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Research Article

DETECTION OF MYCOPLASMA BOVIGENITALIUM ANTIBODIES BY INDIRECT-ELISA IN COWS WITH VARIOUS REPRODUCTIVE DISORDERS

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ABSTRACT

During present investigation, a total of 135 cows comprising 85 diseased (15 abortion/still-birth, 17 metritis, 6 cervicitis, 25 repeat breed, 22 anoestrus) and 50 apparently healthy, were tested for *Mycoplasma bovigenitalium* infection by indirect – ELISA. *M. bovigenitalium* specific ELISA-titre (≥1:160) was detected in 30 out of 85 (35.3%) diseased and 11 out of 50 (22%) apparently healthy cows. Higher percentage of cows having anoestrus (45.55%) and abortion/still-birth (80%) proved positive for *M. bovigenitalium* ELISA-Ab as compared to repeat breed, cervicites and metritis cases (16 to 17.64%).

Key words: *Mycoplasma bovigenitalium*, cows, indirect-ELISA, reproductive disorders

Mycoplasma bovigenitalium, a potential genital pathogen of bovines, is known to cause a variety of reproductive disorders viz. granular vulvovaginitis, abortion/still-birth, seminal vesiculitis and sperm abnormalities. This organism was first isolated from genital tract of infertile cows having bursitis and salpingitis (Edward *et al.*, 1947). In India, it was first reported from the genital tract of cow having the history of repeat breeding (Jayaraman, 1961) and has been reported continuously from various bovine reproductive disorders (endometritis, cervico-vaginitis, cervicitis, repeat breed, abortion, low fertile bull-semen) as well as from apparently healthy bovines (Volintir *et al.*, 1970, Misra *et al.*, 1975, Ball *et al.*, 1978, Pal *et al.*, 1982, 1984, Garg *et al.*, 1988, Sharma *et al.*, 1997). In the present study, cows having reproductive disorders as well as healthy cows were tested for *M. bovigenitalium* infection by indirect- ELISA.

MATERIALS AND METHODS

Specimen: Serum samples from 135 cows including 85 diseased (16 abortion/still birth, 17 metritis, 6 cervicitis, 25 repeat breed, 22 anoestrus) and 50 apparently healthy cows were collected. In case of abortion/still birth cases, paired serum samples were collected from dam at an interval of three weeks.

Production of hyperimmune serum: A polyclonal hyperimmune serum against *M. bovigenitalium* (PG-11) was raised in rabbits (New Zealand white) and bovine calves (4-6 months age), respectively (Chander, 1999).

Indirect ELISA: To perform this test, the procedure described earlier by Garg *et al.* (1997) was followed. The sonicated whole cell *M. bovigenitalium* (PG-11) antigen diluted (1:25) in carbonate–bicarbonate buffer (pH 9.2) was centrifuged at 10,000 g for 30 minutes and protein of supernatant was adjusted so that each well receives 4.8 µg protein. After adding 100 µl of *M. bovigenitalium* antigen (1:100) in each well, the microtitre plates were incubated at 37° C for 2 h followed by overnight incubation at 4° C. After three washings with PBS-tween-20, diluted test serum (100 µl) was added to each well and incubated at 37° C for 1 h. The phosphate buffer saline (pH 7.6) containing 0.05% Tween-20 was used as serum and conjugate diluent. The horse-radish-peroxidase (type VI) labelled rabbit anti-cow IgG (M/s Dakopatts, Denmark) was used as enzyme-antiglobulin conjugate. Optimum dilution of coating conjugate was 1:2000. The substrate used was orthophenylene diamine-

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Table 1
Seroprevalence of *M. bovigenitalium* ELISA- Ab in cows with various reproductive disorders and apparently healthy

ELISA titre	Number of animals							
	Apparently Healthy	Diseased						Total
		Anoestrus	Repeat breeders	Cervicitis	Metritis	Abortion/Still-birth		
						1-3D	21D	
0 to 1:40	29	10	14	3	9	6	2	38 (49.35)*
1:80	10	2	7	2	5	2	1	17 (20)00
1:160	5	6	0	0	1	3	2	09 (10.58)
1:320	6	2	4	0	2	4	3	11 (12.94)
1:640	0	1	0	1	0	0	5	07 (8.23)
1:1280	0	1	0	0	0	0	2	03 (3.52)
ELISA+ve	11 (22)	10 (45.55)	4 (16)	1 (16.66)	3 (17.64)	7 (46.66)	12 (80)	30 (35.30)

* Figure in parenthesis shows percentage, D = day

dihydrochloride (OPD, Sigma, USA), which was prepared fresh in 30 % hydrogen peroxide solution with 0.05M citrate buffer. Absorbance values were measured at 492 nm in a Organon Teknika Reader 530. With each test plate, known positive and known negative sera as controls were included. The maximum absorbance of reference negative serum was considered as cut-off value for calculating ELISA end titre. Eliminating a cross-reacting titres upto 1:80, the serum samples showing ≥1:160 ELISA titre were considered positive.

RESULTS AND DISCUSSION

The results of seroprevalence of *M. bovigenitalium* antibodies in cows with various reproductive disorders and normal health are depicted in the Table 1. *M. bovigenitalium* specific ELISA-titre (≥1:160) was detected in 30 out of 85 diseased (35.30%) and 11 out of 50 (22%) apparently healthy cows. A higher rate of prevalence of *M. bovigenitalium* ELISA-Ab was recorded in genitally diseased cows in comparison to that in apparently healthy cows. Data regarding seroprevalence against *M. bovigenitalium* using ELISA in cows with various reproductive disorders is scanty. Higher seroprevalence of specific *M. bovigenitalium* ELISA antibodies in genitally diseased cows (19.1%) and mastitic cows (9.4%) than that in healthy cows (8.2%) has been recorded earlier (Kumar and Garg, 1996, Garg *et al.*, 1999). Disease-wise specific seroprevalence recorded against *M. bovigenitalium* in diseased cows ranged from 16 to 80% where the highest incidence was seen in cows showing anoestrus (45.45%) or abortion/still birth (80%) conditions as compared to repeat breed, cervicitis and metritis cases (16-17%). These finding are in agreement with Garg *et al.* (1999) who also reported higher incidence of *M. bovigenitalium* antibodies using ELISA in aborted cows (30.5%) as compared to repeat breed condition (11%). The results of present study indicate that amongst various reproductive disorders in cows, seroprevalence of *M. bovigenitalium* infection was more in cases of anoestrus and abortion.

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