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RESEARCH ARTICLE

INVESTIGATION ON BOVINE JOHNE'S DISEASE IN AN ORGANIZED DAIRY FARM OF MHOW IN MADHYA PRADESH

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ABSTRACT

In this study, serum and fecal samples collected from 14 dairy animals (11 cattle and 3 buffaloes) of an organized dairy farm located at Mhow, Indore, Madhya Pradesh were screened by ELISA and acid fast staining, respectively for the presence of MAP infection. Majority of cattle (80.0%) including a suspected buffalo were positive by Indigenous ELISA kit and 36.6% bovines were found positive for MAP by acid fast staining. Study revealed that Bovine Johne's disease was major health problem (by clinical and laboratory examination) in the dairy herd located at Mhow, Madhya Pradesh and need attention to control the disease.

INTRODUCTION

Mycobacterium avium subspecies *paratuberculosis* (MAP), the cause of Bovine Johne's disease (BJD) in dairy cattle and is characterised by chronic enteritis followed by weakness and emaciation. MAP is major infectious disease of ruminants and also infects other animals and primates (Soltys *et al*., 1967, Fawcett *et al*., 1995, De Lisle *et al*., 2005) inflicting heavy economic and production losses. MAP can also infect non- ruminant (pigs, dogs, horses, cat, etc.) - (Miranda *et al.* 2011, Glanemann *et al*., 2008, Mitchell *et al*., 2005, David *et al*., 2006), free ranging animals (Rabbits, blue bulls, bison, deer) (Kumar *et al*., 2010, De Lisle *et al*., 2005; Singh *et al*., 2012), primates (baboons, gibbon and cotton-top tamarins) and human beings (Chiodini *et al*., 1984, Singh *et al*., 2011a). MAP has been frequently reported in cattle, buffalo, goat and sheep farms (Shroff *et al*., 2013, Singh *et al*., 2007b, 2008, 2009, 2013a, b, c) in India. Though MAP accounts for increased culling rate, high morbidity and high economic and production losses in dairy farms, but impact of the BJD has neither been realize nor is in the priority for control in India. Present study was undertaken to estimate prevalence of MAP infection in the clinical cases of BJD in a dairy farm located in Mhow, Madhya Pradesh.

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MATERIALS AND METHODS

History

A total of 11 cattle and 3 buffaloes of an organized dairy cattle farm, located at Mhow, Mathya Pradesh were Sampled for screening of MAP infection by Indigenous ELISA and acid fast staining. The 11 serum (10 cattle 1 buffalo) and 11 fecal (8 cattle and 3 buffaloes) samples submitted to Microbiology lab of Central Institute for Research on Goats, Makhdoom were screened by Indigenous ELISA and acid fast staining (ZN staining), respectively.

Enzyme-linked Immuno-sorbent Assay

Indigenous ELISA kit (i-ELISA) was developed first time for screening of goats against JD (Singh *et al*., 2007a). Protoplasmic Antigen (PPA) was harvested from Indian 'bison type' genotype of MAP isolated from a terminal case of JD in a Jamunapari goat (Singh *et al*., 2007b).Whole cell sonicated lysate was centrifuged and standardized at 0.1 ug protein per well of microtiter plate. Serum samples were used in 1:50 dilution and anti-species horseradish peroxidase conjugate (Sigma) in 1:5000 dilution. Serum of culture positive and negative animals were used as positive and negative controls, respectively. Optical densities (OD) were transformed to S/P ratios and animals were negative (0.00-0.09), suspected (0.10-0.24), low positive (0.25-0.39), positive (0.40-0.99) and strong positive (1.0-

10.00) category for the status of JD (Collins, 2002). Animals in positive and strong positive category were considered positive for the bio-presence of MAP. Later on this goat based ELISA was adapted to screen bovine serum samples against MAP infection, since MAP 'Indian Bison Type' (the source of antigen for ELISA) was major bio-type infecting of Indian domestic livestock inculting cattles (Singh *et al*., 2009, 2010).

Direct microscopy

Two grams of feces was collected directly from 11 animals (8 cattles and 3 buffalo) in polythene bags. Fecal samples were triturated and centrifuged at 4500 rpm for 45-60 min. at room temperature. The upper layer was discarded and smears prepared from middle layer were stained by Ziehl neelsen method and examined under 100X of microscope (Nikon E200). Presence of small short pink coloured rods indistinguishable to MAP were considered positive.

RESULTS

Screening by ELISA

Of 11 serum samples (10 cattle and 1 buffalo) collected from organized dairy cattle farm, Mhow, MP were screened by indigenous ELISA wherein 81.1% (8) animals were found positive. Sero-presence of MAP was 80.0% in cattle. One buffalo clinically suffering from BJD o screening by ELISA and was also found positive (Table 1).

Screening by microscopy

Of 11 fecal samples screened from organized dairy cattle farm, 36.3% (4) were positive in microscopy. Specie-wise bio-presence of MAP was 50.0 (4) and 0.0% (0) in cattle and buffaloes, respectively (Table 1).

Table 1. Prevalence of MAP infection in an organized dairy cattle farm, Mhow (MP) using Indigenous ELISA and acid fast staining

Sn	Animal no.	Indigenous ELISA	Acid fast staining
1	37C	Negative	NA
2	38C	Positive	Negative
3	5C	Positive	Negative
4	7C	Positive	Positive
5	8C	Positive	NA
6	9C	Positive	Positive
7	10C	Positive	Positive
8	31C	Negative	Negative
9	3C	Positive	Positive
10	39C	Positive	NA
11	4B	Positive	Negative
12	20 B	NA	Negative
13	X B	NA	Negative
14	36C	NA	Negative
Total=14		9 (81.1)	4 (36.3)

*figures in parenthesis are in percent; NA- not available

DISCUSSION

Due to lack of awareness, unavailability of appropriate facilities and also lack of willingness of owners there is often lot of struggling by animals in collection of blood samples, that's why screening of large ruminants is often met with difficulties. Johne's disease is a major health concern in domestic and wild animals (Manning *et al*., 2001, Chiodini *et al*., 1984, Collins, 1994). MAP has been frequently reported from cattle, buffaloes, bison, blue bulls in India (Singh *et al*., 2007b, 2008, 2011b, Kumar *et al*., 2010). Serum samples were collected from cattle and buffaloes of organized cattle farm, Mhow on the basis of their poor physical and health status with respect to BJD. Animals were weak and suffering from diarrhoea. In ELISA, indigenous protoplasmic antigen from MAP 'India bison-type' strains of goat origin was used for the screening of cattle. Of the 11 (10 cattle and 1 buffalo) serum samples screened by Indigenous ELISA, prevalence of MAP was 81.1 (9) and species wise prevalence was 80.0 (10) and 100% (1) in cattle and buffalo, respectively. Of 11 (8 cattle and 3 buffaloes) fecal samples, prevalence of MAP by acid fast staining was 36.3% (4) and species wise prevalence was 50.0 (4) and 0.0% (0) in cattle and buffaloes, respectively (Table 1). Of 14 animals, both serum and fecal were collected from 8 animals (7 cattle and 1 buffalo) and 6 other collected samples were either serum or, fecal. The two tests (Indigenous ELISA and Acid fast staining) were used for screening of all 14 animals for MAP infection. The two tests in combination detected 50.0 (4), 12.5 (1), 37.5 (3) and 0% (0) animals in and positive, and negative, positive only in ELISA and category by two tests positive only by acid fast staining respectively (Table 2). Screening of 8 clinical cases of BJD in dairy cattle by ELISA and acid fast staining, 87.5 and 50.0% were detected positive, respectively. The one animal which was negative in two test must be false positive. Similarly 3 (37.5%) animals positive in ELISA were negative in AFB were false negative. And none of animal positive in microscopy was missed by ELISA. Therefore this study showed higher number of clinically animals positive in ELISA than by acid fast staining. It has further confirmed the superior sensitivity of ELISA over acid fast staining in large ruminants (Marquardt *et al*., 1980) but, in small ruminants shedding (by acid fast staining) was often seen prior to peripheral immune response (Lybeck *et al*., 2011). Complete elimination of the infectious agent is not practicable in the country due to ethical reasons. Therefore, transmission of the MAP infection from adult infected adult animals to susceptible young calves should be prevented by adopting better management practices such as segregation of healthy and diseased animals and proper treatment and disposal of waste from affected animals. However, use of therapeutic vaccine may be better option to control BJD in the country since slaughter of cows is banned due to religious reasons. In conclusion, study showed that Indigenous ELISA and acid fast staining could serve as useful tests for screening of adult cattle herds for the diagnosis of MAP infection by laboratories that lack suitable facilities and equipment for other costly tests, particularly in developing countries like India.

Table 2. Comparative evaluation result of Indigenous ELISA and acid fast staining

Test	Comparison			
	1	2	3	4
ELISA	+	-	+	-
Microscopy	+	-	-	+
Total (8)	4 (50.0)	1(12.5)	3 (37.5)	0 (0.0)

*figures in parenthesis are in percent

Conflict of Interest

No conflict of interest to declare.

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