

Brief Communication

BOVINE MYCOPLASMAS:
IDENTIFICATION OF 100 STRAINS ISOLATED FROM SEMEN
OF BULLS

In 1970, *Blom & Ernø* reported on the frequency of mycoplasmas in semen of breeding bulls. The material originated mainly from the annual sampling for brucellosis control of all Danish artificial insemination bulls supplemented with material from exported and imported bulls. Mycoplasmas were cultivated in 158 cases (7.8 %). Of these strains, 100, selected at random, were freeze-dried for subsequent species diagnosis. The identification was postponed until the classification scheme of bovine mycoplasmas was better defined and greater personal experience had been gained in routine diagnostic work. The work has now been completed, and the results are presented below.

Materials and methods. The isolates were studied according to the following scheme: (1) All strains were examined for catabolism of glucose and arginine, phosphatase activity and production of film and spots. Indirect immunofluorescence was also performed with all strains using antisera against *Mycoplasma bovis genitalium* and *M. agalactiae* subsp. *bovis*. (2) Glucose positive strains were tested for sensitivity to digitonin. (2a) Digitonin resistant strains were examined by immunofluorescence using antisera against *Acholeplasma modicum* and *A. laidlawii*; tests for hydrolysis of aesculin and arbutin were also performed with these strains. (2b) Digitonin sensitive strains were examined by immunofluorescence using antisera against *M. bovirhinis* and bovine serogroups 7 and L.

For details of materials and methods, the reader is referred to previous publications, summarized by *Ernø* (1974).

Results. (1) A total of 85 strains were identified by immunofluorescence as *M. bovis genitalium*. All were glucose and arginine negative, and all were film and spot positive. Phosphatase activity was demonstrated in all but 2 strains. (2) The remaining 15 strains were all glucose positive and arginine negative; 13 were digitonin resistant (genus *Acholeplasma*), and 2 were digitonin sensitive (genus *Mycoplasma*). (2a) In the genus *Acholeplasma*,

1 strain, aesculin and arbutin negative, was diagnosed as *A. modicum*. Twelve strains were identified as *A. laidlawii*, 10 being phosphatase negative and 2 phosphatase positive. All were arbutin negative, while 4 were found to be aesculin positive. One strain of *A. laidlawii* was film and spot positive. (2b) The digitonin sensitive, glucose positive and arginine negative strains were diagnosed as *M. bovirhinis*. Both were phosphatase negative, but they differed in respect to film and spot formation.

Discussion. It appears that indirect immunofluorescence is of great practical value in the identification of mycoplasmas cultivated from semen of bulls, as all isolates could be diagnosed by this method. It is interesting to note that none of the 85 strains of *M. bovirhinis* reacted with antisera against *M. agalactiae* subsp. *bovis*, although these 2 species are biochemically very much alike.

As compared with other diagnostic methods, immunofluorescence has the advantage that one may utilize the primarily isolated colonies. If the colonies are tested with antiserum against *M. bovirhinis* and *A. laidlawii*, the diagnosis will be made in the vast majority of cases. The more time-consuming work may then be reserved to isolates not belonging to any of these species. Furthermore, it is a great advantage that immunofluorescence gives the possibility of revealing infections with more than one species.

As to the question of biochemical variation within species it is noteworthy that no variations were found in regard to the catabolism of arginine and glucose, and also that the digitonin test did unquestionably separate achleplasmas from mycoplasmas. The species *M. bovirhinis* was very homogeneous as the only variation seen was the lack of phosphatase activity in 2 strains. A greater tendency to variation was seen for *A. laidlawii*, but all strains were arbutin negative.

It is in accordance with the literature that *M. bovirhinis* is the species which is most prevalent in semen samples from bulls, although *A. laidlawii* has also often been encountered. Other mycoplasmas recovered from this region include *M. agalactiae* subsp. *bovis*, *M. arginini*, *M. gateae* and ureaplasmas (*T-mycoplasmas*). In Hungary, *A. modicum* was isolated by *Stipkovits et al.* (1973) from the seminal vesicle of a bull with primarily intestinal and respiratory symptoms.

In Denmark, the isolation and identification of mycoplasmas from semen of bulls are of diagnostic interest, mainly in 2 situations, viz. when the semen originates either from imported animals or from young bulls with abacterial seminal vesiculitis.

Henning Ernø,
FAO/WHO Collaborating Centre for Animal Mycoplasmas,
Institute of Medical Microbiology,
University of Aarhus, Denmark.

REFERENCES

- Blom, E. & H. Ernø:* Mycoplasmas in semen of Danish breeding bulls. Proc. 11. Nord. Vet. Congr., Bergen 1970, p. 254.
- Ernø, H.:* Bovine mycoplasmas. Cultural, biochemical, immunological and pathogenicity studies. Thesis. Aarhus 1974.
- Stipkovits, L., J. Bokori, Z. Horváth & L. Molnár:* Incidence of group 6 bovine *Mycoplasma* in Hungary. *In vitro in CSSR*, 1973, 76—83 (Reports presented at the Conferences on Taxonomy and Physiology of Mycoplasmas held in Brno 1970 and 1972).

(Received April 17, 1975).

Reprints may be requested from: H. Ernø, Institute of Medical Microbiology, Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark.