Isolation of *Mycoplasma bovoculi* from genitally diseased bovines and its experimental pathogenicity in pregnant guinea pigs

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Thirteen strains of *M. bovoculi*, 6 from frozen bull-semen (3.5% of 168), 3 from neat bull-semen (3.0% of 100), one each from heart blood and stomach contents of aborted foetuses of 85 (1.18%) bovine-abortions, one each from stomach contents and pooled internal organs of 9 (11.1%) stillborn calves, were isolated. All the isolates were resistant to ampicillin and sensitive to spiramycin, vibramycin, demeclocyclin, oxytetracycline, lincomycin and tylosin. However, variation in resistance to tetracycline, erythromycin, neomycin, kanamycin and streptomycin was observed. The gross lesions like congestion of lungs, liver, kidney and spleen were noted only in stillborn calf. However, significant microscopic lesions were encountered in internal tissues of both the aborted bovine fetuses and stillborn calf. Thickened alveolar wall, congestion of blood vessels, mesenchymal cell proliferation along with infiltration of lymphocytes and macrophages were observed in lungs. The liver showed mild infiltration of lymphocytes, macrophages in hepatic triad and necrosis of hepatic cells. The kidney tissues had focal lymphocytic infiltration in the interstitium. One strain of *M. bovoculi* (isolate # SBC-7/84,10) isolated from a stillborn calf was found abortigenic upon experimental inoculation in pregnant guinea pigs.

**Keywords:** *Mycoplasma bovoculi*, Bovine reproductive disorders, Experimental pathogenicity

*Mycoplasma bovoculi* was taxonomically characterized in 1973\(^1\). It is considered an important predisposing factor for establishment of *Moraxella bovis* in bovine eyes. Besides, *M. bovoculi* has also been reported to be present in the genital tract of male and female bovines\(^5\). Existence of *M. bovoculi* in the genital tract of bovines in India\(^2,6\) is a finding not being reported elsewhere\(^2\). This paper describes *M. bovoculi* associated reproductive disorders, viz., abortion, stillbirth, low fertility semen recorded during a long-term (1982-98) investigation.

**Materials and Methods**

Eighty-five abortions and 9 stillbirth cases that have occurred on 4 animal farms (Haryana Agricultural University, Animal Breeding Farm; Progeny testing Farm; Govt. Livestock Farm; Model Exotic Animal Farm) situated in the cities of Hisar and Bhiwani of Haryana State were investigated. Heart-blood, stomach contents, pieces of heart, liver, lung, kidney and spleen from aborted fetuses/stillborn calves and the cervical mucus or vaginal swabs of aborted dams were collected within 12 hr of abortion/stillbirth and subjected to microbiological and pathological examinations. Besides, 168 frozen bull-semen straws, 100 neat bull-semen and 30 extended bull-semen were also tested for mycoplasmas. Both the media, viz., Standard Liquid Medium (SLM) and Standard Solid Medium (SSM) used for isolation of mycoplasma have been described earlier\(^8\). Pure cloned colonies of mycoplasmas were obtained by the filter-cloning technique\(^7\) and subjected to biochemical and serological identification\(^9\). The tests used included the digitonin test; the catabolism of glucose, galactose, arginine and urea; the reduction of tetrazolium chloride; phosphatase activity; production of 'film and spot' and the growth inhibition test using the paper disc technique. Antibiotic sensitivity of the isolates was recorded, employing the *in vitro* single disc diffusion technique\(^10\). The collected samples from abortion and stillbirth cases were also examined for known abortifacient agents, viz., *Brucella, Leptospira, Campylo-
bacter, Coxiella and Trichomonas (virus etiology not suspected) as described elsewhere.

Gross pathological lesions were recorded during postmortem examination of aborted fetuses and stillborn calves. Various tissues (lung, liver, kidney, spleen, heart) were collected, fixed in 10% formal saline and processed conventionally for histopathological examination.

Experimental pathogenicity of M. bovoculi in pregnant guinea pigs by genitourinary challenge was determined as per Taylor-Robinson. Briefly, in a group of 6 pregnant guinea pigs (36 days pregnancy) devoid of any mycoplasma, 4 guinea pigs were inoculated intravaginally with 0.5 ml (10^5 cfu/ml) of freshly propagated M. bovoculi (isolate # SBC-7/84, IO) culture and 2 guinea pigs inoculated with 2 ml sterile SLM and served as control. Parameters of infection were clinical signs including rectal temperature, recovery of inoculated organisms from vagina and detection of haemagglutinating-M. bovoculi-antibodies at regular intervals. The guinea pigs from infected and control groups were sacrificed when aborted or parturated normally. Tissues from genital (ovary, fallopian tube, uterus, vagina) and visceral (lung, liver, heart, spleen, kidney) organs were collected for cultural recovery and observing histopathological changes.

Results

One of the 85 (1.18%) bovine abortions and 1 of 9 (11.1%) stillborn calves, proved positive for Mycoplasma bovoculi. Besides, 6 of 168 (3.5%) frozen bull-semen and 3 of 100 (3.0%) neat bull-semen also proved positive for M. bovoculi. However, none of the 30 extended bull-semen was found positive for M. bovoculi. The detail of these cases along with number of M. bovoculi strains isolated is given in Table 1. All the samples of neat bull-semen found positive for M. bovoculi had 60-70% sperm motility.

The abortion in the cow was in the last trimester of gestation (6.5 months) while stillborn cow-calf was of almost full gestation (9 months). Two strains of M. bovoculi, one each from the heart-blood and stomach contents of foetus from an aborted cow (case # BA-72/84), were isolated. Two more strains of M. bovoculi were from the stomach contents and internal organ pool of a stillborn cow-fetus (case # SBC-7/84, IO). The deep vaginal swabs of aborted dam and cow delivering stillborn calf, however, were found negative for mycoplasmas. Remaining 9 strains of M. bovoculi were from either frozen or neat bull-semen. No other significant bacteria were isolated from any of the M. bovoculi-positive samples.

All the 13 isolates of M. bovoculi fermented glucose and galactose except 2 isolates from aborted foetus, which were galactose negative (BA-72/84 HB, BA-72/84 SC). Only 2 isolates (FS-126/88, FS-136/88) from frozen bull-semen reduced tetrazolium. All the isolates failed to hydrolyse urea and deaminate arginine. None of the isolates produced ‘film-spot’ but all produced phosphatase. Four strains—2 each from the aborted foetus and stillborn calf were serogrouped at the earstück FAO/WHO Collaborative Centre for Animal Mycoplasmas, University of Aarhus, Denmark. However, remaining 9 isolates from frozen and neat semen of bulls were identified in our laboratory by disc growth inhibition test using hyperimmune rabbit antiserum against type strain (=NCTC-10141) of M. bovoculi.

All the 13 M. bovoculi isolates were found resistant to ampicillin (10 μg) but sensitive to spiramycin (30 μg), vibramycin (30 μg), demeclocycline (30 μg), oxytetracycline (15 μg), lincomycin (2 μg) and tylosin (15 μg). Only one isolate from abortion was found resistant to tetracycline (15 μg). Five isolates (2 from abortion, 3 from frozen semen) to erythromycin (15 μg); 3 isolates (2 from abortion, 1 from stillbirth) to neomycin (30 μg); 2 isolates (1 each from abortion and stillbirth) to kanamycin (30 μg) and 4 isolates (2 each from abortion and frozen semen) to streptomycin (10 μg) were resistant, whereas remaining isolates were sensitive to these antibiotics.

Postmortem examination of stillborn calf (SBC-7/84) revealed gross congestion of kidney, liver, lung and spleen whereas other internal organs appeared

<p>| Table 1 — Details of M. bovoculi positive cases of bovine abortion, stillbirth and semen of bull |</p>
<table>
<thead>
<tr>
<th>Source of sample</th>
<th>No. of sample</th>
<th>Samples cultured</th>
<th>M. bovoculi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow-foetus (6.5 months)</td>
<td>1</td>
<td>HB</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SC</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IO</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VS</td>
<td>-</td>
</tr>
<tr>
<td>Cow-foetus (9 months)</td>
<td>1</td>
<td>HB</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SC</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IO</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VS</td>
<td>-</td>
</tr>
<tr>
<td>Cow-bull, frozen semen</td>
<td>6</td>
<td>FS</td>
<td>+</td>
</tr>
<tr>
<td>Cow-bull, neat semen</td>
<td>3</td>
<td>NS</td>
<td>+</td>
</tr>
</tbody>
</table>

HB= heart blood, SC= stomach contents; IO= internal organs from aborted foetus/stillborn calf, VS= vaginal swab from aborted cow, FS=frozen bull-semen, NS= neat bull-semen.
normal. The aborted foetus (BA-72/84) did not show any significant gross lesion. Significant microscopic lesions were observed in the tissues of internal organs of aborted bovine foetuses and stillborn calf. Lung tissues revealed thickened alveolar wall due to oedema, mesenchymal cell and macrophage proliferation. The interlobular septae wall was thickened due to serofibrinous exudation and mild infiltration of mononuclear cells. The liver section showed necrosis of hepatic cells along with mild infiltration of lymphocytes and macrophages in the hepatic triad. The kidney tissues had focal lymphocytic infiltration in the interstitium and spleen tissues exhibited infiltration of lymphocytes and macrophages in the spleenic capsule along with oedema and reticuloendothelial hyperplasia.

In the pregnant guinea pigs, challenged with an isolate of M. bovoculi (# SBC-7/84,10), rectal temperature was found elevated; ranging 102 to 105.60°C from 2-day postchallenge up to the day of abortion. Guinea pig no.1, 2 and 4 of infected group aborted at 9, 10 and 16-day post-challenge, respectively. The aborted foetuses were dead weighing ca. 45 g each whereas guinea pig no. 3 aborted at 8-day postchallenge with one live (wt. 80 g) and one dead (wt. 50 g) fetus. Both the guinea pigs of control group littered normally at 22-day post-challenge with foetuses of 85 g body weight. Serologically, M. bovoculi haemagglutinins were noted to be 1:10, 1:40 and 1:20 in guinea pig no.1, 2 and 4, respectively. The culture of challenged M. bovoculi was reisolated at regular intervals from the vaginal secretions and genital organs (ovary, uterus, vagina) of several aborted guinea pigs on postmortem examination. Besides, the M. bovoculi organisms were also recovered from the pool of internal organ of foetuses but not from their placenta.

Histopathology of genital organs of the aborted guinea pigs were: mild to moderate degeneration of corpus luteum and granulosa cells along with the cystic dilatation of graffian follicles in ovarian tissues; mild infiltration of lymphocytes and macrophages in lamina propria of fallopian tube; mild infiltration of neutrophils, lymphocytes, macrophages and plasma cells in the endometrium and similar type of infiltration in lamina propria/ connective tissue layer of vagina. Besides, moderate infiltration of neutrophils, lymphocytes and plasma cells along with necrosis and oedema in connective tissues of foetal placenta were noticed. Histopathological lesions in tissues of visceral organs of aborted guinea pigs and their foetuses were: interstitial pneumonia due to lymphocytic/macrophagic infiltration and proliferation of mesenchymal and sepal cells in the alveolar septae along with lymphoid cells accumulation at the periphery of bronchioles; mild lymphocytic and macrophagic infiltration in hepatic triad; mild reticular cell hyperplasia, depletion of lymphocytes in malpigian corpuscles and hemosiderin laden macrophages in the spleenic tissues and no appreciable change except congestion in pericapular area of the kidney.

Discussion

Mycoplasma bovoculi is prevalent in bovines throughout the world and many reports are now available regarding its isolation from cases of infectious bovine conjunctivitis (IBC)\(^{14-16}\). It is also known to produce conjunctivitis in calves\(^{17}\) and has also been demonstrated to play an important role in the pathogenesis of Moraxella bovis ocular infection in cattle\(^{18}\). Only in India, M. bovoculi has been reported from the genital tract of male and female bovines\(^{19}\). Previous such reports include its isolation from the vaginal discharge of an aborted buffalo\(^3\) and preputial wash of bulls\(^4\) in Punjab and from semen and prepuce of buffalo-bulls and cervico-vaginal mucus\(^5,6\) in Uttar Pradesh. In the present study, however, M. bovoculi strains have been isolated from the heart blood and stomach contents of an aborted foetus of a cow, the stomach contents and internal organ pool of a stillborn cow-foetus and frozen/feat semen of bulls. Thus, this is the first report on the isolation of M. bovoculi from the aborted cow-foetus and stillborn calf. The isolation of M. bovoculi from frozen and neat semen of bull indicated the possible transmission of M. bovoculi from bull to cow during natural service or artificial insemination.

Pathological lesions of mycoplasmal abortion in bovines are less reported but the recorded histopathological lesions in lung, liver, kidney and spleen tissues of aborted and stillborn foetuses in the present study are similar to those reported earlier in aborted cow foetuses associated with M. mycoides s.s. mycoides LC\(^{19,20}\) and Ureaplasma diversum\(^{19,20}\). The moderate decomposition of expelled fetuses and accumulation of serosanguineous fluid in the peritoneal and thoracic cavities as described previously\(^{21}\) were not recorded in the present study. The experimental intravaginal challenge of pregnant guinea pigs with an isolate of M. bovoculi (# SBC-7/84,10) from the stillborn foetus resulted in abortion suggesting a possible role of M. bovoculi in bovine abortion. In the previous
experimental studies, this isolate *M. bovoculi* (# SBC-7/84,IO) has proved pathogenic in rabbit fallopian tube organ culture and hamster tracheal ring organ culture and mastigogenic in rat mammary gland model. The probable etiologic role of *M. bovoculi* in abortion/stillbirth was strengthened by the successful periodic isolation of inoculated strain from the vagina of aborted guinea pigs, presence of *M. bovoculi*-hemagglutinins in aborted guinea pigs along with significant histopathological picture of genital and visceral organ of infected guinea pigs. However, further such experimental studies in homologous host (cow/buffalo) with genital isolates of *M. bovoculi* are recommended to establish it as a bovine abortifacient agent.

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**References**