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Brief communication

MYCOPLASMOSIS: CERVICAL AND UTERINE INOCULATION OF HEIFERS WITH A DANISH STRAIN OF MYCOPLASMA BOVIGENITALIUM

The "K" strain of Mycoplasma bovigenitalium has proved to be pathogenic for the mammary gland of cows, for the genital tract of bulls, and for calves (*Ernø* 1967, *Blom & Ernø* 1967, *Ernø* 1969). Antibodies can be demonstrated in the blood following infection of the mammary gland, and after intravenous and intraperitoneal inoculation of calves with or without clinical signs of infection.

In the United States 2 different bovine isolates of Mycoplasma were studied in regard to their pathogenicity for the reproductive tract of heifers (*Hirth & Mosher* 1966). One isolate, tentatively named M. agalactiae var. bovis, produced marked lesions and long standing infertility in heifers inseminated with semen containing the mycoplasmas. Preliminary investigation of the second isolate from the prepuce of a bull indicated that this isolate did not survive well in the genital tract, as it could be recovered for only very short periods of time, and only from some of the heifers. The American group of workers has not reported on the serologic response of cattle to genital mycoplasmosis.

This communication reports the results of cervical and uterine inoculation of heifers with the "K" strain of M. bovigenitalium.

Materials and methods. Twelve gynaecologically normal, virgin heifers were used for the experiment. They were free of strongylosis, brucellosis, leptospirosis and vibriosis. Mycoplasmas could not be isolated by the vaginal tampon test on 3 attempts at an interval of 3—4 days. In the fall of 1967 all animals were inoculated, being in heat synchronized by enucleation of the corpus luteum. Three heifers were inoculated intracervically (nos. 1—3), and 9 heifers (nos. 4—12) intrauterinally, all of them receiving 10^4 colony forming units (c.f.u.) of a lyophilized culture which had been rehydrated in sterilized homogenized 2 % milk. To prove the pathogenicity of the mycoplasmas the content of 1 ampoule was rehydrated in 10 ml of milk from a cow; this cow was inoculated through the teat canal, in 1 quarter,

and a typical mycoplasma mastitis developed with a rapid multiplication of the organisms.

The heifers nos. 4 and 7, nos. 5 and 6, nos. 8 and 9, no. 10, no. 12, were slaughtered 2, 3, 5, 12 and 20 weeks p.i., respectively. The remaining 4 heifers (nos. 1, 2, 3 and 11) were reinoculated (6 weeks p.i.) apically in the right uterine horn, while in heat. Nos. 1 and 3 received a freeze-dried culture, as with the first inoculation, while nos. 2 and 11 were inoculated with 1 ml of a 72-hour-old broth culture containing 5×10^5 c.f.u. Heifer no. 3 and heifers nos. 1, 2 and 11 were slaughtered 6 and 14 weeks after the last inoculation, respectively. Gynaecological examinations were performed once a week; blood samples for differential countings and serologic tests, i.e. CF and IHA, were drawn twice a week. Vaginal tampon tests for the cultivation of mycoplasmas were also performed twice a week. The tampons were soaked for 24 hrs. in peptone broth with penicillin, whereupon 0.1 ml was transferred to 1.7 ml of a semisolid medium (Brain Heart Infusion supplemented with horse serum, yeast extract and DNA). After 3 and 6 days of incubation, plating onto the same solid medium was done, and the plates were incubated for 10 days. Post mortem the genital organs were examined macroscopically; histopathologic examination and cultivation were performed from representative areas of the ovaries, salpinges, uteri, cervices and vaginas.

Results. All heifers cycled normally, and gynaecological examinations did not reveal any abnormalities except in heifer no. 7 which developed an ovarian cyst and a hydrometra. No significant changes in the haemograms were noticed. Neither gross nor histopathologic lesions were present. Mycoplasmas were cultured post mortem from the vagina of heifer no. 7 which had been slaughtered 2 weeks p.i. Before slaughtering mycoplasmas were isolated on 4 occasions from heifers no. 10 (27/11, 4/12, 7/12 and 11/12, i.e. appr. 6—8 weeks p.i.), once (4/12) from nos. 3 and 12, 8 weeks p.i. Only heifer no. 12 produced antibodies, demonstrable by indirect haemagglutination; this animal was negative until December 14 (8 weeks p.i.), when the titer was 4; January 4, 8, 11 and 15 the titres were 16; during the remaining period of observation, 8 weeks, it was constantly 8. Complement fixing antibodies were never demonstrated in any of the animals.

Conclusion and discussion. The "K" strain of M. bovigenitalium does not seem to produce lesions in the reproductive tract of heifers; the strain may, however, survive latent for a long period of time and may elicit a weak, though specific, formation of antibodies.

As to the question why the "K" strain — unlike the "Donetta" strain — seems to be primary apathogenic for the female genital tract while pathogenic for the mammary gland, seminal vesicles and joints, it is worth observing that the "K" strain of M. bovigenitalium is much more fastidious to cultivate and propagate than is the "Donetta" strain of M. agalactiae var. bovis. One might therefore suspect that the udder, seminal vesicles, and the joints — in contrast to the reproductive tract — can supply the "K" strain with such growth conditions that a critical level of microorganisms is attained necessary to elicit a reaction from the tissue. In this connection it is worth mentioning that the 2 strains in question produce lesions of principally identical character — at least in the udder — with eosinophilia as the outstanding feature.

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