Study of immunomodulating effects of *Streptococcus pyogenes* B-7612 bacteria on cellular and humoral immunity of pets

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Abstract. For the first time, a study was conducted of the effect of living bacteria of the *Streptococcus pyogenes* avirulent strain B-7612, on the cellular and humoral immunity indicators of domestic animals (dogs) with signs of immunodeficiency. Lyophilizates of live bacteria was administered intradermally to dogs according to the scheme of 0,02-0,04 ml. for 25 days with an interval of 5 days. At the beginning of the experiment and for every 5 days, all animals were sampled to determine immunity parameters: the number of formed elements (leukocytes, leukocyte formula), protein, albumin, globulin, phagocytic number, phagocytic index, phagocytic activity, bactericidal activity of blood serum, lysozyme activity of blood serum. Blood was taken using blood collection systems. Streptococcus pyogenes strain B-7612 has been found to stimulate the cellular immunity, resulting in an increase in the total number of leukocytes (granulocytes, monocytes and lymphocytes), activation of neutrophilic phagocytic activity of the blood, with simultaneous growth of phagocytic index and phagocytic number, the activity of humoral factors is activated, which indicates an increase in the natural stability of the immune system. In the blood of experimental animals there were no significant changes in the amount of total protein, albumin and globulins compared with the control.

1 Introduction

A promising area is the search for effective immunotherapeutic drugs. Experimental and clinical studies have shown that the development of immunotherapeutic biologics based on bacteria and viruses for the treatment of immunodeficiencies and oncological diseases is promising, due to their possible high efficiency and sufficient safety. Among the variety of bacteria, non-pathogenic or attenuated strains of *Lactobacillus sp., Bifidobacterium sp., Micobacterium bovis, Listeria monocytogenes, Salmonella typhimurium, Clostridium novyi-NT, and Streptococcus pyogenes* are available (Forbes et al., 2018; Kramer et al., 2018; Torres et al., 2018; Lukasiewicz and Fol 2018).

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Bacteria and viruses are immunopotentiators or biological response modifiers that are often used as immunomodulators, to reduce the risk of cancer relapse or increase the effectiveness of chemotherapy and radiotherapy, and reduce side effects, but they can also be used to prevent infectious complications of immunodeficiency conditions (MacEwen 1990).

With regard to immunostimulatory, antiviral and anti-cancer mechanisms, *Streptococcus pyogenes* is well known, since these bacteria are able to act on the adaptive and innate parts of the immune system, enhance the antitumor and antiviral activity of the immune system, and also directly suppress tumor growth. This opens up the possibility of creating new immunotherapeutic drugs. *Streptococcus pyogenes* produces a number of substances: streptolysin S, arginine deiminase, streptokinase, cell wall components that exhibit complex biological activity, as demonstrated in in vitro and in vivo studies, experiments on laboratory animals, and the clinic trials (Russia, Japan, Germany, USA, etc.). In particular, it was shown that injections of this type of bacteria can lead to complete regression of some types of cancer. At the same time, the action of live bacteria is higher (Chereshnev and Morova 2006; Maletzki et. al., 2008; Suvorova, 2017).

Studies at the Perm Medical Institute showed high titers of antistreptokinase and antistreptolysin-O in the group of healthy people, and low titers in the group of patients with a confirmed diagnosis of neoplasms. The authors suggest that the causes of malignant tumors may be associated with the disappearance of non-pathogenic strains of *Streptococcus pyogenes*, which had an oncoprotective effect. For more than 20 years for treating thrombosis, cancer and different viral diseases at the Perm Medical Institute (Russia), a non-pathogenic live culture of bacteria Streptococcus pyogenes strain Gurov (isolated in 1938) used, which was introduced at a certain concentration and dose intradermally in the forearm. Given the pronounced antitumor, antiviral and thrombotic effect with almost complete absence of side effects, A.A. Morova and V.A. Chereshnev proposed a hypothesis according to which, in the course of a long evolutionary symbiotic relationship between group A streptococci and a person, it became possible not only to preserve long asymptomatic carriage of bacteria, but also to maintain homeostasis in the human body due to the action of certain bacterial enzymes, which prevented the formation of malignant neoplasms, blood clots and pathogenic viruses (Chereshnev and Morova 2006).

A similar hypothesis was proposed by Okamoto et al. To explain the experimentally reported antitumor activity of *Streptococcus pyogenes* and the streptolysin S produced by them. The authors note that a long history of the coexistence of people and Streptococcus pyogenes could lead to the formation of mutually beneficial (mutualistic) relationships. Destruction of tumor cells can occur due to non-pathogenic strains of *Streptococcus pyogenes* that are normally present in the human body, which are capable of producing streptolysin-S. Once in the bloodstream, the cells of these bacteria and / or their anti-cancer factor make the normal tissue of the human body a very unfavorable place for the attachment and activity of cancer cells. Thus, strains of Streptococcus pyogenes that produce streptolysin-S can be natural inhibitors of cancer in humans (Okamoto et al., 1967).

Recently, the complete genome of the *Streptococcus pyogenes* GUR strain (Gurov) strain genome type emm111 and a derivative of the GURSA1 strain with the inactivated emm gene were sequenced. The GURSA1 strain showed higher anticancer activity on cancer cell cultures and on vaccine tumor models compared to the original GUR strain (Suvorova et al., 2017).

Thus, it can be concluded that bacteria of the *Streptococcus pyogenes* species produce a whole range of substances, some of which are activators of human and animal cellular and

humoral immunity, while others inhibit the growth of cancer cells, as shown in in vitro and in vivo studies. (Yang et al., 2006; Bobek et al., 2006; Maletzki et. Al., 2008).

The development of a veterinary immunomodulating drug based on a non-pathogenic strain of *Streptococcus pyogenes* is promising. A drug based on these bacteria can be an immunomodulator for the treatment of immunodeficiency in domestic and farm animals. This drug has potential antiviral and anticancer properties due to the above-described mechanisms of antitumor, antiviral activity of bacteria *Streptococcus pyogenes*.

A new non-pathogenic strain of *Streptococcus pyogenes* strain B-7612 was found by Kvadro-Biotech LLC. Preliminary tests showed its harmlessness for laboratory and farm animals and it potential antiviral, thrombolytic and anticancer effects in laboratory animals, dogs and volunteers.

The purpose of this study: to study the immunomodulatory effect of live bacteria of the *Streptococcus pyogenes* B-7612 strain on the body of dogs.

For this, the following tasks were set:

- To study the effect of the strain of *Streptococcus pyogenes* B-7612 on the cellular link of the immunity of dogs.
- To study the effect of the *Streptococcus pyogenes* B-7612 strain on the humoral link of dog immunity.

2 Materials and methods

We used a lyophilized culture of live bacteria, strain Streptococcus pyogenes B-7612 in 0.5 ml ampoules (RF patent No. 2658606, RF patent No. 2624068). Strain *Streptococcus pyogenes* B-7612 has been deposited in the State Committee for the Ministry of Food Industry-Obolensk (Federal Budgetary Institution of Science, State Scientific Center for Applied Microbiology and Biotechnology).

The work was carried out on the basis of the laboratory of diseases of young animals of the Siberian Federal Scientific Center of Agrobiotechnology of the Russian Academy of Sciences and a shelter for homeless animals in Krasnoobsk (Address: 630501 Novosibirsk Region, Krasnoobsk, PO Box 8.).

In the experiment, mongrel dogs were used at the age of 2-3 years, weighing 10-20 kg, with signs of immunodeficiency states, divided according to the analogy principle into 2 groups (n = 10).

All dogs were kept in a shelter for stray animals in Krasnoobsk, in open-air cages for 5 heads. Each animal was assigned an individual serial number (in accordance with the group number and serial number).

The basic rules for keeping and caring for experimental animals corresponded to the "Sanitary Rules for the Maintenance of Experimental Biological Clinics (Vivariums)" and orders "On measures to further improve organizational forms of work using experimental animals" and "On amendments to the order of the USSR Ministry of Health No. 775 dated 12.08.77 ".

The following conditions were maintained in the enclosures: ambient temperature 5 - 10 ^{o}C; light mode of natural lighting; relative air humidity 30 - 70%;

Watering of animals was carried out using semi-automatic drinkers. As the main ration, we used ready-made pelleted feeds from Kiteket.

Before the start of all experiments, the animals were kept in quarantine for 14 days in accordance with the "Requirements for the treatment of animals" GOST R ISO 10993-2-2009 (Order of Rostekhregulirovanie dated 03.09.2009 No. 315-st).

During this period, the animals were monitored for signs of abnormal health. A detailed clinical examination was carried out before the formation of the groups.

The animals of the experimental group were injected intradermally with 0.5 ml of saline diluted with a lyophilisate of live bacteria *Streptococcus pyogenes* B-7612 in doses of 0.02-0.04 ml. Animals of the control group were injected with 0.9% "Isotonic sodium chloride" in similar dosages.

At the beginning of the experiment and every 5 days, blood samples were taken from all animals to determine the parameters of immunity: the number blood cells (leukocytes, leukocyte formula), protein, albumin, globulins, opsonophagocytic reaction, bactericidal activity of blood serum, lysozyme activity of blood serum. Blood was collected using blood collection systems.

The counting of blood cells was performed on a veterinary semi-automatic hematological analyzer "Mindray BC-2800 Vet".

The dynamics of changes in blood biochemical parameters was studied on a biochemical analyzer "Erba Mannheim", using kits for biochemical studies produced by CJSC "Vector-Best" and LLC "Olveks-diagnosticum".

Investigated the phagocytic activity of neutrophils. Changes in the phagocytic index, phagocytic activity, and phagocytic number were assessed (Dobrynya et al., 2017).

The dynamics of the bactericidal activity of blood serum (BABS) and lysozyme activity of blood serum (LABS) was assessed using the nephelometric method (Dobrynya et al., 2017; Saruhanov et al., 2012).

Determination of protein fractions of blood serum was carried out by the turbidimetric method (Kondrahin et al., 1985).

Results are presented as mean and SE (x ± SE, n = 5). The significance of differences between the samples was assessed by the ANOVA method and the Mann-Whitney U-test, P = 0.05. * - denotes a significant difference from the control variant.

3 Results and Discussion

The criteria for assessing the effect of the *Streptococcus pyogenes* B-7612 strain on blood parameters characterizing the work of the cellular component of immunity were the dynamics of the leukocyte formula of dogs in the experimental and control groups, as well as the change in the opsonophagocytic reaction (OCR) parameters during the entire experiment.

The results of the dynamics of the total number of leukocytes in blood samples of experimental and control animals are presented in Table 1.

Table 1. Dynamics of the number of leukocytes (%), x ± SE, n = 5.

Groups	Duration of experiment (days)					
	1	**5**	**10**	**15**	**20**	**25**
Experimental	5.19±1.2	7.41±2.3	5.21±0.4	10.31±0.9 [a]*	13.64±2.8[a]*	14.32±3.1 [a]*
Control	4.82±0.67	5.37±1.8	5.44±0.8	6.07±1.1	5.3±0.5	6.73±1.7
norma	5.5-18.5*10^9/л					

By the 5th day, there is an insignificant increase in the total number of leukocytes after the first injection of *Streptococcus pyogenes* B-7612 (Table 1); by the 10th day of the experiment, their number decreases, not differing from the same indicator of the control group, and then significantly increases by the 15-25th day compared with the control and the initial value in the experimental group (Table 1). It should be noted that a similar increase in the number of leukocytes was recorded in mice with grafted tumors after injection of live *Streptococcus pyogenes* strain 591 (Maletzki et al., 2008).

This situation is also revealed with a more detailed examination of the leukocyte formula. Throughout the experiment, the percentage of granulocytes (neutrophils and basophils) increased and remained elevated until the end of the experiment (Table 2).

Table 2. Dynamics of the number of granulocytes (%), x ± SE, n = 5.

Groups	Duration of experiment (days)					
	1	**5**	**10**	**15**	**20**	**25**
Experimental	45.57±15.7	65.23±8.3	71.84±16.1	78.63±5.8	75.32±9.1	64.57±11.2
control	58.94±28.9	60.18±13.4	76.41±12.2	65.44±15.0	77.05±10.3	71.86±7.7
norma	35.0-78.0 %					

A somewhat different picture can be noticed in relation to the dynamics of the number of monocytes (Table 2).

Table 3. Dynamics of the number of monocytes (%), x ± SE, n = 5.

Groups	Duration of experiment (days)					
	1	**5**	**10**	**15**	**20**	**25**
Experimental	1.18±0.3	1.46±0.2	1.9±0.4	2.74±1.5	1.03±0.2	2.53±0.9
Control	2.95±1.0	1.74±0.5	2.2±0.7	1.14±0.1	2.82±0.6	1.37±0.4
norma	1.0-5.0 %					

By the middle of the course of application of *Streptococcus pyogenes* B-7612, at the end of the study, the number of monocytes in the blood samples of the experimental group of animals increases. But there were no significant differences from the control group. Granulocytes, monocytes, and macrophages are the effectors of innate immunity, and they are the first to respond to the introduction of bacteria, as demonstrated in a study in mice (Maletzki et al., 2008).

On the 10th day of the experiment, a significant increase in the number of lymphocytes is observed. By the 15th day of the experiment, the number of lymphocytes in the blood of the animals of the experimental group continues to decrease, and by the 20-25th day it again significantly increases. No significant differences in eosinophils were found between the control and experimental groups, but in the experimental group there was a tendency towards a decrease in the level of eosinophils by the end of the experiment (Tables 4 and 5).

Table 4. Dynamics of the number of lymphocytes (%), x ± SE, n = 5.

Groups	Experiment duration (days)					
	1	**5**	**10**	**15**	**20**	**25**
Experimental	10.31±4.1	13.80±4.2	20.93±3.0 [a]*	15.72±1.4	23.49±4.7 [a]*	25.18±5.1 [a]*
Control	12.58±2.5	10.32±1.3	13.36±1.9	16.27±3.2	12.93±2.6	15.42±3.8
Norma range	13.0-30.0 %					

Table 5. Dynamics of the number of eosinophils (%), x ± SE, n = 5.

Groups	Experiment duration (days)					
	1	**5**	**10**	**15**	**20**	**25**
Experimental	4.41±2.0	3.21±1.7	2.58±0.8	2.8±0.4	1.4±0.3	1.9±0.5
Control	3.75±1.4	3.96±2.1	2.64±1.3	3.51±0.5	4.7±1.1	3.68±1.6
Norma range	0-5.0 %					

According to the results of studies of the leukocyte picture, it can be concluded that the use of *Streptococcus pyogenes* B-7612 has a rather pronounced stimulating effect on leukopoiesis, due to an increase in lymphocytic and granulocytic fractions.

When assessing the effect of the strain *Streptococcus pyogenes* B-7612 on the parameters of phagocytosis, the following results were obtained (Table 6).

Table 6. Indicators of the opsonophagocytic reaction of animals in the experiment, x ± SE, n = 5.

Days	Phagocytic index, %	Phagocytic number	Phagocytic activity
Experiment			
1	40±1.3	2.35±0.63	0.25±0.02
5	46.1±1.28	3.11±0.17	0.28±0.01
10	51.5±1.83	3.03±0.29	0.39±0.03
15	67.5±1.89 [a]*	4.18±1.41	0.36±0.02
20	78.4±1.73 [a]*	3.64±0.30 [a]*	0.57±0.04 [a]*
25	85±1.74 [a]*	5.13±0.77 [a]*	0.81±0.05 [a]*
Control			
1	36.0±2.57	2.00±0.10	0.23±0.01
5	38.3±1.44	2.15±0.15	0.15±0.01
10	27.1±2.84	3.40±0.39	0.30±0.02
15	43.2±3.58	2.95±0.52	0.29±0.03
20	39.6±2.14	2.14±0.31	0.28±0.04
25	44.9±4.72	1.97±0.10	0.19±0.01
Norma	65-95	5-10	0.1-1

As can be seen from the data presented, the phagocytic blood index of the dogs of the experimental group significantly increased by the 25th day in comparison with the control. This indicator reflects the percentage of active leukocytes involved in phagocytosis to the total number of counted neutrophilic leukocytes.

It should be noted that this indicator begins to increase already on the 10th day of the experiment. Further, the positive dynamics of changes in the phagocytic index of neutrophils was traced throughout the experiment.

Phagocytic number - the average number of phagocytosed bacteria per one active leukocyte. In the experimental group, positive dynamics was observed throughout the experience, while in the control group there was a certain downward trend.

The results obtained indicate that the *Streptococcus pyogenes* B-7612 strain activates the phagocytic activity of neutrophils and increases their absorption capacity.

Also, in the experimental group, there is an increase in phagocytic activity, which is a criterion for assessing the digestive ability of neutrophils.

It was found that by the 25th day of the experiment, this indicator in the animals of the experimental group increases significantly, while in the control, its fluctuations are practically not traced.

Analyzing the results obtained, it can be concluded that the strain *Streptococcus pyogenes* B-7612 has a stimulating effect on the cellular link of the immunity of dogs, expressed in an increase in the total number of leukocytes (mainly granulocytes, monocytes and lymphocytes), activation of the phagocytic activity of blood neutrophils, with a simultaneous increase in the phagocytic index and phagocytic number (the number of absorbed microbial cells).

The effect of the *Streptococcus pyogenes* B-7612 strain on the humoral link of immunity was assessed by the dynamics of serum bactericidal activity (BABS), serum

lysozyme activity (LABS) and the number of globulin fractions in the blood serum at the beginning and end of the experiment (Tables 7 and 8).

Table 7. Dynamics of bactericidal activity of serum (%), x ± SE, n = 5.

Groups	Duration of experiment (days)					
	1	5	10	15	20	25
Experimental	42.07±4.0	48.41±5.8	63.10±7.9	80.89±5.5 [a]*	77.26±8.1	86.54±10.4 [a]*
Control	34.66±5.1	40.3±7.2	41.98±6.0	47.05±4.7	38.9±2.5	43.3±5.5

On the first day of research, the BABS indicator in the experimental group was lower than the control value. Moreover, in both groups, this indicator did not exceed the lower limit of the norm (70%), which indicates a low nonspecific immunity of animals. However, by the 5th day of the experiment, an increase in BABS was noted in the experimental group. By 15-25 days, the growth of BABS in the experiment exceeded 70%.

At the same time, in the control group by the 15th day, there was a slight increase in bactericidal activity and then a decrease by the 25th day.

A similar trend can be noticed when studying the effect of *Streptococcus pyogenes* B-7612 on the lysozyme activity of blood serum.

Table 8. Dynamics of serum lysozyme activity (%), x ± SE, n = 5.

Groups	Duration of experiment (days)					
	1	5	10	15	20	25
Experimental	25.03±2.5	29.14±3.9	37.73±4.1	50.36±5.0 [a]*	47.30±3.8 [a]*	64.12±8.2 [a]*
Control	21.87±1.8	15.96±2.1	32.63±0.4	24.52±1.2	28.83±2.3	19.10±0.7

On the first day of the study, the indicator of lysozyme activity of blood serum (hereinafter, LABS) of the experimental and control groups were approximately at the same level. The increase in the LABS level occurs throughout the experiment in the experimental group. The peak of LABS growth is observed in the experimental group by the 25th day.

On the 10th day of the experiment, the level of LABS in the control group increased to decrease, but then a decrease followed by 15-25 days of the experiment.

Thus, based on the data presented, we can conclude that the *Streptococcus pyogenes* B-7612 strain stimulates an increase in the natural resistance of the immune system.

The results of studying the effect of *Streptococcus pyogenes* B-7612 on the protein composition of blood serum are presented in Tables 9 and 10.

Table 9. Indicators of the protein composition of the blood serum of the animals of the experimental group (g / l), x ± SE, n = 5.

	Duration of experiment (days)					
	1	5	10	15	20	25
Total protein	65.6±4.1	68.2±3.8	69.4±3.71	65.2±1.39	61.7±2.28	63.1±5.17
Albumin	25.21±1.7	27.38±2.3	28.1±1.1	26.9±1.14	27.7±3.62	27.4±1.8
α-globulin	5.5±1.26	4.0±0.53	5.1±1.03	4.5±1.0	5.3±1.74	5.8±0.7
β-globulin	11.64±1.9	10.74±2.3	11.26±1.5	9.3±1.17	10.5±3.13	12.5±4.85
γ-globulin	10.2±2.54	9.12±1.7	11.4±3.4	13.71±2.4	12.5±3.8	14.73±4.1

Indicators of total protein in the experimental group did not significantly change in comparison with the initial indicators and the control group (Tables 9 and 10).

A similar picture is observed with respect to the albumin and α-globulin fractions.

Table 10. Indicators of the protein composition of the blood serum of animals of the control group (g / l), x ± SE, n = 5.

	Duration of experiment (days)					
	1	**5**	**10**	**15**	**20**	**25**
Total protein	61.9±5.7	64.6±4.7	63.2±6.0	68.9±3.1	65.1±5.1	63.2±5.28
Albumin	24.11±2.1	29.48±3.8	27.2±2.2	28.6±2.0	25.3±2.13	26.9±2.4
α-globulin	6.4±0.3	5.1±0.62	7.2±2.6	5.6±2.1	6.4±2.63	3.9±1.2
β-globulin	13.45±2.8	12.84±3.27	12.15±2.4	13.1±2.8	14.2±2.24	11.6±1.67
γ-globulin	7.1±1.35	8.20±2.8	10.3±2.5	9.81±2.77	10.1±1.5	9.27±1.8

Regarding β-globulins and γ-globulins, there were no significant differences between the experimental and control groups.

In addition, increased levels of interleukin-2 and interferon gamma were recorded (data not shown). Interferon gamma levels remained three times higher than normal for two weeks after the last injection.

Previously, preliminary studies of the antitumor activity of the *Streptococcus pyogenes* B-7612 strain were carried out on 1 dog and 1 cat with spontaneous breast cancer (data not shown). The drug was injected into the area around the tumor in a total dose of 0.1-0.2 ml once a week.

The dog is in complete remission after 2 months of vaccinations, the tumor has not been detected for 5 months. The cat showed a partial response, a 50% reduction in the linear size of the tumor, and stabilization over 18 months. This preliminary study showed the potential anti-cancer activity of *Streptococcus pyogenes* strain B-7612.

The use of bacteria for immunotherapy in pets was first pioneered by Dr. Tracy and Dr. Beeba at the Huntington Cancer Research Laboratory when they succeeded in completely eliminating giant sarcomas in dogs with a mixture of heat-killed *Streptococcus pyogenes* and *Serratia marcescens* bacteria, which produced the red pigment prodigiosan. It is now known that prodigiosan has both immunomodulatory and cytotoxic effects on cancer cells (Vernon 2018).

Currently, some bacteria have been proposed for veterinary immunotherapy: Corynebacterium parvum, Mycobacterium bovis (BCG), Salmonella typhimurium (VNP20009), Listeria monocytogenes, Clostridium novyi-NT, Streptococcus pyogenes (OK-432), as well as analogs of the bacterial components encapsulated in liposomes. These immunomodulators can affect both humoral and cellular immunity (Bergman 2014). The combination of surgery with immunotherapy with a mixed bacterial vaccine based on inactivated bacteria Streptococcus pyogenes and Serratia marcescens has been shown to prolong the life of cats with breast cancer twice as compared to a group of cats that only received surgery (MacEwen 1990).

The use of live attenuated and genetically modified bacteria in veterinary immunotherapy may be a more effective strategy, as demonstrated by the results of studies using *Salmonella typhimurium* and *Listeria monocytogenes* in dogs. Thus, *Salmonella typhimurium* strain VNP20009 was administered 1-2 times a week at doses ranging from 1.5 x 105 to 1 x 108 CFU / kg intravenously to 41 dogs with spontaneous malignancies. Of these, 4 had complete remission, 2 had partial remission, and 10% had stabilization. In another study, 17 dogs with osteosarcoma were injected intravenously with the attenuated bacteria Listeria monocytogenes, which stopped the growth of metastases and prolonged the overall survival of the dogs compared to historical controls (Andersen and Modiano 2015). Intratumoral administration of viable *Clostridium novyi-NT* spores resulted in complete remission in 3 dogs and partial remission in 3 out of 6 subjects (Roberts et al., 2014).

4 Conclusion

Streptococcus pyogenes strain B-7612 administered to dogs according to the scheme 0.02-0.04 ml intradermally for 20 days with an interval of 5 days, has a stimulating effect on the cellular link of immunity, which is expressed in an increase in the total number of leukocytes (mainly granulocytes and lymphocytes), activation of the phagocytic activity of blood neutrophils, with a simultaneous increase in the phagocytic index and phagocytic number (the number of absorbed microbial cells).

Streptococcus pyogenes strain B-7612 affects the activity of humoral factors (bactericidal activity of blood serum, lysozyme activity of blood serum), i.e. stimulates an increase in the natural resistance of the immune system.

After injection of the *Streptococcus pyogenes* strain B-7612, no significant differences in protein composition (total protein, albumin and globulins) were observed in the blood of experimental animals compared to the control.

The anticancer effect of the *Streptococcus pyogenes* strain B-7612 may be associated with the activation of the effector cells of the immune system.

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References

1. K.L. Anderson, J.F. Modiano, Progress in Adaptive Immunotherapy for Cancer in Companion Animals: Success on the Path to a Cure, Veterinary Sciences, **2**, **4**, 363-387 (2015) DOI: 10.3390/vetsci2040363
2. P.J. Bergman, Immunotherapy in Veterinary Oncology, The Veterinary clinics of North America. Small animal practice, **44**, **5**, 925-939 (2014) doi: 10.1016/j.cvsm.2014.05.002
3. V. Bobek, D. Pinterova, K. Kolostova, M. Boubelik, J. Douglas, P. Teyssler, J. Pavlasek, J. Kovarik, Streptokinase increases the sensitivity of colon cancer cells to chemotherapy by gemcitabine and cis-platine in vitro. Cancer Letters, **237**, **1**, 95-101 (2006) DOI: 10.1016/j.canlet.2005.05.030
4. V.A. Chereshnev, A.A. Morova, If a friend is considered an enemy. Warnings of clinical endoecology, Ecologia I Jizn, **10**, 64-68 (2006)
5. Y.M. Dobrynya, L.D. Timchenko, I.V. Rjepakovskiy, N.I. Bondareva, S.I. Piskov, Substances from Medusomyces Gisevii (tea mushroom) on white immunity rats in antibiotically associated conditions dysbacteriosis, Veterinarnaya patologiya, **3**, 22-30 (2017)
6. N.S. Forbes, R.S. Coffin, L. Deng, L. Evgin, S. Fiering, M. Giacalone, C. Gravekamp, J.L. Gulley, H. Gunn, R.M. Hoffman, B. Kaur, K. Liu, H.K. Lyerly, A.E. Marciscano, E. Moradian, S. Ruppel, D.A. Saltzman, P.J. Tattersall, S. Thorne, R.G. Vile, H.H. Zhang, S. Zhou, G. McFadden, White paper on microbial anti-cancer therapy and prevention. Journal for Immunotherapy Cancer, **6**, **1**, 78 (2018) doi: 10.1186/s40425-018-0381-3
7. M.G. Kramer, M. Masner, F.A. Ferreira, R.M. Hoffman, Bacterial Therapy of Cancer: Promises, Limitations, and Insights for Future Directions Frontiers in Microbiology, **9**, 16 (2018) doi: 10.3389/fmicb.2018.00016

8. K. Lukasiewicz, M. Fol, Microorganisms in the Treatment of Cancer: Advantages and Limitations. Journal of Immunology Research, 8 (2018) Doi: 10.1155/2018/2397808
9. E.G. MacEwen, Spontaneous tumors in dogs and cats: Models for the study of cancer biology and treatment. Cancer and Metastasis Reviews, **9**, 125-136 (1990) Doi: 10.1007/BF00046339
10. C. Maletzki, M. Linnebacher, B. Kreikemeyer, J. Emmrich, Pancreatic cancer regression by intratumoural injection of live Streptococcus pyogenes in a syngeneic mouse model. Gut, **57**, 483-491 (2008) DOI: 10.1136/gut.2007.125419
11. H. Okamoto, S. Shoin, S. Koshimura, R. Shimizu, Studies on the Anticancer and Streptolysin S-Forming Abilities of Hemolytic Streptococci. Japanese Journal of Microbiology, **11**, **4**, 323-336 (1967) doi:10.1111/j.1348-0421.1967.tb00350.x
12. N.J. Roberts, L. Zhang, F. Janku, A. Collins, R.Y. Bai, V. Staedtke, A.W. Rusk, D. Tung, M. Miller, J. Roix, K.V. Khanna, R. Murthy, R.S. Benjamin, T. Helgason, A.D. Szvalb, J.E. Bird, S. Roy-Chowdhuri, H.H. Zhang, Y. Qiao, B. Karim, J. McDaniel, A. Elpiner, A. Sahora, J. Lachowicz, B. Phillips, A. Turner, M.K. Klein, G. Post, L.A.Jr. Diaz, G. J. Riggins, N. Papadopoulos, K.W. Kinzler, B. Vogelstein, C. Bettegowda, D.L. Huso, M. Varterasian, S. Saha, S. Zhou, Intratumoral injection of Clostridium novyi-NT spores induces antitumor responses. Science translational medicine, **6**, **249** (2014) doi: 10.1126/scitranslmed.3008982
13. V.Ya. Saruhanov, N.N. Isamov, I.M. Kolganov, Method for determining the lysozyme activity of blood in farm animals, Selskohozyaystvennaya biologiya, **2**, 120-122 (2012)
14. M.A. Suvorova, A.N. Tsapieva, E.G. Bak, V.A. Chereshnev, E.P. Kiseleva, A.N. Suvorov, M. Arumugam, Complete Genome Sequences of emm111 Type Streptococcus pyogenes Strain GUR, with Antitumor Activity, and Its Derivative Strain GURSA1 with an Inactivated emm Gene. Genome Announcements, **5**, **38**, (2017) doi: 10.1128/genomeA.00939-17
15. W. Torres, V. Lameda, L.C. Olivar, C. Navarro, J. Fuenmayor, A. Perez, A. Mindiola, M. Rojas, M.S. Martínez, M. Velasco, J. Rojas, V. Bermudez, Bacteria in cancer therapy: beyond immunostimulation. Journal of Cancer Metastasis and Treatment, **4**, 4 (2018) Doi: 10.20517/2394-4722.2017.49
16. L.F. Vernon, William Bradley Coley, MD, and the phenomenon of spontaneous regression. Immunotargets and therapy, 7, 29–34 (2018) doi: 10.2147/ITT.S163924
17. W.S. Yang, S.O. Park, A.R. Yoon, J.Y. Yoo, M.K. Kim, C.O Yun, C.W. Kim, Suicide cancer gene therapy using pore-forming toxin, streptolysin O. Molecular Cancer Therapy, **5**, **6**, 1610-1619 (2006) DOI: 10.1158/1535-7163.MCT-05-0515