Demonstration of Circulating Antibodies of *Mycobacterium avium* Subspecies *paratuberculosis* in Cattle of Rupandehi District, Nepal

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ABSTRACT

Paratuberculosis, caused by Mycobacterium avium subspp. paratuberculosis, is a chronic intestinal infection of global importance in mainly domestic and wild ruminants. The main objective of the study was to find out the seroprevalence of Paratuberculosis in cattle of Rupandehi district. The research was conducted from October 2016 to December 2016. A total of 184 blood samples were collected from Jugular vein of cattle and tested by Enzyme Linked Immunosorbent Assay (ELISA). The Paratuberculosis Indirect Screening Test Kit was developed by ID. Vet, France. Cattle with history of chronic diarrhoea and emaciation were taken as study population along with other cattle in close association with them. Overall seroprevalence in Rupandehi district was found to be 4.89%. No significant relation of paratuberculosis was found with age, breed, parity, body condition score and location. Higher prevalence was found in cattle of older age and low body condition score. The result of this study reports the presence of bovine paratuberculosis in cattle of Rupandehi district.

Keywords: ELISA, Paratuberculosis, Seroprevalence

INTRODUCTION

Paratuberculosis, also known as Johne's disease, is a chronic intestinal infection of global importance in mainly domestic and wild ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). This infection can cause significant economic losses in cattle primarily related to decreased value at slaughter (Kudahland Nielsen, 2009), reduced milk production and premature culling (Raizman *et.al.,* 2009), and eventual death (Kudahl *et.al.*, 2007). Other economic losses resulting from the presence of paratuberculosis in cattle herds include decreased fertility, decreased fat and protein content in milk, reduced feed efficiency, and increased incidence of mastitis (NeilsenandToft, 2009).

The diagnosis of paratuberculosis is divided into two parts- the diagnosis of clinical disease and the detection of subclinical infection- and the latter is essential for control of the disease at the farm, national or international level. In cattle, clinical cases can be diagnosed without difficulty because chronic diarrhea in adult animals is indicative of the disease. In the laboratory, a diagnosis can be made by isolating the agent from feces or necropsy materials, by histological study of the lesions, and polymerase chain reaction (PCR) assays. By using the Ziehl-Neelsen staining method (ZN-test), acid-alcohol resistant bacteria are observed in fecal smears and at the

end portions of the small intestine, however, serum-ELISA has been referred as superior to the ZN-test to confirm the presumptive diagnosis of clinical paratuberculosis in cattle (Weber *et.al.,* 2009). Subclinical cases can be diagnosed by isolating the bacteria from the feces, serological tests, or allergy tests (Lilenbaum *et.al.,* 2007). Hendrick *et.al.,* (2005) found that serum- and milk-ELISAs may be potentially useful and convenient methods in detecting subclinical paratuberculosis in lactating dairy cattle, with sensitivities of 73.6% (95% CI = 61.9-83.3%) and 61.1% (95% CI = 48.9-72.4%), and specificities of 87.5% (95% CI = 84.7-90.0%) and 94.7% (95% CI = 92.6-96.3%), respectively, compared to fecal culture.

Intradermal skin tests carried out in cattle of organized farms of Kathmandu and Pokhara valley showed an overall prevalence of 3.22% (0.9-10.13%) (Annual Reports of Central Animal Disease Investigation and Research Laboratory, Kathmandu, 1982-1988). Absorbed ELISA was conducted in 115 serum samples from buffaloes and 98 serum samples from cattle of different districts of Western Nepal which revealed 39 samples (33.9%) and 27 samples (27.5%) positive to antibodies against paratuberculosis (Joshi and Joshi, 1999). Similarly, rectal scrapings were collected from 28 buffaloes and 26 cows and 4 scrapping smears and 3 cultures were positive for acid fast organisms (Jha, 2003/2004). In recent years (2006-2008), studies on paratuberculosis were conducted by using different diagnostic tests conducted at Animal Health Research Division, NARC, Khumaltar and overall prevalence of 7.10 percent (3.5-9.09%) were detected in DTH, 4.55 percent (4.25-4.85%) in fecal smear, 0.70 percent in fecal culture and 5.34 percent (4.54-5.82%) in AGID in cattle of Kathmandu and Chitwan district. Also, Acharya (2016), found 12.17 percent animal level prevalence and 19.36 percent herd level prevalence of paratuberculosis in cattle of Western Chitwan.

Johne's disease shows some histopathological analogies with Crohn's Disease (CD), a human chronic inflammatory bowel disease of still unresolved etiology, and MAP appears to be related to the etiology of CD as either a causative agent or one of the factors of a multi-etiological syndrome (Atreya *et.al.,* 2014). This association has aroused great scientific interest since the 1980s, when Chiodini *et.al.,* (1984) isolated MAP and its DNA from biological tissues in CD patients. Based on these observations, the link between MAP and CD has been widely investigated through direct and indirect methods for MAP detection (Chiodini *et.al.,* 2012).

MAP prevalence estimation is a key element to assess the disease impact, and for the design of control programs (Verdugo *et.al.,* 2014), therefore, the objective of this study was to determine the seroprevalence of MAP infection using serology from cattle from Rupandehi district and compare it according to age, sex, breed, parity, location etc. These findings will be used by the concerned government and non-government bodies and organizations to monitor the disease, make necessary plans and policies and intervention programs.

Although Johne's disease has been reported in various districts of Nepal, no previous studies have been carried out to estimate its prevalence in Cattle of Rupandehi District. No study has been conducted to determine the associated risk factor for Johne's disease in smallholder dairy cattle in Rupandehi District. Rupandehiis at high risk due to import of animals and animal products from free border of India. MAP prevalence estimation is a key element to assess the disease impact and for the design of control programs.

MATERIALS AND METHODS

A cross sectional study was carried out in Rupandehi district, Nepal, a total of 184 serum samples was collected purposively from female cows between October 2016 and December 2016. Blood was collected from jugular vein and serum was separated and stored at -20^{o}C ELISA test was performed according to the manufacturer's protocol (ID Screen® manufactured by ID Vet, France). Data entry, management and analysis was done using program Microsoft Office Excel 2007. The difference in prevalence according to location, age, parity, breed, body condition score parameters was compared statistically by a Chi-square ($\chi 2$) analysis and Fisher Exact analysis using software OpenEpi version 3 with significance level defined at the $p<0.05$.

RESULT

Seroprevalence of MAP

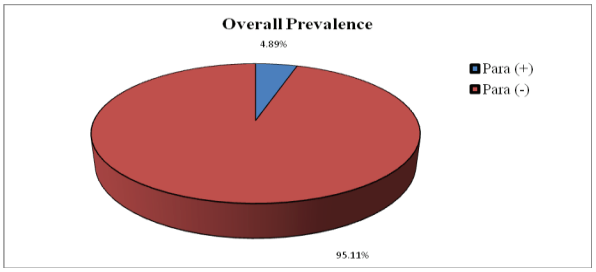


Figure 1: Seroprevalence of MAP in Rupandehi District

Prevalence by Breed

Table 1: Prevalence of MAP by Breed

Breed	Total sample	Positive cases	Seropositivity%
Jersey and Jersey Cross	135	6	4.44%
Others	49	3	6.12%

Out of 135 serum samples of Jersey and Jersey Cross, 6 serum samples (4.44%) were positive whereas in other breeds (Holstein, Holstein Cross, Brown Swiss, Sindhi, Local), 3 serum sample were positive (6.12%) out of 49 samples. There was no significant difference in prevalence according to breed ($p=0.891$; $p>0.05$).

Prevalence by Age

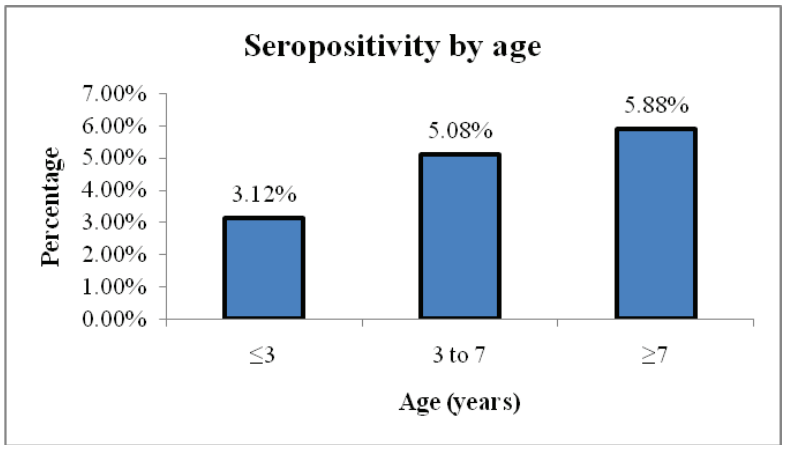


Figure 2: Prevalence of MAP by Age

Out of 32 serum samples of less than equal to 3 years age group, 1 serum sample (3.12%) was positive, out of 118 samples between 3 to 7 years age group, 6 serum samples were positive (5.08%) and 2 serum samples (5.88%) were positive of greater than equal to 7 years age group out of 34 samples. There was no significant difference in prevalence among the age groups (p=0.8625; p>0.05).

Prevalence by Parity

Table 2: Prevalence of MAP by Parity

Parity	Total sample	Positive cases	Seropositivity%
<5	147	7	4.76%
≥5	37	2	5.40%

Out of 147 serum samples of cattle of parity less than fifth, 7 serum samples (4.76%) were positive whereas in cattle of parity more than equal to fifth, 2 serum samples were positive (5.40%) out of 37 samples. There was no significant difference in prevalence according to parity (p=0.754; p>0.05).

Prevalence by BCS

Out of 122 serum samples of cattle having BCS less than 2.5, 7 serum samples (5.74%) were positive whereas in cattle having BCS more than equal to 2.5, 2 serum samples were positive (3.22%) out of 62 samples. There was no significant difference in prevalence according to BCS (p=0.72; p>0.05).

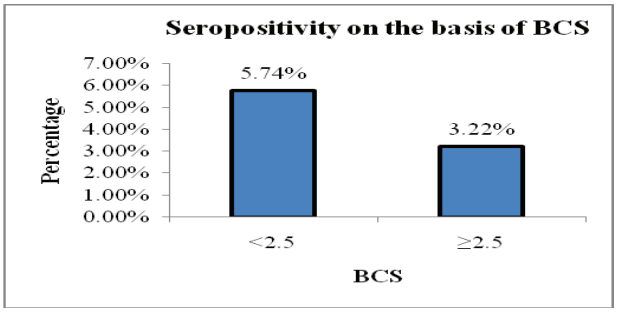
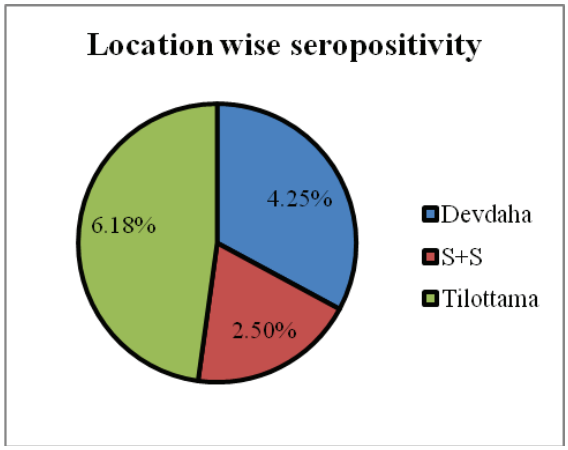


Figure 3: Prevalence of MAP by BCS

Prevalence by Location



Out of 97 serum samples from Tilottama Municipality, 6 serum samples (6.18%) were positive, out of 47 samples from Devdaha Municipality, 2 serum samples were positive (4.25%) and only 1 serum sample (2.50%) was positive from Sainamaina Municipality and Saljhandi VDC (S + S) out of 40 samples. There was no significant difference in prevalence among location (p=0.643; p>0.05).

Figure 4: Prevalence of MAP by Location

DISCUSSION

Paratuberculosis causes important economic losses in ruminants (Nielsen andToft, 2009; Coelho *et. al.*, 2007). Although the presence of paratuberculosis has been known for a long time in Nepal, there are a few studies related to prevalence of paratuberculosis. To our knowledge this is the first report on estimation of sero-prevalence of MAP in Rupandehi district. In the present study, overall seroprevalence of Paratuberculosis in Rupandehi district was found to be 4.89%. This finding is greater than overall prevalence of 3.32% in cattle of organized farms of Kathmandu and Pokhara valley done by Intradermal Skin Test as reported in Annual Reports of Central Animal Disease Investigation and Research Laboratory, Kathmandu, 1982-1988. However, this result is less than findings of Acharya, 2016 *i.e.,* 12.17% prevalence at individual animal level in cattle of Western Chitwan. In Western Nepal, Joshi and Joshi (1999) found 37.5% (27/98) samples positive to antibodies against MAP using absorbed ELISA. In recent years (2006-2008), studies on paratuberculosis were conducted by using different diagnostic tests conducted at Animal Health Research Division, NARC, Khumaltar and overall prevalence of 7.10 percent (3.5-9.09%) were detected in DTH, 4.55 percent (4.25-4.85%) in fecal smear, 0.70 percent in fecal culture and 5.34 percent (4.54-5.82%) in AGID, in cattle of Kathmandu Valley and Chitwan District. The differences in prevalence may be due to the geographic variations, location of farms, sampling procedures and the use of different detection methods. This is supported by research of McKenna *et.al.* (2004) which regards that prevalence of paratuberculosis can be affected by the factors such as climate, nutrition, region and housing conditions and diagnostic methods.

No significant relation of Paratuberculosis was found with age, breed, parity, body condition score and location. Higher prevalence of Paratuberculosis was found in cattle of Tilottama municipality of Rupandehi district than in other locations. Cattle of older age were found to have higher seropositivity against Paratuberculosis than those of younger age and higher prevalence was found in cattle with low body condition score. The observed greater prevalence with higher parity is in line with the chronic nature of the infection which implies that the cows are relatively

old (typically 2-5 years old), before showing symptoms of the disease (Chiodini *et.al.*, 1984). This result is similar to the findings of Jakobsen *et.al.,* (2000) in Danish dairy cattle, Higher prevalence in other breeds can be observed than in Jersey or Jersey cross breeds, which differs from finding of Jakobsen *et.al.,* (2000) which shows the Jersey cattle having higher risk of being seropositive against Paratuberculosis.

CONCLUSION

This study is the first to report and estimate the seroprevalence of paratuberculosis in cattle of Rupandehi district and hence will certainly contribute in the management and eradication of the disease from this region. This study shows no any significant relation between the disease and various factors such as age, breed, parity BCS, location etc.

However, the validity and accuracy of this research could be challenged through increased sample sizes. Enhanced surveillance using confirmatory techniques with sufficient sample size is a prerequisite to confirm the findings. Further epizootiological investigations on Paratuberculosis in other farm animals at the country level are important to monitor and determine the magnitude of Paratuberculosis infection in order to estimate its economic impact on animal industry.

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