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***Brief Communication***

# MYCOPLASMOSIS: EXPERIMENTAL SEMINAL VESICULITIS. DEMONSTRATION OF LOCALLY OCCURRING ANTIBODY

1. Infections with M. bovigenitalium are often characterized by the presence of leucocytes with eosinophilic granules. *Parsonson* (1970) holds that the persistance of mycoplasmas in the genital tract of bulls provides a constant source of antigen which can combine with locally produced antibodies to form antigen-antibody complexes which attract eosinophils.

2. In experimental mycoplasmal infections of the genital tract of bulls a significant increase in serum antibody could be demonstrated, but as the titers were low and the maximum titers were reached early, it was concluded that it would not often be possible to make a clinical diagnosis on the basis of examination of serum. It was suggested that serological examination of seminal plasma could be of diagnostic value (*Ernø* 1972).

The present communication reports the results of an experiment designed to elucidate aspects of the above questions, i.e. appearance of mycoplasmal antibodies at the site of inoculation and the possible diagnostic value of serological examination of seminal plasma. The latter problem was elucidated also by titration of 40 random samples of semen from normal AI bulls.

*Inoculation.* Twin bulls, nos. 185 and 186, 2½ years old, were inoculated in the left vesicular glands with 5×2 ml of a 48-hour-old broth culture of M. bovigenitalium, strain "K", containing $10^8$ colony forming units (c.f.u.) per ml. The bulls were slaughtered 40 days after inoculation (p.i.).

*Immunological tests.* Serum and seminal plasma were examined by 2 serological tests, viz. indirect hemagglutination (IHA) and tetrazolium reduction inhibition (TRI). Secretion from the vesicular glands were tested by IHA and also by indirect immunofluorescence to demonstrate possible appearance of mycoplasmal antibodies within the known immunoglobulin classes (*Ernø & Aalund* 1972).

*Results*

The results obtained in one of the experimental bulls (no. 186) are summarized in Table 1, and the results of serological examination and cultivation of vesicular secretions from both bulls are seen in Table 2.

Table 1. Experimental vesiculitis induced by M. bovigenitalium (bull no. 186).

| Days p.i. | Clinical changes | Pus cells in semen | Colony forming units/ml of semen | Antibodies in seminal plasma and serum: titer (IHA) sem.pl. | titer (IHA) serum | titer (TRI) sem.pl. | titer (TRI) serum |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 1024 | 0 |
| 4 | 0 | 0 | $10^4$ | 256 | 8 | 256 | 0 |
| 7 | 0 | 0 | $8\times10^7$ | 32 | 64 | 128 | 0 |
| 18 | 0 | 0 | $4\times10^5$ | 64 | 64 | 256 | 0 |
| 21 | (+) | 0 | $10^8$ | 128 | 64 | 256 | 0 |
| 32 | + | + | $2\times10^8$ | 512 | 128 | 256 | 0 |
| 35 | + | ++ | $4\times10^8$ | 256 | 128 | 8 | 0 |
| 40 | + | ++ | NT | NT | 128 | NT | 0 |

NT: Not Tested.

Table 2. Serological examination of vesicular secretion (post mortem). Indirect hemagglutination.

| Bull no. | Left vesicular gland: c.f.u. per ml of secretion | Left vesicular gland: titer (IHA) | Right vesicular gland: c.f.u. per ml of secretion | Right vesicular gland: titer (IHA) |
|---|---|---|---|---|
| 185 | $10^7$ | 0 | $8\times10^2$ | 0 |
| 186 | $3\times10^3$ | 5, 120 | 0 | 0 |

Antibodies did appear very early in seminal plasma as measured by IHA. The TRI test was not applicable, as metabolic inhibiting factors were present before inoculation. Antibodies were demonstrable by IHA in 18 of the 40 random semen samples. The average titer of positive samples was 1:400. In bull 186 antibodies were demonstrable in secretion from the inoculated gland. In bull 185 antibodies were not found in either of the

glands, but it is important to note that in the inoculated gland of this animal a high count of mycoplasmas was found, which may have neutralized possible antibody present. Antibodies of all known bovine immunoglobulin classes (IgM, IgA, IgG-1, IgG-2) were present in the secretion of the inoculated gland of bull 186.

*Conclusions*

1. Antibody may be present locally at the site of inoculation, with which one of the prerequisites for the correctness of the theory of *Parsonson* is fulfilled. 2. Serological examination of seminal plasma is not of diagnostic importance, as antibody often occurs in seminal plasma of healthy bulls.

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