riocreux F., Stede Y.V., Driessche, E.V., Schoubroeck, L.V., Czaplicki, G., Quinet, C., Hooyberghs, J. and Houdart, P. 2012. Seroprevalence of *Neospora caninum*, paratuberculosis and Q fever in cattle in Belgium in 2009-2010. *Vet. Rec.*, **171:** 477.

Woodbine, K.A., Schukken, Y.H., Green, L.E., Ramirez-Villaescusa, A., Mason, S., Moore, S.J., Bilbao, C., Swann, N. and Medley, G.F. 2009. Seroprevalence and epidemiological characteristics of Mycobacterium avium subsp. paratuberculosis on 114 cattle farms in South West England. *Prev. Vet. Med.*, **89:** 102-109.

Yadav, D., Singh, S.V., Singh, A.V., Sevilla, I., Juste, R.A., Singh, P.K. and Sohal, J.S. 2008. Pathogenic 'Bison–type' Mycobacterium avium subspecies paratuberculosis genotype characterized from riverrine buffalo (*Bubalus bubalis*) in North India. *Comp. Immunol. Microbiol. Infect. Dis.*, **31:** 373–387.