Acta vet. scand. 1971, *12*, 451—453.

Brief Communication

MYCOPLASMOSIS: EXPERIMENTAL MASTITIS. DEMONSTRATION OF ANTIBODY IN MILK

According to several authors specific antibodies can be demonstrated in serum of cows with mycoplasma mastitis. For practical reasons, and for pathogenetic studies, it would be of interest if presence of antibody in milk could be established. To elucidate this and to examine the pathogenicity of the type strain, PG 11, of M. bovigenitalium, the experiment described below was conducted.

Inoculation. Cow no. 151, 3 years old, in late lactation, was inoculated in the left and right hind quarters with 10^8 and 10^6 colony forming units (c.f.u.), respectively, of strain PG 11. Two weeks later both right quarters were inoculated with 10^5 c.f.u. of strain "K" of M. bovigenitalium known to be pathogenic to the mammary gland (*Ernø* 1967). The cow was slaughtered 46 days after inoculation (p.i.) with strain "K".

Serological tests. Serum and whey were examined by 3 tests, viz. indirect haemagglutination (IHA), tetrazolium reduction inhibition (TRI) and growth inhibition (GI).

Results

a. Inoculation of strain PG 11 did not cause clinical symptoms. Mycoplasmas were not recovered from milk. During the observation period of 16 days, examination of blood and milk samples did not reveal antibody formation. The course of disease resulting from infection with strain "K" did not seem to be altered by a previous inoculation with strain PG 11 (Table 1).
b. On the 3rd day p.i. milk from quarters inoculated with strain "K" contained 1000 c.f.u. of mycoplasmas per ml. Four days p.i. the number of mycoplasmas had increased to 10^6 c.f.u. per ml, antibody was demonstrated in milk of inoculated quarters by IHA and a clinical mastitis was evident. A rise of antibody in serum was not yet demonstrable (Table 1). On the 9th day p.i. antibody was found also in milk from non-inoculated quarters along with a decrease of the mycoplasma

Table 1. Experimental mastitis induced by M. bovigenitalium.

Days after inoculation with strain "K"	Antibodies in milk as measured by indirect haemagglutination				Colony forming units per ml of milk (RR)	Clinical mastitis (RF and RR)	Elevation of temperature	Antibodies in serum as measured by indirect haemagglutination
	RF[1])	LF[2])	RR[3])	LR[4])				
0	<5	<5	<5	<5	0	0	0	16
3	<5	<5	<5	<5	5×10^3	0	0	NT
4	32	<5	64	<5	5×10^6	+	2°C	NT
5	128	<5	512	<5	10^8	+	2°C	16
9	128	64	256	32	5×10^7	+	0	NT
12	2048	2048	2048	128	10^5	+	0	NT
16	1024	1024	256	512	2×10^5	+	0	NT
23	1024	256	256	512	0	0	0	2048
46	64	512	128	32	0	0	0	1024

NT: Not tested. [1]) Inoculated with strain "K". [2]) Not inoculated".
[3]) Inoculated with strains PG 11 and "K". [4]) Inoculated with strain PG 11.

count in milk from inoculated quarters. Three weeks p.i. no signs of mastitis were evident though the yield of milk never reached the preinoculation level.

c. TRI antibody was also demonstrable in milk. Preinoculation titers were < 10 and a rise of antibody was not demonstrable until 9 days p.i. when a titer of 16 was found in milk from all quarters. Sixteen days p.i. the titers were of the same order as found by IHA; the antibodies persisted throughout the period of observation but a great variance in titers from quarter to quarter was found. The highest titer (2048) was found in milk from the left front quarter; this quarter never revealed symptoms of disease. The serum titer was never higher than 64.

d. GI antibody was demonstrated in milk 12 days p.i. in both healthy and inflamed quarters. Later samples were intermittently positive. Antibody in blood could not be demonstrated by the GI test until 3 weeks after inoculation; all subsequent samples were positive. These results were achieved with strain PG 11 as antigen; with strain "K" no significant inhibition was seen except for inhibition of the film and spot formation in a zone of 5 mm. — The fact that strain PG 11 is a more sensitive test organism than strain "K", is seen also in cross titrations of rabbit hyperimmune sera.

e. The haemograms showed a severe leucopenia with extreme neutropenia 5 days p.i. Three weeks p.i. the total leucocyte count

was again within normal limits — the lymphocyte count was subnormal while the percentage of eosinophilic granulocytes increased to 21.5.

It thus appears that antibody is demonstrable in milk of cows with experimentally induced mycoplasma mastitis. The early appearance of antibody in inoculated quarters is of great pathogenic interest, as this phenomenon is consistent with the hypothesis that the early eosinophilic response to M. bovigenitalium infection is due to antibody-antigen reactions (*Parsonson* 1970, *Ernø & Blom* 1972). Regarding the presence of antibody in milk from the 2 healthy quarters (LF and LR) the data of this experiment do not allow any conclusions as to the origin of these antibodies. A local production is possible as mycoplasmas actually were recovered in small numbers from milk of these quarters on days 5 and 6 p.i. with strain "K".

Henning Ernø
Institute of Medical Microbiology,
University of Aarhus,
Aarhus, Denmark.

REFERENCES

Ernø, H.: Mycoplasmosis: Demonstration of pathogenicity of a Danish strain of mycoplasma. Acta vet. scand. 1967, *8*, 184—185.

Ernø, H. & E. Blom: Mycoplasmosis: Experimental and spontaneous infections of the genital tract of bulls. Accepted for publication in Acta vet. scand. 1972.

Parsonson, I. M.: Mycoplasma bovigenitalium: Experimental induction of genital disease in bulls. Ph. D. Thesis. Cornell University. 1970.

(Received July 7, 1971).