Improving salmonellosis etioprophylaxis in calves using immunomodulators of various pharmacological classes

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\textbf{Abstract.} In order to improve the immune response to salmonellosis in vaccinated calves, we tried applying the 0.01\% solution of miramistin combined with an inactive emulsified vaccine. Timogen was used as the control medicine because it is well known for its immunomodulatory properties regulating the cellular and the humoral immunity. The research was carried out at a large farm in Lipetskaya oblast that has a high salmonellosis incidence rate among calves. This research uses the epizootological, the clinical, the immunological, the hematological, and the molecular genetic methods. We ascertained that the application of the 0.01\% solution of miramistin and timogen to calves for the purposes of salmonellosis etioprophylaxis combined with an inactive emulsified vaccine promoted high cellular immunity. These medicines increased the levels of lymphocytes and their T-cells by 7.0\% and 12.5\%; their B-populations by 2.8\% and 5.1\%, the neutrophil phagocytic activity by 5.9\% and 13.0\%, the phagocytic number by 7.2\% and 15.0\%, the phagocytic index by 7.0\% and 18.0\% respectively. The improvement of humoral immunity factors comprised 3.0\% and 7.2\% for blood serum bactericidal activity; 3.0\% и 4.3\% for blood serum lysozyme activity; and 1.7\% and 2.0\% for blood serum complement activity. The O-agglutinins and H-agglutinins to salmonella antigen increased by 1.5 and 2 times respectively, and the disease rate decreased by 40.0\% and 80.0\%, while promoting the survival rate by 80.0\% and 87.0\% respectively, which shows that the 0.01\% solution of miramistin has better immunomodulatory properties.

\section{1. Introduction}

Among other etiological agents of infection pathologies, salmonella can only be rivalled by the causative agents of virus diarrhea in terms of its ability to cause various clinical problems in cattle. The salmonella infection can be clinically represented by intestinal, septic and reproductive disorders, and the intestinal diseases of young animals can lead to immense economic damages due to cattle culling and mortality. Salmonellosis is the key cause of diet-related toxic infections among people, and the majority of its strains are resistant to antibacterial medicines [1-4].

Cattle are the largest infector of salmonella, its circulation in clinically healthy animals is about 10\%, from 2\% in Europe to 16\% in North America. Around the world, over 140 salmonella serotypes are most widely spread. In Africa, 76 serotypes can be found, and in North America 49 serotypes. S.
Montevideo and S. dublin are most often registered in North America, and S. typhimurium is observed in Africa, Asia, and Australia. S. typhimurium and S. enteritidis together comprise about 50% of all isolates found in humans suffering from clinical conditions around the world [5-7].

In our country, about 12 common salmonella serotypes are isolated from cattle samples every year. The 3 serotypes that are registered more often than any other include S. dublin, S. typhimurium and S. enteritidis. Therefore, taking into consideration the spread of salmonella in animal farming, the main goal of veterinary specialists is taking some preventing actions, including the etioprophylaxis. Living and inactive vaccines are used in order to prevent salmonellosis among younger cattle in our country and abroad. However, this cannot eliminate outbreaks of salmonellosis, which indicates the low immunity protection level of the vaccinated animals. May authors point out that the general immunodeficiency of young animals caused by the intensive animal farming technologies inevitably leads to the emergence of various disorders and thus reduces the adaptive abilities of animals and increases their vulnerability against various infections [8].

That is why the actions to improve the etioprophylaxis of salmonella infections is a challenge. It requires the changing animal vaccination schemes, the proper selection of vaccines, and the application of immunomodulators to improve the immune status of young animals and thus increasing the potency of vaccines [9].

Promoting the use of immunoadjuvant medicines applied together with inactive salmonella vaccines to calves is a key objective of contemporary veterinary medicine. Selecting a proper immunomodulator from the range of medicines of various groups in order to promote the natural resistance of animal organisms, treat the physiological immunodeficiency, promote high-level immune protection, and prolong the post-vaccination immunity period is the most difficult aspect of this work. The range of immunomodulators of various pharmacological classes used in veterinary practice is quite extensive, but we think that the surfactants of the quaternary ammonium compounds group are the most promising due to their complex antimicrobial, antiviral and antimycotic properties [10-14]. Miramistin is one of these medicines, and in addition to the abovementioned, it also has immunoadjuvant properties [15].

At salmonella affected farms, it is crucial that the calves obtain high group immunity that would protect them from diseases and prevent further culling by the age of 2 months (when nursery groups are formed). The etioprophylaxis can help solve this problem, but its success largely depends on the selection of the vaccine taking into consideration the epizootic situation at the farm. Inactive vaccines that have a relatively low immunological potency are nonetheless often used because they make it possible to implement preventive immunization of immunosuppressed animals while using the living vaccines in this situation might lead to a disease outbreak.

2. Materials and methods

In order to improve calves’ specific immunity against salmonellosis, we analyzed the effects the 0.01% solution of miramistin has on specific and non-specific immune factors when it is used together with the inactive emulsified cattle salmonellosis vaccine. Timogen, a synthetic polypeptide (sodium alpha glutamyl tryptophan) that is famous for its immunomodulatory properties regulating the responses of cellular and humoral immunity was selected as the control medication. Timogen increases the expression of differentiation receptors in lymphocytes, regulates the amount of T-helpers, cytotoxic T-lymphocytes and their proportion in organisms suffering from immune deficiency conditions of various etiology.

The research was carried out at a large farm in Lipetskaya oblast that has a high rate of salmonellosis incidence among calves. This research used the epizootological, the clinical, the pathoanatomical, the bacteriological, the immunological, and the molecular genetic, and hematological methods. In order to conduct the experiment, 4 groups of calves were formed. Each contained 15 animals of the Holstein-Friesian breed, 45-48 kg each and aged 20 days. They were produced by parents that were not immunized against salmonellosis and were matched using the analog principle. The animals of the first, second and third groups were treated with an inactive emulsified cattle
salmonellosis vaccine (produced by OJSCM BelVitunipharm) at a dose of 1 cm³ intramuscularly. The procedure was performed two times at an interval of 10 days. The animals of the second group received timogen (produced by ZAO MBSPC CYTOMED) at a dose of 1 ml subdermally along with the vaccine, and the animals of the third group received 2 ml of the 0.01% solution of miramistin each. The 0.01% solution of miramistin (produced by LLC Infamed) was mixed with the vaccine in one syringe directly before injecting the medicine to the animal. The animals of the fourth group were left intact.

We monitored the animals in the experiment up to their 90th day of life. We recorded the disease incidence rates, the number of culled animals and their survival rate up to the 90th day of life. The biomaterial from the culled animals was analyzed through the polymerase chain reaction method to salmonella infection using the SAL-KOM salmonellosis test system (produced by InterLabService, Moscow).

In order to assess the immunomodulatory properties of the 0.01% solution of miramistin, materials were sampled from the animals in the experiment after 30 days of the second vaccine injection to define the titer of specific anti-salmonellosis agglutinins to the O-antigen and the H-antigen in the agglutination assay (AA) using the standard procedures. The antibody level was expressed in geometric average titer in base 2 logarithms (log₂). We also defined the content of T-lymphocytes and B-lymphocytes, the bactericidal, lysozyme and complement activity of the calf blood serum (BSBA, BSLA, and BSCA respectively), along with the phagocytic activity of leucocytes (PAL), the phagocytic number (PN), and the phagocytic index (PI). In order to prepare the O-antigens and the H-antigens for the tube agglutination, we used the collection strain of Salmonella enteritidis No. 11272 (strain certificate No 100451, Scientific Centre for Expert Evaluation of Medicinal Products named after L. A. Tarasevich, Moscow). The antigen was prepared in the lab using standard procedures: the elution of agar culture of Salmonella enteritidis (10 billion microbial bodies per 1 ml of physiological solution) was used as the antigen. The H-antigen was received by inactivating the culture with 1% solution of formalin, and the O-antigen was received by heating the material for 30 minutes at 70°C.

Lymphocytic cells were isolated in the single-stage density gradient of verografin: T-lymphocytes were isolated using the random rosetting method with sheep erythrocytes (T-ROK); B-lymphocytes were isolated in the EAC-ROK reaction on the receptor for the third component of the complement.

The amount of T-lymphocytes in the blood serum of the animals in the experiment was defined using E-rosetting. Plastic centrifugal tubes were filled with 0.1 ml of the 0.5% sheep erythrocyte suspension and incubated at 37°C for 15 minutes. Then the tubes were centrifuged at 1000 rpm for 5 minutes and kept cool (+4°C) for 16-18 hours. After the incubation, we added 50 mcl of the 3% (fresh) solution of glutaraldehyde to each of the tubes without stirring the residues and kept them at room temperature for 20 minutes in order to bind the rosettes. The binding was stopped by adding an excessive amount of cold distilled water. The supernate was removed by adding 0.2 ml of the buffer. Then the mixture was carefully resuspended 8-10 times with a pipette and smeared over degreased slides. The smeared films were bound with methanol for 5-6 minutes and then stained using the Romanovskiy-Giemsa method. The rosetting reaction was analyzed under immersion using the microscope. We estimated the percentage of rosette-forming T-cells by the number of lymphocytes that bound at least three sheep erythrocytes and free lymphocytes. We recorded the data only if the number of cells exceeded 200. In order to define the amount of B-lymphocytes in calf blood, we used the EAC-rosetting method: we mixed equal proportions of the EAC-complex and lymphocytes (100 mcl each) in centrifugal tubes. Then we incubated the suspension at 37°C for 30 minutes and stirred it twice during this time. Then we centrifuged the suspension at 1000 rpm for 5 minutes. The rosettes were bound by adding 50 mcl of the fresh 3% solution of glutaraldehyde for 20 minutes at room temperature. The binding was stopped by adding an excessive amount of cold distilled water. Then the water was removed and the residue was resuspended 10-12 times with a pipette in 200 mcl of the buffer. After that, we smeared the suspension on slides, bound the film with methanol for 5-6 minutes and stained using the Romanovskiy-Giemsa method. We registered the lymphocytes that bound at least three indicator erythrocytes.
We used nephelometry in order to assess the bactericidal activity of the blood serum (BSBA). We put 4.5 ml of the aseptic meat infusion broth (MIB) in tubes and added 1 ml of the serum examined to each of them. Then we added 0.1 ml of a one-day broth culture of *Escherichia coli* to all of the tubes. We used the tube with MIB and without the serum for the control purposes. We thoroughly stirred the content of each of the tubes and sampled 2 ml from each using an aseptic pipette in order to measure the optical density of the mixture. The mixture left in the tubes (MIB + serum + germ culture) was kept in the thermoregulated chamber at 37°C for 3 hours. Thus, there are 2 readings: the first one characterizes the optical density of the MIB with the germ culture and the serum directly after they were mixed, and the second one characterizes the optical density of the same mixture after 3-hour incubation in the thermocontrolled chamber. A 24-hour broth culture of *Escherichia coli* was used in the test (collection strain of *E. coli* No. 25922 strain certificate No. 240533 produced by the Scientific Centre for Expert Evaluation of Medicinal Products named after L. A. Tarasevich, Moscow). In order to prepare the germ culture, we required the daily elution of the agar culture of up to 2 billion microbial cells per 1 ml of the antiseptic physiological solution on the FEK-56 photoelectric colorimeter (red drum scale, extinction 0.3).

In order to define the lysozyme activity (BSLA) of the blood serum, we added 0.1 ml of the blood serum examined in each of the experiment tubes with a micropipette and added 1.4 ml of the standard test-germ culture, the 4-billion microbial suspension of *Micrococcus lisodeicticus* and then shook the tubes. Then we placed the mixture in the thermoregulated chamber for 1 hour at 37°C. After that, we shook and measured the tubes again. We also recorded the readings of FEK-56 in light transmission units (black scales). In order to prepare the 4 billion microbial mixture of *Micrococcus lisodeicticus*, 0.6 mg of culture acetone powder were thoroughly pounded with a pestle in 1 ml of the buffer. The suspension was standardized according to its optical density measured using FEK against the phosphate buffer solution using the green color filter No. 6 (wavelength of 540 nm) in the pans with the optical path length of 3 mm.

In order to define the complement activity of the blood serum (BSCA), the centrifugal tubes were filled with 3 ml of the work solution and 0.3 ml of the serum. This experiment allows to monitor and control the hemolytic system and the 100% hemolysis. The control and experimental tubes, along with the erythrocytic mixture and the hemolytic serum (diluted) were placed in the thermocontrolled chamber for 30 minutes at the temperature of 37°C. After this time, the hemolytic serum was mixed with the erythrocytic suspension in equal proportions. The received hemolytic system was poured in all of the tubes, including the control ones at a dose of 2 ml each. The content of the tubes was stirred and placed in the thermocontrolled chamber for another 30 minutes at 37°C (we stirred the tube content again after the first 15 minutes). After that, the samples were centrifuged (except the 100% hemolysis control ones) at 3000 rpm for 10 minutes. The supernatant fluid was colometered using FEK-56. On a workday, we received sheep erythrocytes and cleaned them with the work solution until the supernatant fluid became clear. Erythrocytes were centrifuged 3 times at 3000 rpm for 10 minutes. We used the 2.5% sheep erythrocyte suspension in our research. In order to carry out the hemolysis, we took the hemolytic serum diluted 4 times. The volume in which the hemolytic serum was diluted (in ml) was the same as the volume of the erythrocytic suspension.

In order to define the phagocytic activity of blood cells, the aseptic centrifugal tubes were filled with 0.5 ml of stabilized examined blood and 0.5 ml of the microbial suspension containing 0.5-1 billion germ cells of *Salmonella enteritidis* per 1 ml according to the optical turbidity standard. The tube with the prepared mixture was carefully shaken and placed into the thermoregulated chamber or on the water bath at 37°C for 30 minutes. Then we smeared the mixture on 3-5 slides, bound the films with methanol and stained using the Romanovskiy-Giemsa method. The number of cells participating in phagocytosis was determined on the 100 phagocytes detected. The phagocytic index was determined as an average of phagocytized germs per one active leucocyte. In order to define the phagocytic index, we used the same blood films as for the phagocytic activity of leucocytes. In the samples prepared using the procedures described above, we counted at least 100 leucocytes and the
number of microbial bodies absorbed by them. The phagocytic index was calculated by dividing the number of phagocytized bacteria by the number of active leucocytes.

The quantitative data were described by the arithmetic mean and the arithmetic mean error. The comparison of the averages between different groups was done using Student's two-sample test. The statistical analysis of the data was carried out in Microsoft Excel.

3. The study of the effect of immunomodulators on immunity of calves during vaccination against salmonellosis

Through our research, we ascertained that the group average background values of anti-salmonellosis agglutinins to the O-antigen and the H-antigen were roughly the same: 1:10 for the O-antigen and 1:26 for the H-antigen, which implicitly prove that the group of animals in question is not suffering from salmonellosis because its diagnostic titer is 1:40 and above (table 1).

Table 1. The titer of specific anti-salmonellosis agglutinins to the O-antigen and the H-antigen on the 30th day of the vaccination in $\log_2$

| Agglutinins      | Before vaccination (Background, $\log_2$ average) n=60 | 30 days after the vaccination, $\log_2$, average |
|------------------|------------------------------------------------------|-----------------------------------------------|
|                  |                                                      | Group No                                      |
|                  |                                                      | 1     | 2     | 3     | 4     |
| O-agglutinins    | 3.3±0.14                                             | 5.3±0.29 | 5.9±0.37 | 6.5±0.32 | 4.3±0.22 |
| H-agglutinins    | 4.7±0.17                                             | 6.9±0.32 | 7.3±0.35 | 8.3±0.28 | 5.0±0.26 |

On the 30th day of the second vaccine injection, the specific antibody titer increased in groups 1-3, and their amount varied depending on the medicines used.

In the first group, where animals were vaccinated only, the O-antigen agglutinin titer was 5.3±0.29 $\log_2$, and the H-antigen agglutinin titer was 6.9±0.32 $\log_2$. In the second group, where animals also received timogen as an immunomodulator, the specific antibody titer values were higher than in the first group and comprised 5.9±0.37 $\log_2$ for the O-antigen and 7.3±0.35 $\log_2$ for the H-antigen. The values of the salmonellosis antibody titer in the third group where the animals received the 0.01% solution of miramistin were the highest and comprised 6.5±0.32 $\log_2$ for the O-antigen and 8.3±0.28 $\log_2$ for the H-antigen.

After analyzing T-lymphocytes and B-lymphocytes in the blood of the calves that received immunomodulators together with the vaccine, we ascertained that these calves have a higher content of lymphocytes 30 days after vaccination, as compared to the values from the intact group (table 2). In the first group, the total amount of lymphocytes on the 30th day of the vaccination was 10.02% more than in the intact group.

In the second and third groups, the total amount of lymphocytes on the 30th day of the vaccination was at its highest: 16.35% and 30.6% more than in the intact group respectively. The second group value was 1.12 times lower than the third group value.

The number of T-lymphocytes on the 30th day of the vaccination in the first group remained virtually the same as in the intact group (the difference comprised only 3.0%). In the groups where calves received timogen and miramistin together with the vaccines, these values were different: the number of T-lymphocytes in the second group was 7.0% higher than in the intact group, and in the third group, this value was 12.5 % higher.

The situation with B-lymphocytes was the same. The increase of B-lymphocytes in the first group was insignificant, only 1.5%, in the second group it was 2.8%, and in the third 5.1% more than in the intact group. Therefore, the highest level of B-lymphocytes was observed in the calves immunized using the 0.01% solution of miramistin.
Table 2. The number of T-lymphocytes and B-lymphocytes in the blood of the calves treated with miramistin and timogen together with the salmonellosis vaccine

| Group No. | Number of animals in group | Total lymphocytes, $x10^9$/l | Quantity of T-lymphocytes, % | Quantity of B-lymphocytes, % |
|-----------|----------------------------|------------------------|---------------------------|---------------------------|
|           |                            | background in 30 days   | background in 30 days      | background in 30 days      |
| 1st       | n=15                       | 3.55±0.16              | 4.17±0.28*                | 31.8±2.33                 | 36.8±1.65*                | 16.1±1.45                 | 19.6±1.92                 |
| 2nd       | n=15                       | 3.47±0.09              | 4.41±0.25**               | 32.3±1.67                 | 40.8±2.43**               | 15.8±0.86                 | 20.9±0.75**               |
| 3rd       | n=15                       | 3.42±0.17              | 4.95±0.38**               | 30.5±1.43                 | 46.3±2.18**               | 18.1±1.74                 | 23.2±3.42**               |
| 4th       | n=15                       | 3.49±0.19              | 3.79±0.25                 | 32.4±2.71                 | 33.8±1.59                 | 16.3±1.80                 | 18.1±0.64                 |

Note: significance of differences *, ** - to the background; ● - to the intact group $P<0.05; 0.01$ respectively.

The results of the analysis of the influence of the 0.01% solution of miramistin on humoral factors of non-specific immune protection for the cattle vaccinated against salmonellosis using inactive emulsified vaccines are shown in table 3.

We ascertained that the level of BSBA in the first group was 1.86% higher, in the second group 3.0% higher and in the third group 7.2% higher than in the intact group. The BSLA and BSCA values correlated with the BSBA values. The increase of BSLA in the first group was insignificant and comprised 1.68%; in the second group, it was 3.0% and in the third group, it was 4.3%. The increase of BSCA in the first group was insignificant and comprised 1.1%; in the second and the third groups, it was 1.7% and 2.0% higher than in the intact group respectively.

Table 3. The bactericidal, lysozyme, and complement activity of the blood serum of the calves that underwent immunization against salmonellosis and received immunomodulatory agents

| Group No. | Number of animals in the group | BSBA, % | BSLA, % | BSCA, % |
|-----------|-------------------------------|---------|---------|---------|
|           |                               | background in 30 days | background in 30 days | background in 30 days |
| 1st       | n=15                          | 69.7±5.12         | 75.95±6.30         | 27.02±2.25         | 29.98±3.28         | 7.03±1.72         | 7.6±0.76         |
| 2nd       | n=15                          | 69.21±4.08        | 77.11±5.10*        | 26.87±3.13        | 31.33±3.42**       | 7.92±1.86        | 8.45±2.15**      |
| 3rd       | n=15                          | 69.88±3.78        | 81.29±5.80**       | 27.19±2.65        | 32.61±4.28**       | 7.51±1.33        | 8.79±1.95**      |
| 4th       | n=15                          | 69.55±4.22        | 74.09±3.49         | 27.11±2.08        | 28.30±2.03         | 7.19±0.62        | 6.80±0.71        |

Note: the significance of differences * - to the background; * - to the intact group $P<0.05$ respectively.

All three values were at their lowest in the first group, where the animals only received vaccines without any immunomodulators; the highest results were observed in the animals treated with the 0.01% solution of miramistin together with the vaccine; the results of those that received timogen were a little bit lower.
The results of the analysis of the phagocytic activity of leukocytes on the 30th day of the experiment show that the 0.01% solution of miramistin has a clear impact on the cellular level of calves’ non-specific immunity (Table 4). The highest increase of PAL was in the group vaccinated together with miramistin treatment and it was 13.0% higher than the same value in the intact group on the 30th day of the vaccination. In the timogen group, PAL increased by 5.9% as compared to the intact group, and the increase of PAL in the calves that only received the vaccine was the lowest - only 2.7% higher than in the intact group.

The phagocytic number in the first group virtually did not change during this period and was only 5.0% higher than in the intact group. In the second group, it was 7.2% higher, and in the third, it was 15.0% higher. The phagocytic index in the first, the second and the third groups was 6.0%, 7.0%, and 18.0% higher than in the intact group respectively.

Thus, the lowest increase of PAL, PI and PN was observed in the first group of animals that received the vaccine only, and the highest was in the second and the third groups. The values of the third group (that was treated with the 0.01% solution of miramistin) were higher than in the second (timogen) group by 7.36%; 7.8% and 11.0% respectively.

Table 4. Phagocytic activity of leucocytes (%), phagocytic number (of microbial elements) and phagocytic index (of microbial elements) of calves after 30 days of their vaccination and immunomodulatory treatment

| Group No | Number of animals in the group | PAL, %  | PN, m.e.  | PI, m.e.  |
|----------|--------------------------------|---------|-----------|-----------|
|          |                                | background in 30 days | background in 30 days | background in 30 days |
| 1st      | n=15                           | 82.52±5.15 | 86.19±7.21* | 6.86±0.52 | 7.41±1.15** | 8.05±2.33 | 8.60±2.95** |
| 2nd      | n=15                           | 81.41±6.65 | 89.44±7.93* | 6.56±0.43 | 7.59±1.46** | 7.93±2.47 | 8.77±3.12** |
| 3rd      | n=15                           | 82.75±6.25 | 96.80±8.42** | 6.79±0.64 | 8.17±1.88* | 7.75±2.18 | 9.61±3.43** |
| 4th      | n=15                           | 81.58±4.20 | 83.49±7.36  | 6.76±0.31 | 7.08±1.62  | 7.86±2.16 | 8.11±1.98  |

Note: the significance of differences *, **, *** - to the background; *, ** - to the intact group

The clinical surveillance of the calves in the experiment up to their 90th day of life showed the following results: in the intact group, the majority of the animals (12, or 80.0%) had some gastrointestinal problems during the nursery period (up to their 60th day of life) that led to severe suffering and culling. In the first group, only 5 animals (33.3%) had some problems before their 60th day of life and 11 animals (73.0%) had some problems until their 90th day of life, and the observed disease severity was medium. Among the calves from the 2nd and the 3rd groups, severe diseases never occurred. In the third group, none of the calves had any health problems before their 60th day of life, and only one animal became ill before its 90th day of life. In the second group, 2 animals (13.0%) became ill before their 60th day of life, and 7 animals (47.0%) had some health problems before their 90th day of life. In these two groups, 0 and 1 animal was culled respectively. During the molecular genetic analysis of the biomaterial (feces) from the diseased calves, the salmonella factor was found (table 5).
Table 5. Calf disease and survival rates in the research groups

| Group No. of animals in the group | Number | Disease incidence over the first 60 days | Total disease incidence at the end of the 90-days period |
|----------------------------------|--------|----------------------------------------|-------------------------------------------------------|
|                                  |        | Animals/Animals| Animals/Animals | Animals | Animals/Animals |
| 1st n=15                         | 5/33.3 | 11/73.0                      | 7                      | 8/53.0       |
| 2nd n=15                         | 2/13.0 | 7/47.0                      | 1                      | 14/93.0      |
| 3rd n=15                         | 0/0.0  | 1/6.0                       | 0                      | 15/100       |
| 4th n=15                         | 12/80.0| 13/87.0                     | 13                     | 2/13.0       |

Table 5 makes it evident that the 4th group where the calves were not vaccinated has the lowest survival rate (13.0%). The highest survival rate (100%) is in the 3rd group that was treated with the 0.01% solution of miramistin in addition to the vaccine. The survival level in the 2nd group where the animals received timogen as an immunomodulator was a little lower (by 7.0%). The survival rate in the 1st group where the animals received the vaccine only was 53.0%, which is 40.0% higher than in the intact group, but 40.0% and 47.0% lower than in the 2nd and in the 3rd groups respectively.

4. Discussion and Conclusion

Many researchers [7, 9] claim that the low level of immune protection against salmonellosis among young cattle is due to the general immune deficiency of the animals that are exposed to a number of adverse factors of technological and environmental nature among others. One of the ways to solve this problem is using immunomodulators to improve animals’ immune responses, including the post-vaccination antibody titers.

We believe that the immunogenic potential of an inactive emulsified cattle salmonellosis vaccine can be significantly increased by applying it together with the 0.01% solution of miramistin. Experiments [16, 17] prove that cationic surfactants can be successfully applied topically, internally and through parenteral injections, which prerequisites the combined use of miramistin and inactive vaccines.

Research shows that treating calves from non-immunized mothers with an inactive salmonellosis vaccine combined with miramistin provides for a significant stimulation of the specific humoral and cellular response. The combined injection of miramistin and the vaccine promoted a firm increase of O-agglutinin titers by 10.0% and H-agglutinin titers by 13.0% as compared with timogen, which proves that this medicine can stimulate the serogenesis.

The highest increase of the total number of lymphocytes and their T and B populations was also evidenced in the calves that received miramistin together with the vaccine, which shows that the animals underwent a significant immune alteration induced by the medicine. It is widely known that lymphocytes play a crucial role in the cellular immunity struggle against intestinal infections [9].

The combination of miramistin and the vaccine also improves the level of non-specific humoral and cellular immunity factors in the calves that received it (BSBA, BSCA, BSLA, PAL, PN, PI).

V. A. Shatrov [18] in his paper on the immunomodulatory properties of cationic surfactants points out that these compounds activate macrophages and lymphocytes, which leads to the increased response of these cells to the antigen, and the immunoadjuvant effect of the medicines of this group does not lie in the absence of macrophages. Surfactants activate the oxygenic metabolism in
macrophages, significantly increase the random migration and the absorbency of these cells stimulating their immune activity.

Having analyzed the data received from the experiments and the literature, we can say that the immunoadjuvant effect of miramistin on top of vaccination is based on the increased permeability of cytosolic membranes of lymphoid cells and the stimulation of their metabolism, as well as the intensified functional activity of phagocyte leading to a more efficient phagocytosis and antigen presentation to the immunocompetent cells. On the other hand, miramistin is a detergent by its chemical structure and thus it has a solubilizing effect on the cell membranes of bacterial structures in vaccines making the antigenic complexes more available for the antigen-presenting cells of the animal organism, which improves the rate and efficiency of the immune response. The research conducted shows that the salmonellosis incidence among the calves of up to 90 days old at the affected farms is 87.0%, and the proportion of fallen and culled animals is so great that the survival rate of the herd is just 13.0%.

The vaccination of young animals with an inactive emulsified cattle salmonellosis vaccine reduces the incidence of the disease among calves by 14.0% the increases their survival rate by 40.0%.

The application of the 0.01% solution of miramistin and timogen to calves on top of an inactive emulsified vaccine promotes cellular immunity, i.e. increases the levels of lymphocytes and their T-cells by 7.0% and 12.5 %, the B-cells by 2.8% and 5.1%, the neutrophil phagocytic activity by 5.9% and 13.0%, the phagocytic number by 7.2% and 15.0%, the phagocytic index by 7.0% and 18.0%. The humoral immunity factors increase as well: the blood serum bactericidal activity by 3.0% and 7.2%, the blood serum lysozyme activity by 3.0% и 4.3%, the blood serum complement activity by 1.7% and 2.0%, the O-agglutinins and H-agglutinins to salmonella antigen by 1.5 and 2 times respectively.

The use of such immunomodulators as timogen and the 0.01% solution of miramistin increases the protective properties of the salmonellosis vaccine, reduces the disease incidence rate by 40.0% and 80.0% and increases the survival rate of the calves by 80.0% and 87.0% respectively, which shows that the 0.01% solution of miramistin has better immunomodulatory properties than timogen. The injection of the 0.01% solution of miramistin and the vaccine in one syringe makes this operation more practical and less traumatizing to animals.

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