

## BIOCHEMICAL PARAMETERS OF BLOOD SERA OF SHEEP VACCINATED AGAINST CONTAGIOUS AGALACTIA

Stegniy B. T. <sup>1</sup>, Bohach D. M. <sup>1</sup>, Vovk D. V. <sup>1</sup>, Bohach M. V. <sup>2</sup>

<sup>1</sup> National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: bogachdenis1@gmail.com

<sup>2</sup> Odesa Experimental Station of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Odesa, Ukraine

**Summary.** The paper presents data on the positive effect of the inactivated vaccine against contagious agalactia of sheep and goats developed in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine) on the biochemical and immunological parameters of sheep blood serum. It has been proved that the vaccine is not reactogenic, does not have immunosuppressive action and corrects the recovery of serum albumin concentration in vaccinated sheep, namely by reducing  $\alpha$ -globulins by 28.5% and  $\beta$ -globulins by 36.8% and has a positive effect on the growth of  $\gamma$ -globulin by 31.5%, activating the humoral level of immunity

**Keywords:** contagious agalactia, vaccine, biochemical parameters, albumins, globulins

**Introduction.** Improvement of existing and development of new means for the specific prevention of infectious diseases is an extremely important task for veterinary science. No less important is finding ways to use vaccines against pathogenic agents from local strains and creating inactivated vaccines. The advantage of such vaccines is the creation of immunity in animals to diseases in the short term, depending on the epizootic situation in the farm, district or region (Ryzhenko et al., 2010; Kosenko and Liubenko, 2001).

In Ukraine, contagious agalactia is recorded only in some districts of the Odesa region, but due to the active development of the sheep industry, the disease can spread to other regions. That is why the directions for studying of epizooty and the development of specific means for prevention are relevant (Stegniy et al., 2018).

Research and correction of the state of general resistance of animals will allow realizing potential, genetically conditioned possibilities of an organism (Perederiy et al., 1995).

The study of immunological parameters is a key point for the detection of immunodeficiency and immunopathological state, primary assessment of the immune state of the organism, as well as in the diagnosis, treatment and prognosis of the disease (Tymoshenko, Maslak and Maslak, 2009; Petrov and Lebedev, 1984).

The level of concentration of total immunoglobulins in the blood serum of animals is a criterion for evaluating the functional activity of the B-cell link of immune system, which allows to determine objectively the state of the immune status of animals (Kosenko et al., 2004).

**The purpose of the work** was to determine the effect of an inactivated vaccine against contagious agalactia of sheep and goats developed in NSC 'IECVM' on the biochemical and immunological parameters of sheep blood serum.

**Materials and methods.** The experimental part of the work was carried out in the conditions of a sheep farm PE 'Borlak' (v. Dmitrivka, Bolgrad district, Odesa region). Two groups of sheep ( $n = 15$ ) were formed in the farm on the principle of analogues, for which the same conditions of feeding, care and keeping were provided.

In the experimental group, sheep were vaccinated with an inactivated vaccine against contagious agalactia of sheep and goats developed in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (NSC 'IECVM'). The vaccine was used twice subcutaneously in the tail fold at a dose of 1 cm<sup>3</sup> with an interval of 30 days.

Control of the vaccine effectiveness was carried out by biochemical and immunological parameters of the blood. For research, blood samples were collected from sheep before vaccination, on the 14<sup>th</sup>, 21<sup>st</sup>, and 30<sup>th</sup> days after vaccination and on the 60<sup>th</sup> day — revaccination.

Biochemical parameters of sheep blood serum reflecting the functional state of the liver were determined using an automatic biochemical analyzer 'IDEXX VestTest' ('IDEXX Laboratories', USA). Additionally, blood samples were tested for circulating immune complexes (Grinevich and Alferov, 1981) and seromucoids (Weimer and Moshin, 1953) in the Laboratory of Biochemistry of the NSC 'IECVM' (Kharkiv, Ukraine).

**Results and discussion.** The state of protein metabolism in the body of animals indicates the nature of biochemical processes that occur in the body under a certain antigenic load.

It was established that inactivated vaccine against contagious agalactia of sheep and goats (NSC 'IECVM') influences and corrects protein metabolism in the body of vaccinated animals that is shown by the obtained results (Table).

**Table** — Biochemical parameters of blood sera of sheep inoculated with an inactivated vaccine against contagious agalactia of sheep and goats (NSC 'IECVM') (n = 15, M ± m)

Parameters	Research period, days	Groups	
		experimental	control
Total protein, g/l	b/v	72.18 ± 2.08	71.95 ± 2.06
	14	72.25 ± 1.04	70.21 ± 2.72
	21	72.98 ± 0.96	72.20 ± 1.09
	30	72.76 ± 2.12	71.39 ± 1.54
	60	73.31 ± 0.90	71.20 ± 2.13
Albumins, g/l	b/v	31.58 ± 2.21	31.14 ± 4.41
	14	33.15 ± 1.82	31.01 ± 2.17
	21	36.39 ± 0.75	32.22 ± 1.96
	30	36.91 ± 2.14	31.85 ± 2.23
	60	36.91 ± 2.09	32.29 ± 1.16
α-globulins, g/l	b/v	13.57 ± 1.12	13.98 ± 2.92
	14	11.96 ± 0.72	13.06 ± 1.73
	21	10.65 ± 2.17	13.72 ± 0.55
	30	9.95 ± 1.92	13.91 ± 2.17
	60	10.06 ± 0.34	13.09 ± 1.24
β-globulins, g/l	b/v	10.75 ± 0.56	10.92 ± 2.02
	14	10.16 ± 1.29	11.72 ± 0.98
	21	7.52 ± 0.48	11.39 ± 1.17
	30	7.22 ± 2.12	11.42 ± 2.62
	60	7.32 ± 0.19	11.05 ± 1.09
γ-globulins, g/l	b/v	16.28 ± 0.53	15.91 ± 2.01
	14	16.98 ± 1.81	15.02 ± 1.01
	21	18.42 ± 2.04	14.87 ± 0.94
	30	18.68 ± 0.16	14.21 ± 1.04
	60	19.02 ± 1.32	14.77 ± 2.07
A/G ratio	b/v	0.8	0.8
	14	0.9	0.8
	21	1.0	0.8
	30	1.0	0.8
	60	1.0	0.8
CIC, mg/ml	b/v	0.25 ± 0.03	0.25 ± 0.01
	14	0.26 ± 0.01	0.25 ± 0.01
	21	0.28 ± 0.02	0.24 ± 0.02
	30	0.29 ± 0.02	0.24 ± 0.02
	60	0.29 ± 0.01	0.24 ± 0.02
Sero-mucoids, mg/ml	b/v	0.26 ± 0.01	0.26 ± 0.01
	14	0.27 ± 0.02	0.26 ± 0.01
	21	0.26 ± 0.01	0.27 ± 0.02
	30	0.26 ± 0.01	0.27 ± 0.02
	60	0.26 ± 0.01	0.27 ± 0.02

Notes: b/v — before vaccination; \* —  $p < 0.05$ , \*\* —  $p < 0.01$ , \*\*\* —  $p < 0.001$  compared to the control group.

According to the biochemical parameters of sheep blood serum, the albumin fraction in the experimental and control groups was almost equal —  $31.58 \pm 2.21$  and  $31.14 \pm 4.41$  g/l, respectively. After vaccination with the

vaccine in the experimental group on the 21<sup>st</sup> day, the content of albumin increased by 12.9% and amounted to  $36.39 \pm 0.75$  against  $32.22 \pm 1.96$  g/l in control. On the 30<sup>th</sup> day after vaccination, the indicator increased by 15.9% against control and was almost at this level —  $36.91 \pm 2.09$  g/l until the 60<sup>th</sup> day of the experiment — after revaccination, which indicates stabilization of homeostasis of sheep organism.

According to the results of the studies, the quantitative content of globulin fractions of proteins, namely α-, β- and γ-globulins before vaccination in the experimental and control groups was almost at the same level with a difference of no more than 1.2%. In the experimental group on the 14<sup>th</sup> day after vaccination, the content of α-globulins decreased by 8.4% and amounted to  $11.96 \pm 0.72$  against  $13.06 \pm 1.73$  g/l in the control. On the 21<sup>st</sup> day, the indicator decreased by 22.4%, that is to  $10.65 \pm 2.17$  against  $13.72 \pm 0.55$  g/l in control. On the 30<sup>th</sup> day of the experiment, the lowest index of α-globulin content was reached —  $9.95 \pm 1.92$  g/l, which is 28.5% less than in the control. Significantly decreased β-globulin content on the 21<sup>st</sup> day after vaccination by 34% and on the 30<sup>th</sup> day by 36.8%, that is  $7.22 \pm 2.12$  against  $11.42 \pm 2.62$  g/l in control. No significant fluctuations were observed after revaccination on the 60<sup>th</sup> day. The content of γ-globulins before vaccination in the experimental group of sheep was  $16.28 \pm 0.53$  g/l, and in the control  $15.91 \pm 2.01$  g/l. Significant increase of the indicator in the experimental group occurred on the 21<sup>st</sup> day, by 23.9%, compared with the control, and on the 30<sup>th</sup> day the content of γ-globulins increased by 31.5% and amounted to  $18.68 \pm 0.16$  against  $14.21 \pm 1.04$  g/l in control. After revaccination, on the 60<sup>th</sup> day the content of γ-globulins increased to  $19.02 \pm 1.32$  against  $14.77 \pm 2.07$  g/l in control, which is 28.8% more.

Based on the analysis of the results of the studies, it was found that hypergammaglobulinemia is the result of increased synthesis of immunoglobulins of all classes. During all immunological reactions, especially the γ-globulin fraction of the protein tends to increase, namely when vaccinated with an inactivated vaccine against contagious agalactia in sheep and goats. It is known that the bulk of the antibodies contain precisely γ-globulins, which provide humoral protection of the body. Increasing their number in the blood serum characterizes the morphological maturity and functional completeness of the body's immune reactive system.

The increase in albumin content on the 21<sup>st</sup> day after vaccination influenced the formation of A/G coefficient which in the experimental group was 1.0. It should be noted that in clinically healthy animals it is 0.8-1.0.

Certain immunosuppressive effect of the vaccine on the body of sheep was evaluated by the level of seromucoids. The preparation did not cause immunosuppressive effects on the body of sheep after vaccination and revaccination.

Seromucoid level in the test and control animals remained almost unchanged and was from  $0.26 \pm 0.01$  to  $0.27 \pm 0.02$  mg/ml during the study period.

The administration of the vaccine against contagious agalactia of sheep and goats (NSC 'IECVM') increases the content of total protein on the 30<sup>th</sup> day by 1.9%, and by 3% on the 60<sup>th</sup> day, and circulating immune complexes — by 20.8% on the 30<sup>th</sup> and 60<sup>th</sup> days.

**Conclusion.** The vaccine against contagious agalactia of sheep and goats is not reactogenic, does not have immunosuppressive action and corrects the recovery of albumin concentration in serum of vaccinated sheep, namely by reducing  $\alpha$ -globulins by 28.5% and  $\beta$ -globulins by 36.8% and has a positive effect on increasing  $\gamma$ -globulins by 31.5%, activating the humoral level of immunity.

#### References

- Grinevich, Yu. A. and Alferov, A. I. (1981) 'Determination of immune complexes in the blood of cancer patients' [Opredelenie immunnykh kompleksov v krovi onkologicheskikh bol'nykh], *Laboratory Science [Laboratornoe delo]*, 8, pp. 493–495. [in Russian].
- Kosenko, M. V. and Liubenko, Ya. M. (2001) 'Immunological preparations in veterinary practice' [Imunolohichni preparaty u veterynarnii praktytsii], *Veterinary Medicine of Ukraine [Veterynarna medytsyna Ukrainy]*, 2, pp. 22–23. [in Ukrainian].
- Kosenko, M. V., Kotsiumbas, I. Ya., Kosenko, Yu. M., Datskiv, O. M. and Lisova, N. E. (2004) 'Control of the influence of veterinary medicines on the state of animals' immunity' [Kontrol vplyvu veterynarnykh likarskykh zasobiv na stan imunitetu tvaryn], *Veterinary Medicine of Ukraine [Veterynarna medytsyna Ukrainy]*, 1, pp. 43–44. [in Ukrainian].
- Perederiy, V. G., Zemskov, A. M., Bychkova, N. G. and Zemskov, V. M. (1995) *Immune Status, Principles of Its Evaluation and Correction of Immune Disorders [Immunnyy status, printsipy ego otsenki i korrektsii immunnykh narusheniy]*. Kyiv: Zdorov'e. ISBN 5311008547. [in Russian].
- Petrov, R. V. and Lebedev, K. A. (1984) 'Diagnostics of immunopathological conditions based on evaluation of the balance in the functioning of the immune system components' [Diagnostika immunopatologicheskikh sostoyaniy na osnovanii otsenki balansu v funktsionirovanii komponentov immunnyy sistemy], *Immunology [Immunologiya]*, 5(6), pp. 38–43. [in Russian].
- Ryzhenko, V. P., Ryzhenko, G. F., Gorbatyuk, O. I., Andriyashchuk, V. A., Belik, S. N., Zhovnir, A. M. and Yushchenko, M. S. (2010) 'Haematological and biochemical indexes of peripheral blood of sheep graft simultaneously against Necrobacteriosis, Colibacteriosis and Salmonellosis' [Hematolohichni ta biokhimichni pokaznyky peryferychnoi krovi ovets, shcheplynykh odnochasno proty nekrobakteriozu, kolibakteriozu i salmonelozu], *The Animal Biology [Biolojiivna tvaryn]*, 12(2), pp. 323–328. Available at: [http://nbuv.gov.ua/UJRN/bitv\\_2010\\_12\\_2\\_53](http://nbuv.gov.ua/UJRN/bitv_2010_12_2_53). [in Ukrainian].
- Stegniy, B. T., Rula, O. M., Muzyka, D. V., Bogach, D. M. and Maiboroda, O. V. (2018) 'Development of the technology for manufacturing domestic vaccine against Contagious agalactia of sheep and goats' [Rozrobka tekhnolohii vyhotovlennia vitchyznianoj vaksyny proty infektsiinoi ahalaktii ovets i kiz], *Veterinary Biotechnology [Veterynarna biotekhnolohiia]*, 32(1), pp. 272–277. Available at: [http://nbuv.gov.ua/UJRN/vbtb\\_2018\\_32\(1\)\\_37](http://nbuv.gov.ua/UJRN/vbtb_2018_32(1)_37). [in Ukrainian].
- Tymoshenko, O. P., Maslak, Yu. V. and Maslak, M. V. (2009) 'The informative biochemical indices of blood serum and urine of goats during different season' [Biokhimichni pokaznyky syrovatky krovi ta sechi kiz u rizni pory roku], *News of Poltava State Agrarian Academy [Visnyk Poltavskoi derzhavnoi ahrarnoi akademii]*, 2, pp. 63–65. Available at: [https://www.pdaa.edu.ua/sites/default/files/visnyk/2009/02/4\\_vet\\_med\\_2\\_2009.pdf](https://www.pdaa.edu.ua/sites/default/files/visnyk/2009/02/4_vet_med_2_2009.pdf). [in Ukrainian].
- Weimer, H. E. and Moshin, J. R. (1953) 'Serum glycoprotein concentrations in experimental tuberculosis of Guinea pigs', *American Review of Tuberculosis*, 68(4), pp. 594–602. Available at: <https://www.atsjournals.org/doi/10.1164/art.1953.68.4.594>.