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Brief Communication

MYCOPLASMOSIS: EXPERIMENTAL MASTITIS. IMMUNOGLOBULIN CLASSES OF MYCOPLASMAL ANTIBODIES IN MILK AND SERUM

It was demonstrated (*Ernø* 1971) that mycoplasmal antibodies appeared in milk of cows with experimentally induced mycoplasma mastitis. The present communication reports the results of an experiment designed to elucidate the appearance of mycoplasmal antibodies within the known immunoglobulin classes.

Inoculation. Cow no. 155, 5 years old, in late lactation, was inoculated in the left front quarter with 10^6 colony forming units (c.f.u.) of M. bovigenitalium, strain "K". The cow was slaughtered 35 days after inoculation (p.i.).

Serological tests. Serum and whey were examined by 3 tests, viz. indirect hemagglutination (IHA), tetrazolium reduction inhibition (TRI) and growth inhibition (GI). Samples were taken every day and every second day for milk and serum, respectively.

Differentiation of immunoglobulin classes. Samples of whey and serum were tested undiluted, diluted 1:10 and 1:20, with a 3-layer indirect immunofluorescence technique employing monospecific rabbit antisera against bovine IgA, IgM, IgG-1 and IgG-2, and a fluoresceinconjugated horse anti-rabbit immunoglobulin (HAR-C, Central Laboratorium van de Bloedtransfusiedienst van het Nederlandsche Roode Kruis). Unfixed agar blocks with mycoplasma colonies (*Rosendal & Black* 1972) were incubated with a drop of the test sample for 30 min. at 22°C in a moist chamber. The blocks were washed twice for 10 min. with PBS in a test tube and then transferred to slides. A drop of monospecific rabbit anti-immunoglobulin was placed on each block which was incubated for 30 min. at 22°C. After washing twice for 10 min. the blocks were again transferred to slides, and a drop of HAR-C was placed on each block. Incubation for 20 min. After washing 2×5 min. the blocks were placed on slides and read by means of a Zeiss Standard (RA) microscope with incident illumination.

Table 1. Experimental mastitis induced by M. bovigenitalium (LF: Left front quarter, inoculated with strain "K". S: Serum. RR: Right rear quarter, not inoculated).

Days after inoculation	Mastitis	Colony forming units per ml of milk (LF)	Antibodies in milk (whey) and serum								
			indirect hemagglutination			growth inhibition in mm			tetrazolium reduction inhibition		
			LF	S	RR	LF	S	RR	LF	S	RR
0	0	0	0	32	2	0	0	0	8	0	8
5	+	3×10^8	256	32	2	0	0	0	0	0	16
9	+	10^9	160	512	32	0	0	0	0	0	8
14	+	3×10^2	1280	1280	160	2	2	0	64	8	8
35	0	0	160	640	80	2	2	1,5	32	16	8

Table 2. Immunofluorescent immunoglobulin differentiation.

Days after inoculation	Mastitis	Fluorescence score											
		milk inoculated quarter				serum				milk non-inoculated quarter			
		IgA	IgM	IgG-1	IgG-2	IgA	IgM	IgG-1	IgG-2	IgA	IgM	IgG-1	IgG-2
0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	+	+	0	0	0	0	0	0	0	0	0	0	0
9	+	+	+	0	0	+	+	0	+	0	+	0	0
14	+	+	+	+	0	+	+	+	+	0	+	+	0
35	0	+	+	+	0	0	+	+	+	0	+	+	0

Results. a. On the 2nd day p.i. milk from the inoculated quarter contained 10^6 c.f.u. per ml of M. bovigenitalium. Five days p.i. (Table 1) the number of mycoplasmas had increased to 3×10^8 c.f.u. per ml, antibody was demonstrated in milk of the inoculated quarter by IHA, and a clinical mastitis was evident. A rise of antibody was not demonstrable neither in serum nor in uninfected quarters. On the 9th day p.i. antibody was found also in serum and in milk from the uninoculated quarter, but still by IHA only. On the 14th day p.i. maximum titers (IHA) were found in milk as well as in serum, growth inhibiting antibodies appeared in milk and serum, and the mycoplasma count was 3×10^2 c.f.u. per ml of milk. Sixteen days p.i. no signs of mastitis were evident, though the yield of milk never reached the

preinoculation level. A weak rise of titers in the TRI test was found in serum and milk from the inoculated quarter 14 days p.i.

b. It appears from Table 2 that the early antibody response in the inoculated quarter may be due to IgA, and that the presence of IgA was demonstrable concurrently with a significant mastitis. IgM appeared in milk and serum 9 days p.i. At this point the secretion of mycoplasmas per ml of milk started to decrease. IgG-1 was demonstrated in milk and serum 14 days p.i. concurrently with the findings of growth inhibiting antibodies. IgG-2 was seen in serum only, and IgA was never demonstrated in milk from the non-inoculated quarter.

It is suggested that IgA is locally produced and that protective antibodies are found mainly in immunoglobulin classes IgM and IgG-1.

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