

Brief Communication

ISOLATION OF MYCOPLASMA CANADENSE FROM
BULL SEMEN

In earlier studies on the occurrence of mycoplasmas in the genital tract of bulls at Danish A.I. (artificial insemination) centres (*Blom et al.* 1983) mycoplasmas were frequently found. Serological identification of a small number (100) of isolates revealed that *Mycoplasma bovis genitalium* was by far the commonest species, while *Mycoplasma canadense* and *Ureaplasma* were not found.

As only one medium (*Ernø et al.* 1967) was used in the survey, it was decided to examine a number of normal semen samples from Danish A.I. bulls by means of different media, in order that the specific growth requirements of the various species belonging to the order Mycoplasmatales could be met. One unprocessed semen sample from each of 155 healthy bulls at 2 A.I. centres were sent to the Laboratory under cooling with ice and examined in 4 different media: The said medium of *Ernø et al.* and 3 media previously used by *Friis & Krogh* (1983), viz., a medium originally intended for cultivation of *Mycoplasma suis pneumoniae*, a *Hayflick's* type of medium containing urea and arginine, and finally a medium based on Medium 199 (Wellcome). The cultivation was made initially in broth followed by plating on solid medium. *Ureaplasmas* were identified by their ability to split urea and by the characteristic colonies after staining with $MnCl_2$. Isolates of species belonging to the genus *Mycoplasma* were identified as crude strains by the disc growth inhibition test (DGI) with antisera for *M. canadense*, *M. bovis genitalium* PG11 (type strain) and further with antiserum for serovar MBG3 of *M. bovis genitalium*. This serovar was found to cross-react with strain PG11 of *M. bovis genitalium* by indirect epi-immunofluorescence, but not by DGI. Peculiarly, strain MBG3 was not inhibited by its own antiserum in DGI, unlike fresh isolates, which generally showed distinct inhibition zones of 3 to 5 mm.

The frequency of occurrence of mycoplasmas in the semen samples is given in Table 1. It appears that approx. 3 out of 4

bulls were found infected and that nearly half the bulls harboured at least 2 different species. The species identification of the isolates is shown in Table 2. Ureaplasmas, which were found in nearly half the animals, was the most frequent species. It is noteworthy that *M. canadense* was found in 38 % of the bulls, while *M. bovis* was not found. The 3 unidentified strains were culturally similar to *M. bovis*, but by DGI they could neither be identified as such nor as *M. bovis*, *M. californicum* or *M. verecundum*.

The strains recovered by using the recipe of *Ernø et al.* were all identified as *M. bovis* and, except for 1, found to belong to the PG11 group; for cultivation of this mycoplasma

Table 1. Isolation of mycoplasmas from semen samples from bulls at 2 Danish A.I. centres.

examined	Bulls		Bulls with			
	positive		1 species*		2 or more species	
	T	%	T	%	T	%
155	119	76.8	52	33.6	67	43.2

T = number of bulls; % = percentage of bulls examined.

* In the event of concomitant occurrence, strains belonging to groups PG11 and MBG3 of *M. bovis* were regarded as one species.

Table 2. Identification of mycoplasmas isolated from semen samples from 155 bulls at 2 Danish A.I. centres.

Total number of isolates	<i>M. bovis</i>				<i>M. canadense</i>		Ureaplasma		Not identified*	
	PG11		MBG3		T	%	T	%	T	%
	T	%	T	%						
219	67	43.2	18	11.6	59	38.1	72	46.5	3	1.9

T = total number of bulls yielding the indicated species (or serovar MBG3).

% = percentage of total number of bulls.

* though culturally similar to *M. bovis*, these strains could not be identified as such, nor as *M. bovis*, *M. verecundum* or *M. californicum*, by DGI.

the medium was excellent. Ureaplasmas were recovered from the enriched *Hayflick's* medium, in which the growth was easily recognised because of the phenol red indicator. The strains of the serovar MBG3 group and of *M. canadense* were recovered from pooled passages of the 3 media of *Friis & Krogh*, for which reason it is unknown which of them did indeed support growth.

The demonstration of *M. bovis genitalium*, *M. canadense** and *Ureaplasma* as frequently occurring microorganisms in semen samples from normal Danish A.I. bulls has revealed a similar situation in this country as was recently reported from England (*Rae* 1982). It appears that the genital tract constitutes a natural reservoir for these species, though their exact localisation has not been determined. It may well be restricted to the preputial cavity. It is noteworthy that although found in calf lungs in Denmark in 1981 (*Friis & Krogh*) *M. bovis* was not identified in the present material.

This is the first demonstration of *Mycoplasma canadense* in Denmark.

N. F. Friis and E. Blom

The State Veterinary Serum Laboratory, Copenhagen, Denmark.

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Reprints may be requested from: N. F. Friis, the State Veterinary Serum Laboratory, Bülowsvej 27, DK-1870 Copenhagen V, Denmark.

* The identification of *M. canadense* was kindly confirmed by Dr. H. Ernø, Institute of Medical Microbiology, University of Aarhus.