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THE CROSS REACTION BETWEEN TWO AGALACTIAE STRAINS LORESTAN AND AIK2 IN SERA OF VACCINATED SHEEP AND GOATS INOCULATED WITH LIVE VACCINE.*

Aarabi, I., Sotoodehnia, A., and Naseri Rad, A.

Summary

A batch of freeze dried live agalactiae vaccine was prepared with attenuated strain AIK40. A group of forteen sheep and goats was vaccinated with one ml of vaccine containing about $1.3x10^9$ viable **Mycoplasma** and was challenged with virulent strain AIK2 7.5 months post vaccination. Sera were prepared after the vaccination and challenge were tested with two Lorestan and AIK2 antigens by growth inhibition test. Results indicated that both antigens had similar reactions against antibodies in vaccinated animals.

Introduction

In some of the countries where agalactiae disease occurs, the use of **Mycoplasma** attenuated strains in preparation of agalactiae vaccine has recently been tried with satisfactory results.

Strain 99M (AIK) isolated from sheep milk was attenuated by forty subcultures on selective agar (1, 2). Further studies showed that the attenuated strain was not only harmless but rather induced a good immunity in animals without any evidence of revers to virulent form (3).

In the present study it was found that there was a cross immunity between Iranian and Turkish strains by growth inhibition (GI) test described by Gourlay and Domermuth (4) and later, with some modifications, were used by Arisoy et al to demonstrate growth inhibition of M.agalactiae (5).

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Materials and Methods

Strains:

The field strain Lorestan had been isolated from sheep milk in south west area of Iran and the virulent strain AIK2 was the second subculture of the field strain 99M. Both strains were used as antigens in growth inhibition tests.

The attenuated strain AIK40 was used for preparation of live vaccine which the experimental animals were vaccinated with.

Vaccination:

The live vaccine was prepared with strain AIK40. One ml of 72 hour old culture in PPLO broth medium supplemented with 20% inactivated horse serum was inoculated subcutaneously behind the shoulder of each of the forteen sheep and goats. Each vaccinal dose contained about $1.3X10^9$ viable organisms per ml.

Animals and Bleeding:

Seven sheep and seven goats were vaccinated with live vaccine. A separate group of ten non-vaccinated sheep was also kept as controls for challenge tests.

Blood for serum preparation was withdrawn before the initiation of the experiments and 18 days, 3 months and 7.5 months post-vaccination, for determination of antibody titres in vaccinated animals. Blood was also taken at 8,17 and 30 days post challenge.

Challenge:

Animals were challenged by subcutaneous inoculation of about 10^5 viable organisms of virulent strain AIK2 about 10000 ID50 (1). Challenge tests were made immediately after the last bleeding post-vaccination.

Serology:

The sera of vaccinated and control animals were tested by growth inhibition (GI) test. Each serum was tested with both Lorestan and AIK2 antigens separately and cheoked according to the interpretation method of Arisoy, et al (5).

Table 1

Reaction of the sera to the G.I.T

Animals No.	Before Vaccination		Post vaccination								Post challenge			
			18 Days		3 Months		7.5 Months		8 Days		17 Days		30 Days	
	L	A	L	A	L	A	L	A	L	A	L	A	L	A
Sheep 603	-	-	0	+	+	+	+	+	0	+	+	+	+	+
" 16	-	-	0	+	++	+	+	+	0	+	+	+	+	+
" 549	-	-	0	+	++	+	+	+	0	+	+	±	+	+
" 609	-	-	0	+	+	+	±	+	0	+	++	+	+	+
" 491	-	-	0	+	+	+	+	+	0	+	++	+	+	+
" 877	-	-	0	+	+	+	+	+	0	+	++	+	+	+
" 610	-	-	0	+	++	+	+	+	0	+	+	+	+	+
Goat 997	-	-	0	+	++	++	+	+	0	+	+	+	+	±
" 1000	-	-	0	+	++	+	±	+	0	+	+	+	+	+
" 992	-	-	0	+	+	+	+	+	0	+	+	+	+	+
" 996	-	-	0	+	+	+	+	+	0	+	+	+	+	+
" 994	-	-	0	+	+	+	±	+	0	+	++	+	+	+
" 865	-	-	0	+	+	+	+	+	0	+	++	+	+	+
" 993	-	-	0	+	+	+	+	±	0	+	+	+	0	+
***														
+4°C	25	39	0	48	23	38	1	56	0	30	31	71	41	39
Room temp	30	42	0	57	6	20	1	41	0	114	21	138	220	810
30°C	+1000	+1000	0	+1000	+1000	+1000	371	+1000	0	+1000	225	+1000	920	+1000
37°C	+1000	+1000	0	+1000	+1000	+1000	+1000	+1000	0	+1000	+1000	+1000	+1000	+1000

*** Culture controls in various temperatures (Colony per drop).

L = Myco. agalactiae Lorestan strain.

A = Myco. agalactiae AIK2 strain.

0 = No tested.

++ = No colony per drop (strong inhibition).

+ = The count per drop didn,t exceed 100. (Inhibition)

± = The count per drop exceed 100 but less than 1000. (Slight inhibition).

- = The count per drop exceed 1000. (No inhibition)

Results

The results of serological tests are summarized in table 1. Which shows the antibody titres in vaccinated sheep and goats inoculated with attenuated live vaccine.

Lorestan antigen showed the reaction with all series of sera as well as AIK2 antigen in comparison with their culture controls.

None of the vaccinated animals showed the symptoms of agalactiae disease during the experiments but in the control group, three animals were slightly affected and developed keratitis or conjunctivitis, one showed arthritis with lameness and in another one both symptoms of slight keratitis and arthritis were observed.

Discussion

AIK40 is a safe and potent strain which is used in Turkey at present time against the agalactiae disease. It was found that there was an antigenic relationship between strains Lorestan and AIK2 when animals were vaccinated with strain AIK40 as a live vaccine.

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