The Recovery of Mycoplasmas from the Genital Tracts of Bulls in Artificial Breeding Units in Ontario

O. Onoviran, R. B. Truscott, N. A. Fish,
C. A. V. Barker and H. Louise Ruhnke*

ABSTRACT

One hundred and thirty-two penial-preputial swabbings, 140 raw and 42 processed semen samples were cultured for mycoplasmas. Mycoplasma or acholeplasma were recovered from 87, 32 and one respectively, while ureaplasmas were recovered from 46, 34 and six respectively.

RÉSUMÉ

Les auteurs ont procédé à la recherche de mycoplasmes dans 132 écouvillons préputiaux, 140 échantillons de sperme frais et 42 ampoules de sperme. Ils isolèrent des mycoplasmes ou des acholéplasmes de 87 écouvillons, 32 échantillons et une ampoule. Quant aux uréaplasmes, ils en retrouvèrent dans 46 écouvillons, 34 échantillons et six ampoules.

A survey of the incidence of mycoplasma (this term as used throughout refers to the genera *Mycoplasma* and *Acholeplasma)* and *Ureaplasma* (T-strains) (20) in the genital tracts of bulls in two artificial breeding units in Ontario was carried out (16). Cultured were 132 penial-preputial swabbings, 140 raw and 42 processed semen samples. The medium and techniques used for the isolation of mycoplasma were as described by Davies (6) and the culture medium and techniques for *Ureaplasma* were based on those described by Gourlay (11) and Shepard (19).

A total of 87 (65.9%) and 46 (34.9%) of the penial-preputial swabbings were respectively positive for mycoplasma and ureaplasma. Corresponding figures for raw semen samples were 32 (23.9%) and 34 (24.3%) and in processed semen one (2.4%) and six (14.3%).

The ureaplasma strains were identified by their ability to metabolize urea while the mycoplasma strains were characterized biologically, biochemically and serologically. Biological properties examined were the requirement for sterol, the production of film and spots, growth at 30 and 37°C and a requirement for 10% carbon dioxide or an anaerobic environment. The biochemical test employed was the ability to ferment glucose. An additional test applied to those strains which could not be typed serologically was sensitivity to sodium polyanethole sulphonate (13). The serological methods used were the disc growth inhibition test (5) and indirect immunofluorescence (17). Antisera against the following reference strains were employed: *M. bovigenitalium, A. laidlawii, M. bovirhinis, M. arginini* and *M. agalactiae* subsp. *bovis.* Isolates not identified with the above sera were further tested using antisera against *M. species* Leach group 7, *M. gateae, M. gallinarum, M. alkalescens* and *M. species*

*Department of Veterinary Microbiology and Immunology (Onoviran, Truscott and Fish) and Department of Clinical Studies (Barker) Ontario Veterinary College, University of Guelph and the Ontario Ministry of Agriculture and Food, Veterinary Services Laboratories, Guelph, Ontario (Ruhnke). Present address of senior author: Federal Department of Veterinary Research, Vom, Nigeria.

Submitted April 1, 1975.

TABLE I. The Degree of Infection with Mycoplasmas and Ureaplasmas Based on the Number of Colonies on Direct Culture

	Light (1-10)[a]		Moderate (11-50)		Heavy (over 51)	
	Myco-plasma	Urea-plasma	Myco-plasma	Urea-plasma	Myco-plasma	Urea-plasma
Penial-preputial swabbings	14%	7%	32%	31%	56%	62%
Raw semen	82%	10%	16%	86%	2%	4%
Processed semen	Not significant					

[a]Average number of colonies per 0.01 ml of specimen

"466" (18). Considering the mycoplasmas, 15.7% were *M. bovigenitalium*, 50% were *A. laidlawii*, 26.9% were similar to "466" and 7.4% were untypable. More of the isolates were identified by indirect immunofluorescence than by growth inhibition tests. It should also be noted that 50.6% and 43.7% of the mycoplasma isolates from penial-preputial washings and raw semen respectively could not be subcultured from primary isolation plates and were therefore not identified.

With respect to isolates of mycoplasma and ureaplasma the significance was difficult to assess because a good proportion of the samples had organisms present in moderate numbers (Table I) and as far as was known there was no clinical manifestation of their presence in the genital tracts of the bulls.

Many workers have isolated mycoplasmas (1, 4, 7, 8, 10, 12, 14) and ureaplasmas (21) from the genital system of cattle. Many isolations have been made from infertility conditions (15) and vaginal disorders in the female (2) and from such conditions as seminal vesiculitis, epididymitis (3) and orchitis (H. L. Ruhnke, unpublished data, 1974) in the male, while their role in mastitis has been well documented (9). Their potential as pathogens has therefore been established. However, the extent to which they may be involved as active pathogens is uncertain.

The results presented indicate that bulls may be a reservoir for mycoplasma and ureaplasma and while no specific recommendations are presented it is suggested that thought should be given to the development of procedures concerned with the collection and processing of semen to reduce or eliminate the presence of mycoplasma and/or ureaplasma and thus reduce the opportunity for these organisms to become disseminated.

REFERENCES

1. **AFSHAR, A.** Genital diseases of cattle associated with mycoplasma. Vet. Bull. **37**: 879-884. 1967.
2. **AFSHAR, A., P. STUART and R. A. HUCK.** Granular vulvovaginitis (nodular venereal disease) of cattle associated with **Mycoplasma bovigenitalium.** Vet. Rec. **78**: 511-519. 1966.
3. **AL-AUBAIDI, J. M., K. McENTEE, D. H. LEIN and S. J. ROBERTS.** Bovine seminal vesiculitis and epididymitis caused by **Mycoplasma bovigenitalium.** Cornell Vet. **62**: 581-596. 1972.
4. **BLOM, E. and H. ERNO.** Mycoplasmosis: Infections of the genital organs of bulls. Acta. vet. scand. **8**: 186-188. 1967.
5. **CLYDE, W. A.** Mycoplasma species identification based upon growth inhibition by specific antisera. J. Immun. **92**: 958-965. 1964.
6. **DAVIES, G.** Isolation of mycoplasma from calf lungs. J. comp. Path. **77**: 353-357. 1967.
7. **ERNO, H.** Mycoplasmosis. Serology of infections in the genital tract of bulls. Infection & Immunity **5**: 20-23. 1972.
8. **ERNO, H., W. N. PLASTRIDGE and M. E. TOURTELLOTTE.** Mycoplasma: Isolation from prepuce and semen of bulls. Acta. vet. scand. **8**: 123-135. 1967.
9. **JASPER, D. E.** Mycoplasmas: Their role in bovine diseases. J. Am. vet. med. Ass. **148**: 1650-1655. 1967.
10. **JASPER, D. E., J. M. AL-AUBAIDI and J. FABRICANT.** Isolation of mycoplasma from preputial washings of bulls. Cornell Vet. **64**: 296-302. 1974.
11. **GOURLAY, R. N.** The isolation of T-strains of mycoplasma from pneumonic calf lungs. Res. vet. Sci. **9**: 376-378. 1968.
12. **HIRTH, R. S., S. W. NIELSEN and M. E. TOURTELLOTTE.** Characterization and comparative genital tract pathogenicity of bovine mycoplasmas. Infection & Immunity **2**: 101-104. 1970.
13. **KUNZE, M.** Natrium-polyanethol-sulphonatals diagnostisches. Hilfsmittel bei der differenzierung von mycoplasmen. Zentbl. Bakt. (Orig.) **216**: 501-505. 1971.
14. **LANGFORD, E. V.** Mycoplasma species recovered from the reproductive tracts of western Canadian cows. Can. J. comp. Med. **39**: 133-138. 1975.
15. **O'BERRY, P. A.** Characterization of mycoplasma of bovine origin and their role in infertility. Thesis. Iowa State Univ. of Science and Technology, Ames, Iowa. 1967.
16. **ONOVIRAN, O.** Studies on mycoplasma flora of the male bovine genital tract. Thesis. Univ. of Guelph. 1974.
17. **ROSENDAL, S. and F. T. BLACK.** Direct and indirect immunofluorescence of unfixed and fixed mycoplasma colonies. Acta. path. microbiol. scand. B. **80**: 615-622. 1972.
18. **RUHNKE, H. L. and O. ONOVIRAN.** Isolation of an apparently new species of **Mycoplasma** from cattle. Vet. Rec. **96**: 203. 1975.
19. **SHEPARD, M. C.** Fundamental biology of the T-strains. **In** The Mycoplasmatales and the L-Phase of Bacteria. L. Hayflick, Ed. pp. 49-65. New York: Appleton-Century-Crofts. 1969.
20. **SHEPARD, M. C., C. D. LUNCEFORD, D. K. FORD, R. H. PURCELL, D. TAYLOR-ROBINSON, S. RAZIN and F. T. BLACK. Ureaplasma urealyticum** gen. nov. sp. nov.: Proposed nomenclature for the human T (T-strain) mycoplasmas. Int. J. syst. Bact. **24**: 160-171. 1974.
21. **TAYLOR-ROBINSON, D., M. H. WILLIAMS and D. A. HAIG.** The isolation and comparative biological and physical characteristics of T-mycoplasmas **of** cattle. J. gen. Microbiol. **54**: 33-46. 1968.