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 anoestrous) 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 anoestrous (45.5%) and abortion/still-birth (80%) proved positive for M. bovigenitalium ELISA-Ab as compared to repeat breed, cervicites and metritis cases (16 to 17.6%).

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 vulvo vaginitis, 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 anoestrous) 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 anticow IgG (M/s Dakopatts, Denmark) was used as enzyme-antiglobulin conjugate. Optimum dilution of coating conjugate was 1:2000. The substrate used was orthophenylenediamine-
Table 1
Seroprevalence of *M. bovigenitalium* ELISA-Ab in cows with various reproductive disorders and apparently healthy

<table>
<thead>
<tr>
<th>ELISA titre</th>
<th>Apparently Healthy</th>
<th>Diseased</th>
<th>Abortion/Still-birth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anoestrous</td>
<td>Repeat breeders</td>
</tr>
<tr>
<td>0 to 1:40</td>
<td>29</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>1:80</td>
<td>10</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>1:160</td>
<td>5</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>1:320</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>1:640</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1:1280</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ELISA+ve</td>
<td>11 (22)</td>
<td>10 (45.55)</td>
<td>4 (16)</td>
</tr>
</tbody>
</table>

* 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 an 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 titre up to 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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