Mycoplasma Species Recovered from the Reproductive Tracts of Western Canadian Cows

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ABSTRACT

Samples of cervico-vaginal mucus from 633 animals from 110 herds were cultured and yielded the following mycoplasmas: T-strain — 88: Mycoplasma bovigenitalium — 79, Mycoplasma spp. (Leach Group 7) — 7, Acholeplasma laidlawii — 4, Mycoplasma bovirhinis — 2 and one not typable.

Uterine exudates and endometrial scrapings from 80 infertile cows in two herds were examined. Four animals were positive, M. bovigenitalium was isolated three times, A. laidlawii and Mycoplasma arginini once each.

Sixty-five normal uterine contents from pregnant cows were examined, one yielded M. bovigenitalium and the same organism was recovered from the fetal kidney. T-strain mycoplasma, M. bovigenitalium and other Mycoplasma spp. appear to be a part of the normal flora of the cervico-vaginal region of clinically normal one and two year old bred heifers in Alberta and Saskatchewan.

Although M. arginini was not recovered from the cervico-vaginal region, a single recovery was made from the uterus of an infertile cow.

RÉSUMÉ

La recherche de mycoplasmes dans des échantillons de mucus cervico-vaginal, prélevés chez 633 taures faisant partie de 110 troupeaux de bovins de boucherie, a donné les résultats suivants: 88 isolements de la souche T, 79 isolements de Mycoplasma bovigenitalium, sept isolements de Mycoplasma sp. (groupe 7 de Leach), quatre isolements d'Acholeplasma laidlawii, deux isolements de Mycoplasma bovirhinis et l'isolement d'un mycoplasme dont on n'a pu préciser l'espèce.

La culture d'écouvillons d'exsudat utérin et de l'endomètre, prélevés chez 80 vaches infécondes appartenant à deux troupeaux, se solda par l'isolement des mycoplasmes M. bovigenitalium, à trois reprises, et A. laidlawii, ainsi que Mycoplasma arginini, une fois chacun.

L'examen du contenu de l'utérus gravide de 65 vaches saines permit d'isoler de l'une d'entre elles, ainsi que des reins de son foetus, M. bovigenitalium. La souche T, M. bovigenitalium et d'autres Mycoplasma sp. semblèrent représenter des constituants de la flore normale de la région cervico-vaginale de taures âgées d'un à deux ans, saines et saillies, en Alberta et en Saskatchewan.

Même si l'auteur ne réussit pas à isoler M. arginini de la région cervico-vaginale, il le recouvra une fois de l'utérus d'une vache infeconde.

INTRODUCTION

Many investigations have been carried out on bovine reproductive tracts for the presence of mycoplasma since the original isolation by Edward *et al* (12) of S and P strains from the genital tracts of 18 of 64 infertile cows. The P strain was later characterized and named *Mycoplasma bovigenitalium* (14). Edward and Fitzgerald (13) also observed that the fastidious P strains were often those isolated from herds where there was evidence of associated disease.

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Submitted June 21, 1974.

Workers in various countries have examined cervico-vaginal mucus, vaginal washings, semen, oviducts and preputial washings for mycoplasma (5, 7, 8, 11, 24, 28, 32, 34, 43). Mycoplasmas have been isolated from feti and placentae (33, 35).

The pathogenicity of mycoplasma for the bovine reproductive tract has been examined by several workers. Hartman *et al* (19) infused *Mycoplasma agalactiae* var. *bovis* into the uteri of eight heifers and observed pathological changes. Bull semen to which the same organism had been added was used to inseminate 12 heifers (21). Four of these failed to conceive after multiple inseminations and at necropsy showed the presence of salpingitis and endometritis. Hirth *et al* (22) added *M. agalactiae* var. *bovis* to semen before processing for artificial insemination and recovered the organism after 18 months of frozen storage. Although *M. bovigenitalium* has been implicated in seminal vesiculitis, epididymitis and lowered fertility in bulls (4, 15) there has been little reported association between infection and reproductive failure in the cow. Hirth *et al* (23) were unable to induce lesions by intrauterine infusion of *M. bovigenitalium*.

Isolations at this laboratory of *M. bovigenitalium* from year old processed and frozen semen obtained from an artificial insemination unit, indicated the need for more information on the incidence of mycoplasmas in the bovine reproductive tract and their effect on reproduction. The following studies were designed to examine the incidence of mycoplasma in the reproductive tracts of local cattle and to examine the effect of these organisms on conception.

MATERIALS AND METHODS

Cervico-Vaginal Mucus

The samples were collected in September, October and November from beef cattle, primarily Herefords. The cervico-vaginal (CV) mucus was collected in a manner similar to that described by Al-Aubaidi and Fabricant (2). The tubes containing mucus were corked and placed in the bottom of a styrofoam container under one or two prefrozen commercial freezer packs to maintain refrigeration. Only a few temperatures were recorded when samples were received and these were between 1°C and 5°C. The samples were in transit for six to 48 hours, with an average of 24 hours.

Storage

Approximately 0.2 g of mucus was added to 1.8 ml of Hayflick broth (27) without inhibitors and stored at –70°C until used. The maximum storage period was 90 days.

Reproductive Tracts

Eighty beef cows in two herds were judged to be infertile on the basis of failure to produce a calf over two breeding seasons. Their reproductive tracts were recovered at slaughter and endometrial scrapings of the uteri were examined for the presence of mycoplasmatales. The uteri of 65 pregnant animals were collected at the time of slaughter and the placental tissue and cotyledons were cultured within six hours of collection.

Media

Agar and broth media as previously described (27) were used for the isolation of mycoplasmatales except that autolysed yeast was replaced by 10% V/V of a 25% fresh yeast extract. Shepard urea broth (38) and in some cases T-strain agar (38) were used throughout the study for the isolation of T-mycoplasma.

Cultural Technique

Approximately 0.2 ml of the stored material was added to 1.8 ml each of Hayflick's and of Shepard's broth. The Shepard's broth was examined daily for three days for color change even though in most of the positive samples this change occurred within 18 hours. In the early stages all T-strain "positives" were inoculated on 5% bovine blood tryptose agar[1] plates which were incubated at 37°C aerobically for 72 hours and examined microscopically to eliminate the possibility of a colour change due to other bacterial or mycological activity and also to Shepard's agar for T-strain colonial growth. In the latter two-thirds of the study a color change with slight turbidity and no mycelial growth after five days' incubation was considered to be due to T-strain mycoplasma. The Hayflick broth was

[1]Difco Laboratories, Detroit, Michigan.

incubated at 37°C aerobically for 18 days and 0.1 ml was transferred to Hayflick agar plates with inhibitors at four, 11 and 18 days. The agar plates were incubated at 37°C aerobically in a humidified incubator.

Typing

All of the isolates were typed using a growth inhibition (GI) test as described by Clyde (10) and modified by Stanbridge and Hayflick (39). Hyperimmune sera were prepared in rabbits using a suspension of mycoplasma grown in rabbit serum broth. The organisms were washed twice in phosphate-buffered saline (PBS), pH 7.3 and resuspended in PBS at a concentration of approximately 30 mg protein per ml. The initial dose was 1 ml of antigen with 1 ml of Freund's incomplete adjuvant (1 ml was given intramuscularly in each thigh). Subsequent doses of antigen alone were given intravenously at day 7 and at 14 day intervals until an adequate antibody response, 3 mm or greater zone of inhibition, was attained against the homologous strain and a negative result against heterologous strains.

The GI typing of some of the isolates was confirmed using a fluorescent conjugate prepared according to the methods of Al-Aubaidi and Fabricant (3) and Cherry (9). Incident and transmitted illumination as described by Baas (6) and Tessler (44) respectively were used throughout.

Type Cultures

The following were supplied from the National Culture Type Collection, London N.W. 9, Great Britain: 10122 — *M. bovigenitalium*, 10116 — *Acholeplasma laidlawii*, 10118 — *Mycoplasma bovirhinis*, 10131 — *M. agalactiae* var. *bovis* (Donetta), 10133 — *Mycoplasma* spp. (Group 7 Leach N29) and 10129 — *Mycoplasma arginini*.

The T-strain used as a reference was supplied by Mrs. L. Ruhnke of the Ontario Veterinary College.

RESULTS

The results of examinations of CV mucus from 633 animals in 110 herds are given in Table I.

Only four uteri of 80 taken from infertile cattle in two herds yielded mycoplasma. A mixed culture of *M. bovigenitalium* and *M. arginini* was recovered from one specimen. *A. laidlawii* was recovered in pure culture from one specimen. *M. bovigenitalium* in pure culture was recovered from two specimens. No T-strains were isolated.

M. bovigenitalium was isolated from the cotyledons and the kidney of one fetus of 65 examined. The kidney was the only fetal tissue examined.

DISCUSSION

Although 133 of the 633 mucus samples tested yielded mycoplasma and one or more strains of this microorganism were present in 45 or 110 herds it is difficult to assess the pathogenicity of the strains. The owners of these animals all stated that they had not encountered a reproductive problem in the previous year in the form of either lowered reproduction, repeat breedings or abortion. The majority of the animals tested were yearling heifers which had been bred for the first time during the previous breeding season, May-July, and the remainder were two year old cattle which had calved and were either unbred or had been recently rebred. If the subsequent reproductive records of these animals had been available for a direct comparison against the isolations, the potential pathogenicity could be accurately assessed. It is apparent, on the basis of these results, that approximately 21% of normal, young, bred female cattle on 40% of the ranches in Alberta and Saskatchewan are carrying one or more strains of mycoplasma. .

The reported incidence of vaginal infection in this study, 21%, is strikingly similar to the recovery rate reported by Hoare (25) of 24% from British cattle and Pan and Ogata (36) of 21.7% from Japanese cattle. There is, however, a marked difference in the strains isolated. Both Hoare and Pan reported only *M. laidlawii (Acholeplasma)* while in this study *M. bovigenitalium* was the predominant large colony type and *A. laidlawii* was only isolated four times from one herd. Pan reasoned that the species difference between his recoveries and those of Albertsen (5) and Edward *et al* (12) was due to the source of material, i.e. normal animals as compared to the infertile animals studied by the other work-

TABLE I. Mycoplasma Species Isolated in 1972 from Cervico-vaginal Mucus of Animals in 110 Herds

	T-strains	*M. bovi-genitalium*	*A. laidlawii*	*M. bovirhinis*	*Mycoplasma* spp. Group 7 (N29)	Untyped	Total positive
Herds sampled 110.....	32	29	1	2	5	1	45
Isolates from 633 animals	88	79	4	2	7	1	133
Herds with mixed infections.....	21	21	1	1	4	1	24
Herds with single infections.....	11	8	0	1	1	0	21

One herd had four types present: T-strain, *M. bovigenitalium*, *Mycoplasma* sp. Group 7 had an untyped strain. Preliminary serological studies indicate the untyped strain is a new species

ers. Pan also speculated that P strains (*M. bovigenitalium*) had not yet reached Japan through the importation of breeding animals. Since Pan's study was based on apparently normal Japanese animals his latter statement is probably true. The importation and exportation of breeding cattle and semen will probably result in those strains of mycoplasma which are endemic to one area or country becoming distributed throughout the trading countries if there are no regulatory controls.

The incidence of mycoplasma infection in human reproductive organs has been related to sexual activity (26, 31). If these organisms are sexually transmitted then maiden heifers should be free of mycoplasma and the incidence should increase with age and breeding periods. Edward and Fitzgerald (13) reported that they were unable to recover mycoplasma from the vagina of maiden heifers and we have no data on maiden heifers. However, in this study there was an overall incidence of 21% in one and two year old heifers, with the incidence in two year olds appearing to be higher than that in one year old animals. This could indicate that the incidence of mycoplasma at different ages is related to the number of times the animal has been bred.

A granular vulvovaginitis in cattle has been associated with experimental infection with *M. bovigenitalium* by Afshar *et al* (1) and by Edward *et al* (12). Hirth *et al* (23) were unable to experimentally produce vulvovaginitis using *M. bovigenitalium*. However, they infused the organism into the uterus while Afshar abraded the vaginal mucosa before depositing the mycoplasma on this tissue. In this study no visible signs of reproductive tract pathology were reported and we assume that the mycoplasma isolated were either a part of the normal vaginal flora, the animals were latent carriers or the animals were asymptomatic, recovered carriers.

The recoveries of mycoplasma from the upper reproductive tract of infertile animals, four out of 80 or 5%, does not compare with that reported by Hoare and Haig (24) and Hoare (25) i.e. 64% and 71% for the oviduct of infertile animals and 24% for the oviducts of normal slaughter animals. The difference between the recovery levels previously reported and those in this study may be due either to the age of the animals, the site studied (e.g. oviducts not included), the type of animal (beef versus dairy) or the media and methods used for culture.

M. bovigenitalium was recovered from the uterine cotyledons and the kidney cells of one live bovine fetus from 60 uteri processed. This could indicate that a fetus can be infected *in utero* and remain viable. However, we do not know whether this infection had occurred recently, had been latent for a period of time or whether fetal death might have occurred later. *Mycoplasma* spp., not *M. bovigenitalium*, have previously been recovered from feti and placental tissue (33, 35).

The high recovery rate of *M. bovigenitalium* without the addition of a specific growth factor, such as mucus or deoxyribonucleic acid, which Edward and Fitzgerald (13) indicated was necessary for the growth of this organism may have been due to the residual mucus carried over in the broth cultures or the strains isolated may not

have required these enrichment factors. These strains might have been similar to the less pathogenic strains previously reported.

T-strain mycoplasma have been recovered from the reproductive tracts of both human sexes and may be associated with human reproductive failure (16). Haynes and Nielsen (20) reported on the isolation of T-mycoplasma from feti and fetal membranes recovered after induced abortion. Taylor-Robinson *et al* (40) in the first reported recovery of T-strain mycoplasma from the urinary and reproductive tracts of cows stated that 11% of the vaginas examined yielded this organism. Further reports have been made by several authors of the recovery of this microorganism from various tissues (17, 18, 30, 37, 41, 42). We recovered 88 T-strains from 633 animals or 14%, a very similar rate. T-strain mycoplasma were not recovered from the uteri of either open or pregnant animals. The results of the fetal examinations would suggest that, as in man, this microorganism does not usually gain entry through the intact placenta or fetal membranes and probably that it cannot penetrate the cervical barrier.

M. arginini was not isolated from the mucus but was isolated from a single uterus. The route of infection is unknown but may be hematological as the organism has been recovered from infections of the eye, nasal passage and lung, where it has been found by the author and others (29).

ACKNOWLEDGMENTS

The excellent technical assistance of Mr. J. Burchak is acknowledged. The assistance of Dr. S. E. Magwood in the manuscript preparation and his encouragement during the survey period is gratefully acknowledged.

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