

Original Research Article

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Seroprevalence of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in Goat Population of Bhubaneswar, Odisha, India

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ABSTRACT

Keywords

Antibodies, Goats, Johne's disease, *Mycobacterium avium* subspecies *paratuberculosis*, Odisha, Paratuberculosis, Seroprevalence, Small ruminants

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Present pilot study was the first attempt to estimate the prevalence of antibodies against *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in the goat population of Odisha. A total of 22 serum samples were collected from goats in and around Bhubaneswar, Odisha and subjected to indirect ELISA by indigenous ELISA (i_ELISA) kit for the detection of antibodies against MAP in the Microbiology Laboratory, Animal Health Division, CIRG, Makhdoom. An apparent prevalence 68.19% (15/22) was recorded by i_ELISA where the S/P ratio of the positive control was considered to be 1(100%). The S/P ratio of the positive samples ranged from 0.4169 to 0.6857. The results indicate the previous exposure of the animals to MAP and suggest the need of detailed systematic sampling on a larger scale to estimate the exact prevalence of MAP in various regions of Odisha to formulate necessary preventive and control measures.

Introduction

Mycobacterium avium subspecies *paratuberculosis* (MAP) infection in ruminants results in chronic inflammatory condition of intestine commonly known as paratuberculosis or Johne's disease characterized by poor body condition, progressive weight loss with or without diarrhoea and emaciation (Patel and Shah,

2010). MAP has been incriminated as the cause of Crohn's disease (CD), a chronic inflammatory bowel condition in human beings (Singh *et al.*, 2016). MAP is the cause of considerable concern to the dairy industry worldwide due to the economic losses caused by reduced milk production and food safety situation as a result of post-pasteurization survival in milk and subsequent transmission to humans (Patel and Shah, 2011). India is

endemic to Johne's disease and there have been reports of the prevalence of MAP from different regions of the country in cattle, buffaloes, sheep, goats and humans (Singh *et al.*, 2007, Ramalakshmi *et al.*, 2016, Bhutediya *et al.*, 2017). Although no such prevalence has been reported from the coastal state of Odisha in eastern India, the possibility of the introduction of MAP in the animals of the state resulting from trans-boundary movement of animals from the neighbouring affected states cannot be ruled out (Ramalakshmi *et al.*, 2016, Bhutediya *et al.*, 2017). Thus, the present pilot study targeted at the investigation of the presence/absence of MAP antibodies in small ruminants of Odisha.

Materials and Methods

Serum samples

A total of 22 serum samples were collected from apparently healthy goats in and around Bhubaneswar with the permission of Institutional Animal Ethical Committee and stored at -20°C in the Department of Veterinary Preventive Medicine, College of Veterinary Science and Animal Husbandry, O.U.A.T, Bhubaneswar.

Indirect ELISA (i_ELISA)

The serum samples were subjected to the detection of MAP antibodies by indirect ELISA using indigenous ELISA (i_ELISA) kit provided by Microbiology Laboratory, Animal Health Division, CIRG, Makhdoom according to Singh *et al.*, (2007) in the Microbiology Laboratory, Animal Health Division, CIRG, Makhdoom.

Briefly, 0.1µg/well of antigen, in 100µl of carbonate-bicarbonate buffer, pH 9.6 (coating buffer) was used for coating in duplicate wells of flat bottom ELISA plates (Alto, Italy), at 4 °C for overnight. Plates were washed thrice

with PBST (phosphate buffer saline and 0.05% Tween-20). Blocking was achieved by 200 µl of 3% skimmed milk powder and incubated at 37°C for 1 h. After incubation, the plates were washed thrice with PBST and 100µl of 1:50 diluted test serum samples in dilution buffer (PBST containing 1% BSA) was added to each well, in duplicates and incubated for 2 h at 37 °C. After incubation three washings (5 min each) were given with PBST and 100 µl of optimally diluted (1:8000) rabbit anti-goat horseradish peroxidase conjugate (Bangalore Genei, India) in PBS was added to all wells and incubated for 1 h at 37 °C. After incubation the plate was washed three times (5 min each) with PBST. Finally 200µl of freshly prepared substrate (orthophenylene diamine dihydrochloride, OPD), 5 mg/plate in substrate buffer (pH, 7.4) was added to each well and incubated in dark for 20 min at room temperature. The absorbance was read at 450 nm in ELISA reader (Multiskan, Thermo-Labsystems). Positive, negative, substrate and conjugate controls were run with serum samples. Serum from the goats tested positive and negative to MAP previously were used as positive and negative controls.

The optical density measured were transformed to Sample /Positive (S/P) ratio by the formula mentioned below and the corresponding status of JD in goats was interpreted as per the instructions of Collins *et al.* (2002). Only the positive and strong positive were considered to be positive (Singh *et al.*, 2014).

Sample: Positive ratio value

$$= \frac{\text{Optical Density of Sample} - \text{Optical density of Negative Control}}{\text{Optical Density of Positive control} - \text{Optical Density of Negative Control}}$$

Results and Discussion

A total of 22 serum samples were collected from apparently healthy goats in and around

Bhubaneswar and were subjected to the detection of MAP antibodies by the indigenous ELISA (i_ELISA) kit. Out of 22 samples in the present pilot study, 15(68.19%) were positive, 6 (27.27%) were low positive and 1(4.54%) were negative for antibodies against MAP in i_ELISA (Table 1). The S/P ratio of the positive control was considered to be 1(100%). The S/P ratio of the positive samples ranged from 0.4169 to 0.6857.

Johne's disease (JD) was reported in India for the first time in 1913 and it continues to be a major health problem in domestic ruminants (Singh *et al.*, 2014). Diagnosis and control of disease in sub-clinically infected animals is extremely challenging, since the disease gets transmitted prior to the development of clinical signs (Mukartal *et al.*, 2016). However, most accurate and informative test results are often based on antibody detection using ELISA test (Singh *et al.*, 2007). A total of 22 serum samples were collected from apparently healthy goats in and around Bhubaneswar and were subjected to the detection of MAP antibodies by the indigenous ELISA (i_ELISA) kit according to Singh *et al.*, (2007).

The detection of MAP infection can be done in two ways viz, detection of antigen (bacilli or nucleic acid of the bacilli) by culture, ZN staining or PCR or detection of antibodies (serological tests). Culture is considered as gold standard but requires 12-16 weeks incubation period.

The easiest method for detection is microscopy but it requires trained personnel. Nucleic acid amplification although sensitive and less time consuming, is comparatively costly. A wide range of serological tests such as enzyme-linked immune sorbent assay (ELISA), agar gel immunodiffusion (AGID), delayed type hypersensitivity (DTH),

interferon-gamma assay (IGA), fluorescence antibody test (FAT) and complement fixation test (CFT) have been successfully used for the detection of MAP infection. However, due to high sensitivity, rapidity and low cost, ELISA has emerged as the most widely used test for the screening of MAP infection in herds and flocks. Indigenous ELISA developed at Central Institute for Research on Goats (CIRG), Makhdoom, Mathura, India, has been widely used indigenous kit for the screening of animals and human samples in India (Singh *et al.*, 2016).

On the basis of S/P ratio (Collins *et al.*, 2002), out of 22 samples in the present pilot study, 15(68.19%) were positive, 6 (27.27%) were low positive and 1(4.54%) were negative for antibodies against MAP in i_ELISA (Table 1).

The detection of antibodies to MAP in the goats of Bhubaneswar, Odisha indicates that the animals have been sensitised by previous exposure to the bacilli (Narang *et al.*, 2017). None of the animals in the present study showed any clinical signs of the disease so the detection of specific antibodies to MAP by ELISA indicates a sub-clinical stage of infection of the animals (Bhutediya *et al.*, 2017). Previously, a total of 157 cattle were tested for JD by single intradermal test in Cuttack, Bargarh, Puri and Jagatsinghpur districts of Odisha out of which none were positive (AICRP report, 2014-15). Thus, present pilot study is the first to report the prevalence of JD in the goat population of Odisha.

India is reported to be endemic to MAP infection and the prevalence is reported to be 63.5 % in Mehasana breed of goats in South Gujarat (Singh *et al.*, 2013), 12.22% in goats of Kerala (Abraham *et al.*, 2014) and 76.0% in the Bannur breed of sheep located in Mandya district of Karnataka using ELISA.

Table.1 Percentage prevalence of MAP in serum samples by I_ELISA

Sl. No.	Category of ELISA result (S/P ratio)	No. of Samples (%)
1.	Strong Positive(1.0 to 10.0)	0
2.	Positive(0.4 to 0.99)	15 (68.19)
3.	Low Positive(0.25 to 0.39)	6 (27.27)
4.	Suspected(0.10 to 0.24)	0
5.	Negative(0.00 to 0.09)	1 (4.54)
6.	Total	22(100)

Besides, prevalence of 37.7% in organized and unorganized dairy cattle herds of West Bengal (Bhutediya *et al.*, 2017), 41.0% in buffaloes in central and North India (Audariya *et al.*, 2016), 2.82% in large ruminants of Krishna district of Andhra Pradesh (Didhugu *et al.*, 2015) and 15.14% in cattle of South West Bangalore (Gupta *et al.*, 2012) was recorded in the bovine population of different regions of India. The difference in prevalence pattern of Johne's disease in the present study could be due to diversity in topography and environment, animal rearing system, and husbandry practices followed in different states of India (Bhutediya *et al.*, 2017). Globally, a prevalence of 16.8% was reported in two dairy goat herds in Chile (Salgado *et al.*, 2002), 1.4% prevalence was noted in the Boer Goat Herds in Missouri, USA (Pithua and Kollias, 2012), 83.0% goats were positive in Ontario, Canada (Bauman *et al.*, 2016) and 24% - 35.9% prevalence was recorded in Burdur, Southwestern Turkey (Celik and Turutoglu, 2017).

The present study reports a high apparent prevalence of MAP infection in the small ruminants of Odisha. In absence of any vaccination being practised against the disease, the detection of specific anti-MAP antibodies confirms the infection of the animals with MAP bacilli. It is possible that in absence of past prevalence of MAP, the introduction of the organism was due to trans-border movement of affected animals from the neighbouring states with reported

incidence of MAP infection (Didhugu *et al.*, 2015; Bhutediya *et al.*, 2017).

The goats in the present study did not show any clinical signs of disease which is not uncommon and is indicative of subclinical infection as in the Satge II or lepromatous stage of Johne's disease (Bharathy *et al.*, 2014; Manning and Collins, 2001).

The bacilli can be transmitted through consumption of milk and milk products and increased level of infection in the animals increases the threat of transmission of the disease from affected animals to healthy animals and human beings. Present study reports the sero-prevalence of JD in goat population of Bhubaneswar, Odisha for the first time. The high level of infection detected in this study indicates the exposure of the animals to MAP and increases the risk of transmission to other animals and humans alike. Hence extensive systematic sampling is advocated along with culture and biotyping to detect the strain of bacilli circulating amongst the animals in order to implement effective preventive and control strategies.

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