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PREVALENCE OF PARATUBERCULOSIS IN CATTLE AND BUFFALOES

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Paratuberculosis is chronic incurable disease of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The disease prevalence is estimated using serological tests that detect MAP - specific antibodies in serum or milk (Cocito *et al.*, 1994; Sivakumar, *et al.*, 2005). Seroprevalence of paratuberculosis infection has rarely been studied in India (Sivakumar *et al., loc. cit*). The present study reports on the prevalence of paratuberculosis in cattle and buffaloes reared in scattered and unorganized small dairy units in the Gujarat state.

Materials and Methods

Cattle and Buffalo sera samples : In the present study, the sera samples submitted to the Project Directorate on Animal Disease management and Surveillance, Bangalore (PD_ADMAS) were used for testing the presence of antibodies specific to MAP. A total of 480 sera samples (including 235 cattle and 245 buffaloes) collected at the village level unorganized dairy farms or individual household dairy units in 5 districts in the Gujarat state were subjected to absorbed - ELISA procedure standardized in our laboratory.

Preparation of Antigens and Sera : The capture antigen (protoplasmic) was prepared from heat - killed filtered MAP strain (316F) grown in Middle-brook liquid medium by sonication and absorbing antigen from whole cell killed Mycobacterium, *phlei* (M. *phlei*) grown in glycerin nutrient broth medium, as dried powder (Sivakumar *et al.*, 2005).

The sera samples were absorbed with M. *phlei,* wherein 40mg of dried powder was added to 1 ml of optimally diluted (1:200) serum sample, mixed well and left at 4°C over night. Further, the sera samples were centrifuged to remove M. *phlei* to obtain pre-absorbed serum (Sivakumar *et al., loc.cit*).

Absorbed ELISA : ELISA was done based on the standardized method of Sivakumar *et al.*, (*loc. cit*). The flat bottomed 96 well plates were coated with 100µl of killed protoplasmic MAP antigens at optimum concentration by incubating the plates at 4°C overnight. After washing thrice in washing buffer (PBST; 0.05% tween in phosphate buffered saline) the uncoated sites were blocked with 3% bovine serum albumin (BSA) in PBST for 1 h at 37°C. Following one wash in PBST, 100µl of pre-absorbed serum was added in duplicate and incubated at 37°C for 2h. Followed by three washings (5 min each), 100µl of optimally diluted (1: 15000) antibovine IgG-HRPO conjugate (Sigma, USA) was added for 1 h at 37°C. The presence of antibodies to MAP was detected by adding 200µl of substrate (12 mg of O-Phenylene Diamine dissolved in 20 ml of substrate buffer containing 5 ml

Table 1 : Results of absorbed - ELISA employed on cattle and buffalo sera for MAP

District	Cattle Sera			Buffalo Sera		
	No. tested	No. positive	% Positive	No. tested	No positive	% Positive
Dahod	75	3	4.0	75	1	1.0
Bharuch	75	6	8.0	75	9	12.0
Vadodara	10	2	20.0	20	1	5.0
Panchmahal	60	3	5.0	60	2	3.3
Ashwa Dang	15	2	13.0	15	1	6.6
Total	235	16	6.8	245	13	5.8

of H_2O_2) solution and incubated for 30 min. at 37°C and the absorbance at 492 nm was taken in ELISA reader (Multiscan Ex USA). The positive and negative control, antigen and antibody control were always included in each plate. The cut off value of ELISA was determined by ELISA ratio (ER), in which absorbance of positive duplicates was divided by absorbance of negative duplicates. An ER value of 2 and more was considered positive for the presence of antibodies specific to MAP.

Results and Discussion

The ELISA results on the sera collected are given in the table. Of the 235 cattle sera tested by absorbed - ELISA, 16 (6.8%) were found to be positive for antibodies specific to MAP. The district-wise percentage of seropositivity was found to be quiet variable that could be directly related to unequal number of sera samples obtained from different districts in the Gujarat state.

The percentage seroprevalence of paratuberculosis observed in cattle in the present study was found to be low when compared to the previous study that reported > 20% seroprevalence in cattle from organized dairy farms in selected parts of India (Sivakumar *et al.*, loc. cit). The ELISA results were supported by the findings of positive AGID, faecal smear examination and faecal PCR in the previous study (Sivakumar, 2003).

The absorbed - ELISA procedure is considered to be specific for paratuberculosis (Milner *et al.*, 1990). The lower prevalence rate of paratuberculosis infection among cattle from scattered and unorganized dairy farms in the present study when compared to the higher rate in the organized farms of previous study suggests that infection rate is high among cattle in the organized farms than in unorganized farms (Cocito *et al.*, *loc. cit*).

Of the 245 buffalo sera samples tested, 5.8% were found to be positive in absorbed - ELISA. The district-wise percentage of seropositivity was found to be quiet variable, which might be related to unequal number of samples from different districts, similar to cattle. The seroprevalance of paratuberculosis among the buffaloes is lower than that observed in buffaloes from organized farms, in which 14.5% seroprevalence was reported in a recent study (Sivakumar *loc.cit*). However,

there is no adequate information regarding the overall prevalence of paratuberculosis among buffaloes in India for comparison.

Among buffaloes, Bharuch district had higher prevalence of MAP followed by Ashwa Dang and Vadodara districts and lower prevalence in Panchmahal and Dahod districts. In conclusion, paratuberculosis infection was found to be prevalent in cattle and buffalo population in unorganized dairy farms in the country, also as in organized farms, but at lower incidence.

Summary

The present study reports 6.8 and 5.8% seroprevalence of paratuberculosis infection in cattle and buffaloes, respectively, from unorganized dairy farms in the Gujarat state. The incidence was lower in rural dairy population, then in organized dairy farms.

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