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Brief Communication

FAILURE TO PRODUCE PNEUMONIA IN CALVES BY INHALATION OF MYCOPLASMA BOVIRHINIS AND UREAPLASMA AEROSOLS

Mycoplasmas which can frequently be isolated from pneumonic tissue of calves in this country are Mycoplasma dispar (M. dispar), M. bovirhinis and Ureaplasma (*Bitsch et al.* 1976). Further, M. bovigenitalium is now and then recovered (*Friis*, unpublished). In previous transmission experiments with Danish field strains of M. dispar it was demonstrated that this species possessed a moderate primary pathogenicity for the calf lung (*Friis* 1980).

The aim of the present work was to examine the pathogenicity of Danish isolates of M. bovirhinis and Ureaplasma.

Colostrum-deprived calves of the Jersey breed were used. They were isolated immediately after birth and brought through their critical first 14 days as described by *Friis & Pedersen* (1979). If the animals were found mycoplasma-free by repeated examinations of swabs from the nasal and preputial cavities they were inoculated at about four weeks old. About 20 ml of culture was applied as an aerosol via a 10-liter reservoir.

The medium used for cultivation of M. bovirhinis was one which had been developed for M. suipneumoniae (*Friis* 1975). The same medium was used for ureaplasmas after enrichment with 20 % horse serum and with urea, 0.5 mg/ml and $MgSO_4$, 0.2 mg/ml. The pH was adjusted to 6.75.

For necropsy the animals were anaesthetized with Mebumal and bled to death from a brachial artery. The following organs were examined for mycoplasmas: Three different lung lobes, trachea, larynx, pharynx, nasal cavity, conjunctival sac, olfactory bulb, cerebrum, pleural, pericardial and peritoneal cavities, liver, spleen, right stifle and hock joints, preputial cavity, and urethra.

Experiments with Mycoplasma bovirhinis

Two strains, Br624 and Br666, both recovered from pneumonic calf lungs, were used each for two calves: I, II and III, IV, respectively. The strains were passed through a 0.45 μm membrane and cloned once from solid medium. They were identified

as M. bovirhinis by disc growth inhibition (DGI) tests against antiserum for the type strain PG43. Cultures in their eighth passage, diluted to approx. 10^{-30} of original tissue and containing 10^8 color-changing units (ccu) per ml were used for inoculation.

No clinical disease was observed p.i., but nasal swabs yielded M. bovirhinis. At necropsy 14 days p.i. the only lesion noted was a $0.5 \times 0.5 \times 0.5$ cm large area of consolidation in the right cardiac lung lobe of Calf II. M. bovirhinis was recovered at titers of 10^2 to 10^6 from nasal cavity, pharynx and larynx of all animals, and at titers of 10^1 to 10^2 from the trachea of three calves. Except for a titer of 10^2 in the right cardiac lobe of Calf III, M. bovirhinis was not isolated from any of the examined lung lobes. Thus, the lung of Calf II with the minute area of consolidation contained no M. bovirhinis at all. Since this consolidation did not even contain either aerobe or anaerobe bacteria, no further attention was paid to it. Of the other tissues examined only the cerebrum of Calf III was found to contain M. bovirhinis, titer 10^1.

It appears that both strains of M. bovirhinis readily colonized the upper part of the respiratory tract, but showed little affinity for normal calf lung tissue; they therefore cannot be regarded as primary lung pathogens.

Experiments with Ureaplasma

Four calves were inoculated with strains which had been recovered from pneumonic calf lungs.

Strains U871 and U937 were used each for one calf: V and VI, respectively. After the filter-cloning process the strains were found, by DGI and MI (metabolism inhibition) tests, to be related to, respectively, Serovar T315 and Serovar A417 of *Howard et al.* (1978). They were used for inoculation in their seventh passage, diluted to 10^{-20} of original tissue, with titers of 10^5 and 10^6 ccu/ml. No respiratory distress was noted in any of the animals, but Calf VI showed a temperature of 39.6°C on day nine. No ureaplasmas were found in nasal swabs six days p.i. Necropsy was performed ten days p.i. In Calf V no lesions were found and no ureaplasmas were recovered. In Calf VI there was a sero-fibrinous inflammation of the right hock joint, and in the right apical lung lobe two areas of consolidation, measuring $1 \times 1 \times 0.25$ cm and $0.2 \times 0.3 \times 0.25$ cm, were noted. From these lesions hemolytic staphylococci were recovered, but no

ureaplasmas. In this calf ureaplasmas were recovered only from the right cardiac lobe (10^3) and from trachea (10^2) and larynx (10^5). It would appear that the minute lung lesions were caused by the hemolytic staphylococci and not by the inoculated ureaplasmas.

Two other calves were inoculated with pooled crude isolates of Ureaplasma: Calf VII with U59, U94 and U128, and Calf VIII with U29, U49, U209 and U223. These isolates were used in their second passage, diluted to approx. 10^{-6} of original tissue. The titers of the pools were 10^5 and 10^7, respectively. Apart from a few days with diarrhoe in Calf VIII no clinical symptoms were observed. No ureaplasmas were found in nasal swabs seven days p.i. Necropsy was performed 13 and 15 days p.i. No lesions were noted and no ureaplasmas were recovered.

It appears that none of the nine strains of Ureaplasma examined have much affinity for a normal bovine respiratory tract, and they are not primary lung pathogens.

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REFERENCES

Bitsch, V., N. F. Friis & H. V. Krogh: A microbiological study of pneumonic calf lungs. Acta vet. scand. 1976, *17*, 32—42.

Friis, N. F.: Some recommendations concerning primary isolation of Mycoplasma suipneumoniae and Mycoplasma flocculare. Nord. Vet.-Med. 1975, *27*, 337—339.

Friis, N. F.: Mycoplasma dispar as a causative agent in pneumonia of calves. Acta vet. scand. 1980, *21*, 34—42.

Friis, N. F. & K. B. Pedersen: Isolation of Mycoplasma bovoculi from cases of infectious bovine keratoconjunctivitis. Acta vet. scand. 1979, *20*, 51—59.

Howard, C. J., R. N. Gourlay & J. Collins: Serological studies with bovine ureaplasmas (T-mycoplasmas). Int. J. syst. Bacteriol. 1978, *28*, 473—477.

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