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MYCOPLASMA AGALACTIAE III – THE COMPARISON OF DIFFERENT SEROLOGICAL TESTS WITH M. AGALACTIAE ANTIGEN §

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The serological diagnosis of sheep and goats agalactia is one of the ways to recognize the disease in affected and carrier animals, and is a prerequisite to eradication. The serological tests are also applied for the detection of antibodies in vaccinated animals.

Since 1923 when Bridré and Donatien (3) isolated and studied the Mycolpasma agalactiae, many investigators tried to find out a serological test to determine and titrate the corresponding antibody in infected sheep and goats sera.

Bridré (4) in 1943 described that agglutinin or precipitin reaction could not be detected in sera of M. agalactiae infected animals.

Zavagli (7) and his co-workers and also Ceccarelli (5a & 5b) have developed a complement-fixation test which is valuable for herd diagnosis but is unreliable as an individual test.

In this communication we will describe the comparison of agglutination, complement fixation and antiglobulin (Coombs test) (6) for demonstration of antibody in sheep and goats sera infected with M. agalactiae or vaccinated with killed micro-organisms by a corresponding antigen.

MATERIALS AND METHODS

1— Methods

a) **Antigen Preparation :** The antigen prepared with culture materials of M. agalactiae, strain, isolated by Dr. F. Entessar, Dept. of Microbiology, Razi State Institute, in the peptic digest of beef heart and liver (V.F. Broth) medium. The medium was supplemented by 1% of (Difco) yeast extract and 300 units per ml of penicillin as an inhibitor and 20% of horse serum. The culture was incubated

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in 37°C incubator for 36 hours while being shaked by a shaker apparatus. Then, the micro-organisms were harvested by a high speed sorvall contenuous-flow centrifuge at 17,000 rpm. The sediment was resuspended in PBS (pH = 7.1) and standardized by 2 X 10 McFarland scale. This antigen was used for all the tests.

b) **Hyperimmune Serum:** Hyperimmune serum was prepared on donkeys. Two donkeys were injected intravenously five times with 5 ml of standard antigen, 5 days after the last injection the donkeys were bled and their collected serum was filtered through the Seitz filter and kept at —20°C.

c) **Antiglobulin Preparation :** The antiglobulin were prepared on rabbits by 5 intravenously inoculation of sheep, goat and donkey globulin. Seven days after the last inoculation the rabbits were bled and the serum after being passed through the Seitz filter was kept at —20°C.

d) **Test Sera :** 10 sheep and 10 goats were inoculated by formolized (2.5%) and saponinized (0.1%) culture of M. agalactiae, from 5 days after inoculation the animals were bled once every two days for 52 days period. These sera as well as serum samples received from naturally infected animals were tested by different serological procedures.

2— **Methods**

a) **Direct Agglutination Test:** Once drop (0.1 ml) of each serum samples was placed on a glass plate, mixed with one drop of the antigen and gently rotated for three minutes. For determination of end-point titers of positive serum, serial two-fold dilutions of the samples were made in 0.85% saline.

b) **Tube Agglutination Test**: Regular saline agglutination or tube agglutination tests were done using antigen as stated above. Volumes of 0.25 ml of antigen were added to each of a number of 10 ml plastic tubes containing 1.25 ml of doubling antiserum dilution made in phosphate buffered saline, pH = 7.2. The tubes were incubated for 2 hours at 37°C and then held at + 4°C overnight. The titer of each serum samples were marked the highest titer being noticed at 21st days after inoculation.

c) **Antiglobulin Test**: For antiglobulin test the method of Adler and DaMassa (1) for enhancement of M. gallisepticum agglutination titers and that of Baharsefat and Yamini (2) for sheeppox antigen was used. 0.25 ml of M. agalactiae antigen was added to 1.25 ml of doubling serum dilutions in 0.85% NaCl. The tubes were incubated 2 hours at 37°C and held overnight at +4°C. Then they were centrifuged in a GEC centrifuge at 3,000 rpm. The sediment (M. agalactiae antigen with attached antibodies) in each of the tubes was washed three times by saline to eliminate free, unattached M. agalactiae antibodies. After the last washing the sediment was resuspended in 0.25 ml of saline. One volume of approximately 0.1 ml of the resuspended sediment was placed on a glass plate, and to it was added 0.025 ml of antiglobulin serum. The plate was rotated gently for three minutes, and the agglutination results recorded.

d) **Complement-Fixation Test :** The classical complement-fixation test has been performed with all of these sera.

RESULTS

Table No I shows the titers of sera of sheep and goats being inoculated with killed M. agalactiae by the different serological tests.

Table No II shows the titers of sera of naturally infected animals which had been received for the diagnosis at the Institute. These animals brought from infected farms in different stages of infection were also subjected to the same different serological tests.

DISCUSSION

According to the table No I, from 5 to 21st days after inoculation the direct Agglutination test is negative, while at the 21st day post-inoculation when the serum has the highest antibody titer, it shows one to two plus in the undiluted or 1/0 dilution. Therefore the direct agglutination test is not a good procedure for assessing the titer of antibody in infected or vaccinated animals. The tube agglutination test, in the contrary, was positive from 5 to 21st day after inoculation and the titer went up from time to time so that at the 21st day post-inoculation it reached to its peak. The result of antiglobulin test is the same as for tube agglutination but this test by enhancing the titer of antibody shows the crypto-agglutinin also. As regards to the complement-fixation test, we have not been able to detect a reasonable titer in all of these sera, however, we had a titer of 1/160 with hyperimmun serum.

In natural infection, figured in table No II, also the tube agglutination and antiglobulin tests were the best for showing the presence of antibody during different stages of contamination.

The experience should be continued to find out the most sensitive test for the detection of M. agalactiae antibody in affected and vaccinated animals. But at the present we recommend the antiglobulin test for this purpose.

SUMMARY

Four different serological tests such as: Direct agglutination, tube agglutination, complement-fixation and antiglobulin have been compared for the detection of antibody in inoculated and naturaly infected animals with M. agalactiae.

The antiglobulin is recommended as the most sensitive serological test for the detection of antibody corresponding to M. agalactiae.

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RESUME

Quatre différents tests serologiques comme: Agglutination Direct, Agglu-

tination en tube, Fixation du Complement et Antiglobuline ont été comparées pour la detection de l'Anticorps dans le sérum des animaux inoculés ou naturellement infectés par M. agalaxiae.

Le test de l'Antiglobuline est recommandé comme un test très sensitif pour la détection de l'Anticorps correspondant au M. agalaxiae.

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Table 1. Results of sera titers of sheep and goats inoculated with killed M. agalactiae

ANIMAL No.	SPECIES	PRE-INOCULATION TITER			DAYS POST-INOCULATION																													
					5 Days			7 Days			11 Days			14 Days			17 Days			21 Days			26 Days			31 Days			38 Days			45 Days			52 Days		
		D	T	AG	D	T	AG	D	T	AG	D	T	AG	D	T	AG	D	T	AG	D	T	AG	D	T	AG	D	T	AG	D	T	AG	D	T	AG	D	T	AG
	Sheep																																			
4715	"	-	-	-	-	10+	20	-	10	20	-	10	20	-	20	40	-	40	80	10	80	320	-	10	20	-	10	20	-	10	10	-	10	10	-	-	-
4719	"	-	-	-	-	20	20	-	20	20	-	20	40	-	20	40	-	80	80	10	320	640	-	10	20	-	10	20	-	10	10	-	10	10	-	10	10
4716	"	-	-	-	-	20	40	-	20	80	-	40	80	-	80	160	10	80	160	10	320	640	10	20	40	10	20	20	-	20	20	-	10	10	-	10	10
4550	"	-	-	-	-	10	10	-	10	20	-	10	20	-	10	20	-	40	80	10	80	320	-	10	20	-	10	10	-	10	10	-	-	-	-	-	-
3864	"	-	-	-	-	20	20	-	20	40	-	20	40	-	40	80	-	160	320	10	160	320	-	10	20	-	10	20	-	10	10	-	10	10	-	-	-
3080	"	-	-	-	-	10	20	-	10	20	-	20	40	-	40	160	-	40	160	-	40	160	-	10	20	-	10	10	-	10	10	-	10	10	-	10	10
645	"	-	-	-	-	20	40	-	40	80	-	40	80	-	40	80	-	80	160	10	320	640	-	20	40	-	10	20	-	10	20	-	10	10	-	10	10
351	"	-	-	-	-	10	20	-	20	40	-	20	40	-	80	160	-	80	160	10	160	320	-	20	40	-	10	10	-	10	10	-	-	-	-	-	-
147	"	-	-	-	-	10	10	-	10	20	-	10	20	-	20	20	-	20	40	10	160	320	-	10	20	-	10	10	-	10	10	-	10	10	-	-	-
194	"	-	-	-	-	10	20	-	20	40	-	20	40	-	20	40	-	80	160	10	160	640	-	10	20	-	10	20	-	10	20	-	10	20	-	-	-
	Goat																																			
3758	"	-	-	-	-	-	-	-	10	10	-	10	20	-	20	40	-	40	80	10	160	320	-	10	20	-	10	10	-	10	10	-	10	10	-	-	-
3768	"	-	-	-	-	10	20	-	10	20	-	20	40	-	20	40	-	40	80	10	320	640	-	20	20	-	10	20	-	10	10	-	10	10	-	-	-
3754	"	-	-	-	-	-	10	-	10	20	-	20	40	-	40	80	10	40	80	10	320	640	10	20	40	-	10	20	-	10	10	-	10	10	-	10	10
3757	"	-	-	-	-	20	40	-	20	80	-	20	80	-	20	160	-	40	160	10	320	640	-	40	40	-	10	20	-	10	20	-	10	10	-	10	10
3755	"	-	-	-	-	10	20	-	20	20	-	20	40	-	40	40	-	40	80	10	320	640	-	10	20	-	10	20	-	10	10	-	10	10	-	-	-
3750	"	-	-	-	-	10	20	-	40	80	-	40	80	-	80	320	-	80	320	10	320	640	-	20	40	-	10	10	-	10	10	-	10	10	-	-	-
3679	"	-	-	-	-	10	20	-	20	80	-	40	160	-	80	320	10	160	320	10	320	640	10	20	40	-	10	20	-	10	20	-	10	10	-	10	10
3732	"	-	-	-	-	10	20	-	10	20	-	20	40	-	20	40	-	20	40	10	160	320	-	20	40	-	10	10	-	10	10	-	10	10	-	-	-
3620	"	-	-	-	-	10	20	-	10	20	-	10	40	-	80	160	-	160	160	10	160	320	-	20	20	-	-	10	-	-	10	-	-	-	-	-	-
1	"	-	-	-	-	10	10	-	10	20	-	10	40	-	20	40	-	20	80	-	160	320	-	10	20	-	10	20	-	10	10	-	-	-	-	-	-

D = Direct agglutination titer T = Tube agglutination titer AG = Antiglobulin titer + = 2+

TABLE II — RESULTS OF THE TITERS OF SHEEP AND GOATS SERA COLLECTED FROM NATURAL INFECTION

SERUM No.	SPECIES	BLEEDING DAYS AFTER SHOWING CLINICAL SYMPTOMS	TITER		
			D	T	AG
1	SHEEP	10 DAYS	-	10	20
2	"	"	-	20	40
3	"	"	-	40	40
4	"	"	-	40	80
5	"	"	-	20	40
6	"	"	-	[illegible]	40
7	"	"	-	[illegible]	40
8	"	"	-	[illegible]	40
9	"	"	-	[illegible]	40
10	"	"	-	20	40
11	"	20 DAYS	10	80	80
12	"	"	10	80	[illegible]
13	"	"	10	80	160
14	"	"	10	40	160
15	"	"	10	80	160
16	"	"	10	40	160
17	"	"	10	160	160
18	"	"	10	80	160
19	"	"	-	40	80
20	"	"	-	40	80
21	"	"	-	40	80
22	"	"	-	20	40
23	"	"	-	20	40
24	"	22 DAYS	10	80	160
25	"	"	-	40	80
26	"	30 DAYS	-	80	80
27	"	"	-	40	80
28	"	"	-	20	40
29	"	"	-	20	40
30	"	"	-	20	20
31	"	"	-	20	40
32	"	"	-	20	40
33	"	"	-	40	80
34	"	"	-	20	40
35	"	"	-	20	20
36	"	"	-	20	40
37	"	"	-	40	80
38	GOAT	10 DAYS	-	20	40
39	"	20 DAYS	10	160	320
40	"	"	10	160	160
41	"	"	-	80	160
42	"	"	-	40	80
43	"	30 DAYS	-	40	40

D = DIRECT AGGLUTINATION TITER
T = TUBE AGGLUTINATION TITER
AG = ANTIGLOBULIN TITER
+ = 2+