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IMMUNE MECHANISMS INDUCED BY A MYCOPLASMA IMMUNOGEN

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Summary

The research was conducted on two sheep batches, one batch was vaccinated against sheep's catching agalactie at the beginning of the experiment and 21 days after. The immune response through antibodies has been determined through ELISA, the indirect version, at the moment of the first vaccination, and than at 14, 28 and 42 days.

Test results have shown that the vaccine against this disease influences positively and meaningfully the specific and non-specific, humoral and cellular immune effectors synthesis. So, in vaccinated animals the concentration of non-specific immune effectors as well as antibodies grows following the first vaccination, amplifies after the second vaccination and reaches a maximum level 42 days after the first vaccination.

Key words: immunity, sheep, Mycoplasma

The structures of the immune system are connected into mechanisms which operate permanently and respond to all the likely pathogens agents by elaborating defense factors. Those factors can be either non-specific, characteristic for the natural resistance of the organisms, or specific, characteristic for the adaptive immune response (6, 3).

The non-specific immune defense mechanisms are achieved with the participation of the complement, properdine, lysosime, polimorphonuclear leucocytes, as well as many molecular mediators elaborated by cells.

Even if mycoplasms induce a mix immune response in the organism of the host, both cellular and humoral, the often investigated component of the immune response is represented by the humoral one. Therefore, the indirect diagnosis requires the usage of serologic tests in order to distinguish the presence of specific antibodies into the blood of infected animals (1, 2, 4, 5).

In the conducted research we have observed the effect of the vaccine against Mycoplasma agalactiae on the cellular and humoral, specific and non-specific immune response in sheep.

Materials and methods

The research has been conducted upon 20 sheep, grouped in two experimental batches. In order to make the hematological and serologic tests, blood samples from all the sheep included in the study has been collected as follows: R_1-the day when the vaccine has been administered; R_2-14 days after the

first vaccination; R_3-28 days after the first vaccination; R_4-42 days after the first vaccination.

From each have been collected two blood samples: one on anticoagulant (EDTA 1%) and the other one without anticoagulant. The blood samples have been processed in the laboratory of the Microbiology and Immunology discipline.

The seric lysosime was determinate using the radial simple diffusion in agarose gel 2% test, into which a culture of *Micrococcus lysodeicticus* has been incorporated.

Seric properdine has been measured using a method taken from the Pasteur Institute in Bucharest. The principle of the method consists of colorimetric measurement of the properdine isolated by complexing it onto inuline and treated with Biuret reactive.

From the blood samples collected on anticoagulant, there have been made microscopic preparates, stained through the May-Grunwald-Giemsa method, in order to determine the leukocyte formula.

Determination of the phagocyte index has been made opposite to a reference stem of *Staphylococcus*. For the results evaluation there has been a comparison made between the number of CFU of the initial suspension of *Staphylococcus* and the number of CFU from the mix between *Staphylococcus* and integral blood in a proportion of 1:1, after an incubation of 60 minutes at 37 Celsius degrees.

Measurements regarding the concentrations of the specific antibodies using ELISA method – the indirect version, have been conducted in the Immunology laboratories of the S.N. Pasteur S.A. Bucharest.

Results and discussions

Analyzing the evolution of the postvaccinal reaction, regarding the immunological specific and non-specific, humoral and cellular parameters, for the sheep lots taken into consideration, there have been noticed important differences from one lot to another and from one immunological parameter to another.

Regarding the seric lysosime, the biggest values have been registered for the experimental batch, vaccinated, mentioning that the maximal value of 16.07 μg/cm^3 has been registered at this lot two weeks after the second vaccination (tables 1 and 2).

Table 1

Values of the seric lysosime for the witness batch (μg/cm^3 serum)

No.	Sampling points			
	Initial level	14 days	28 days	42 days
1	13.4	13.8	14.7	14.6
2	12.0	12.9	13.9	14.2
3	12.1	12.3	14.1	15.1
4	13.8	14.5	15.6	14.3
5	12.6	13.6	15.2	12.9

6	12.2	12.8	14.9	12.9
7	13.3	14.1	13.8	14.8
8	13.6	14.5	15.9	13.7
9	12.0	13.0	15.0	13.9
10	12.1	13.6	14.8	14.5
$\bar{x}$ ±Sx	**12.71±0.23**	**13.51±0.24**	**14.79±0.22**	**14.09±0.24**
C.V.	**5.76**	**5.52**	**4.71**	**5.30**

Legend: $\bar{x}$ = arithmetic mean; Sx = standard deviation; C.V. = variability coefficient

Table 2

Values of the seric lysosime for the experimental lot (µg/cm^3 serum)

No.	Sampling points			
	Initial level	**14 days**	**28 days**	**42 days**
1	13.5	14.1	16.8	16.6
2	13.8	14.9	16.1	14.9
3	12.7	15.1	15.9	15.8
4	13.1	13.9	14.8	16.7
5	14.0	15.7	16.3	15.6
6	13.2	14.4	16.5	14.4
7	13.7	14.0	15.7	17.1
8	12.0	14.2	15.9	16.2
9	12.2	13.1	16.8	14.9
10	14.7	15.0	15.9	14.6
$\bar{x}$ ±Sx	**13.29±0.26**	**14.44±0.23**	**16.07±0.56**	**15.68±0.95**
C.V.	**6.24**	**5.16**	**3.48**	**6.09**

Legend: $\bar{x}$ = arithmetic mean; Sx = standard deviation; C.V. = variability coefficient

The properdine, as a principal factor of the non-specific resistance, develops at the two batches, through the experimental period, a characteristic evolution (table 3 and 4). While at the beginning of the experiment there are no significant differences, the values of properdine being almost the same and without individual variations (C.V.-under 5%), 28 days after the vaccination, there are noticed significant differences between batches, differences that are maintained until the end of the experimental period.

Table 3

Values of the seric properdine for the witness lot (mg/100 cm^3 serum)

No.	Sampling points			
	Initial level	**14 days**	**28 days**	**42 days**
1	15.5	16.8	17.6	18.5
2	14.8	15.5	18.5	19.2
3	16.5	17.6	18.9	20.4
4	17.6	18.9	20.8	22.8
5	16.8	18.5	19.1	23.5
6	16.1	18.2	16.9	22.6
7	15.9	17.9	20.9	20.6
8	16.2	15.5	17.5	18.2

9	16.5	15.8	18.2	19.5
10	15.7	16.3	18.9	23.7
$\bar{x}$ ±Sx	**16.16±0.24**	**17.10±0.40**	**18.73±0.38**	**20.90±0.66**
C.V.	**4.74**	**7.50**	**6.68**	**10.00**

Legend: $\bar{x}$ = arithmetic mean; Sx = standard deviation; C.V. = variability coefficient

Analyzing the dynamics of seric properdine compared with that of the lysosime, we notice that in case of properdine the values grow until the end of the experimental period, the highest values being registered 42 days after the vaccination (23.09 ± 0.49 mg/100 cm^3 serum).

Table 4

Values of the seric properdine for the experimental lot (mg/100 cm^3 serum)

No.	Sampling points			
	Initial level	**14 days**	**28 days**	**42 days**
1	16.3	18.9	19.7	22.9
2	15.1	19.3	21.4	23.5
3	15.4	15.7	18.9	21.4
4	15.9	16.8	19.8	19.8
5	16.0	16.4	20.5	24.2
6	14.9	19.7	22.4	24.6
7	15.2	19.2	22.7	23.5
8	15.8	20.1	21.8	22.9
9	16.1	15.5	18.8	25.2
10	16.3	16.4	22.9	22.9
$\bar{x}$ ±Sx	**15.70±.16**	**17.80±0.56**	**20.89±0.44**	**23.09±0.49**
C.V.	**3172.8626**	**10.07**	**7.06**	**6.78**

Legend: $\bar{x}$ = arithmetic mean; Sx = standard deviation; C.V. = variability coefficient

Regarding the cellular immune response, there has been noticed that, at a concentration of 100 µg/ml, the soluble *Mycoplasma agalactiae* antigen has stimulated the lymphocytes obtained from the inoculated batch, those from the witness batch remaining nonstimulated ($p<0.05^*$). At a concentration of 50 µg/ml, the differences between the stimulation indexes of the two lots are statistically unimportant, although at the inoculated batch the stimulation index has a greater value (5.59 ± 2.07).The phenomenon is justified given the fact that for the witness batch greater value index have been obtained opposite those obtained consecutively to the stimulation using the antigen in higher concentrations (100 µg/ml).

Results obtained after hematological investigations (leukocyte formula) are presented in table 5.

The total leucocytes number has grown for both batches considered for the experiment, the growth being progressive and much obvious for the experimental one. The biggest values have been obtained at the end of the experimental period (R_4).

While for the animals from the witness batch there hasn't been noticed a semnificative growth in the lymphocyte numbers and the ratio between lymphocytes and neutrophyles is not reversing even after the second vaccination, for the animals in the experimental batch there has been noticed an increase in the number of lymphocytes, after the first vaccination, as well as after the second antigenic stimuli, and the ratio between lymphocytes and neutrophiles is reversed.

Table 5

Structure of the leucocytes populations (medium values)

BATCH	HARVEST POINT	TOTAL LEUCOCYTES	FROM WHICH %				
			L	N	E	B	M
WITNESS	R_1	2000.2	45.30	41.44	5.51	0.36	7.33
	R_2	2625.0	47.12	39.04	6.46	0.12	7.03
	R_3	4318.1	45.29	46.17	3.54	0.37	4.88
	R_4	4702.4	46.08	45.67	4.74	0.37	3.44
EXPERIMENT	R_1	2643.0	46.96	38.57	7.93	0.22	6.41
	R_2	3409.4	50.07	35.81	7.18	0.39	6.93
	R_3	5605.1	44.96	46.01	3.79	0.38	4.96
	R_4	6138.5	50.98	39.98	4.42	0.44	4.20

The accentuated lymphocytosis in case of the animals from the experimental batch can be explained through the effects of the vaccine components, which had as a result the stimulation of the cellular proliferation. Data from table 5 is correlated with the other immunological parameters which define the immune status of the animals.

Phagocyte index has shown decreased values for the animals in the witness lot throughout the entire experimental period. Minimal values have been observed at the beginning and at the end of the experimental period (tables 6 and 7).

Table 6

The values of the phagocyte index for the witness batch.

No.	Sampling points			
	Initial level	14 days	28 days	42 days
1	18	17	25	30
2	12	19	13	28
3	21	25	19	21
4	24	41	41	19
5	15	19	38	22
6	17	24	24	18
7	12	22	26	19
8	14	18	31	24
9	22	20	24	31
10	14	24	27	17
$\bar{x}$ ±Sx	**16.9±4.25**	**22.9±6.5**	**26.7±7.8**	**20.1±7.3**
C.V.	**25.17**	**28.73**	**29.38**	**36.66**

Legend: $\bar{x}$ = arithmetic mean; Sx = standard deviation; C.V. = variability coefficient

Data obtained regarding the phagocyte index is correlated with the results of the leukocyte formula, specifying that, in case of the leukocyte formula, the maximal values registered at the second gathering of blood samples, maintain their values until the end of the experiment.

Table 7

Values of the phagocyte index for the experimental lot (%)

No.	Sampling points			
	Initial level	14 days	28 days	42 days
1	24	40	35	28
2	21	25	34	24
3	12	30	41	39
4	17	28	40	42
5	15	26	37	30
6	22	41	44	28
7	19	21	42	31
8	14	37	39	32
9	21	31	29	30
10	15	22	43	28
$\bar{x}$ ±Sx	**18.0±3.9**	**30.1±7.2**	**38.4±4.7**	**31.2±5.4**
C.V.	**22.06**	**23.77**	**12.16**	**17.34**

Legend: $\bar{x}$ = arithmetic mean; Sx = standard deviation; C.V. = variability coefficient

Antibodies concentration, expressed through optical density units using a multiphase spectrophotometer, has grown progressively for all the animals, from the witness batch as well as those from the experimental batch, increased values were registered for the lot which was vaccinated (table 8).

Table 8

Concentration of antibodies for the vaccinated lot (O. D.)

No.	Sampling points			
	Initial level	14 days	28 days	42 days
1	201	391	294	935
2	202	385	323	897
3	227	302	433	944
4	310	300	315	413
5	200	279	584	1196
6	271	392	460	916
7	174	320	657	877
8	258	691	650	1087
9	170	512	526	905
10	224	397	505	953
$\bar{x}$ ±Sx	**223.7±14.05**	**396.9±39.17**	**474.7±42.50**	**912.3±63.68**
C.V.	**19.86**	**31.21**	**28.31**	**22.07**

Legend: $\bar{x}$ = arithmetic mean; Sx = standard deviation; C.V. = variability coefficient

The highest medium value (912.3 ± 63.68 optical density units) has been registered for the experimental lot 28 days after the first vaccination, in succession of the second antigenic stimuli.

Compared with the experimental batch, in the witness batch which did not receive the vaccine, the values were lower. Therefore, 14 days after the second vaccination, the difference between the witness and experimental batch has been of 225.7 O.D. units.

Taking into consideration the superior results for the batch where the vaccine was administered, regarding the increase of antibodies concentration, as well as the growth in lymphocyte numbers and in the concentration of non-specific humoral effectors, we consider that the vaccine stimulates general immune reactivity in sheep.

Conclusions

- The vaccine against contagious agalactie in sheep has induced in the immunized sheep a specific and non-specific, humoral and cellular response, which lasts for at least two months.
- The vaccine stimulates the synthesis of lysosime and seric properdine, but the growth is unimportant.
- Hematological tests have shown progressive growth in total leucocytes, maximal values have been registered for sheep belonging to the experimental batch.
- Anti-Mycoplasma vaccine has induced in sheep an immune response, primary and secondary, through antibodies, for all the animals included in this study. The level of antibodies increases after the first immunization (primary immune response), it amplifies after the second immunization (secondary immune response) and reaches the highest levels 42 days after the first vaccination.
- Relaying on the data obtained, we recommend the revaccination of animals four months after the last immunization in order to stimulate immune reactivity.

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