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# The development of preparations for specific prevention and treatment of anaerobic enterotoxemia and escherichiosis in calves


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## ABSTRACT

The preparations for specific prevention and treatment of combined anaerobic enterotoxemia and escherichiosis in calves have not been developed. Taking it into account, the research on the development of combined vaccine, polyvalent hyperimmune therapeutic and preventive serum against these infections in calves was carried out.

When developing biological preparations, inactivated antigens of Clostridium perfringens of Serotypes A, C, D and Escherichia coli that synthesize adhesive antigens K99, A20were used. Harmlessness, antigenic and immunological activity, the therapeutic effectiveness of the developed biological preparations were determined in experiments in laboratory animals. On the farms with a high incidence of these infections, harmlessness and immunological effectiveness of the vaccine administered into down-calving cows and calves aged 15–18 days and therapeutic and preventive properties of hyperimmune serum in the treatment of calves aged 1–20 days were studied. After administering the vaccine into pregnant cows 50–60 days before calving and newborn calves aged 15–18 days for preventive purposes, the incidence of calves on the farms reduces by 4.6 times, their survival increases by 16.6%. The application of the hyperimmune serum obtained by hyperimmunization of stud bulls with inactivated antigens of C. perfringens and E. coli bacteria for therapeutic purposes allow you to cure 93.0% of calves diseased with anaerobic enterotoxemia and escherichiosis that is 13.6% higher than in the case of treatment of diseased calves with gentamicin and streptomycin. In the group of the calves treated with preventive hyperimmune serum, 29.6% of the animals fell ill, and 5.7% of the animals died, whereas in the control group82.7% of intact calves became ill and 17.3% of diseased calves died. Based on the results of laboratory and on-farm experiments on testing the developed biological preparations, normative documents regulating their production and application were developed and approved by the *Scientific Council* of the FGBI "Federal Centre for Animal Health" and the *Rossel khoznadzor* with the aim to implement them in veterinary practice. The novelty of the developed therapeutic and preventive preparations is confirmed by the invention patents of the Russian Federation No. 2428202 and No. 2523389.

**Keywords:** anaerobic enterotoxemia, escherichiosis, vaccine, therapeutic hyperimmune serum

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## INTRODUCTION

The practice of cow–calf farms that produce milk both in our country and abroad shows that gastrointestinal diseases of mixed etiology in calves cause significant economic loss.

In many cases, gastrointestinal diseases of bacterial etiology in newborn calves are caused not by individual pathogens, but a group of pathogenic bacteria. The start of the disease, coverage range of cow population, its severity and outcome depend on the health status of the calf, the level of its resistance and the conditions in which it grows after birth. At the same time, the level of colostral immunity is of special importance which provides newborn calves with protection from infectious agents for the first 10 days. The high resistance of newborns depends on the level of immunoglobulins in colostrum of dams. Defective, unbalanced feeding of pregnant cows causes colostrum immunodeficiency and hypoglobulinemia that lead to disease.[2,3,6]

In most cases, gastrointestinal diseases are caused by combined E. coli, C. perfringens, Salmonella, Streptococci bacteria and others. Anaerobic enterotoxemia of young animals is caused by C. perfringens which are divided into six types: A, B, C, D, E, F; they differ from each other in antigenic structure and toxins they produce. In calves, anaerobic enterotoxemia is caused by the causative agents of Serotypes A, C, and D.[1,3,8,9,10,11,12,13,14] Escherichiosis in calves in most cases is caused by enterotoxigenic E. coli strains that synthesize adhesive antigens K99, A20.[2,7,10,15,16] In the Russian Federation, to prevent livestock from escherichiosis the vaccine against escherichiosis "Coli-Vac" is produced, but preparations for specific prevention from anaerobic enterotoxemia in calves have not been developed.

The analysis of scientific works and the results of our multi-year research indicate that on the farms with a high incidence of gastrointestinal diseases anaerobic enterotoxemia in calves is most commonly observed in combination with escherichiosis.[5]

As a result, the purpose of our research was to develop a combined vaccine, polyvalent hyperimmune therapeutic and preventive serum against anaerobic enterotoxemia and escherichiosis in calves and to study their effectiveness in laboratory and on-farm experiments.

## MATERIALS AND METHODS

The experiments on the study of diseases in young animals were conducted in the laboratory of FGBNU "FCTRB-VNIVI" and cow–calf farms with a high incidence of anaerobic enterotoxemia and escherichiosis in calves. The combined vaccine against anaerobic enterotoxemia and escherichiosis in calves was prepared on the basis of the antigens of C. perfringens strains No. 28 (Type A), No. 392 (Type C), No. 213 (Type D) and E. coli enterotoxigenic strains: "KV-1 and PZ-3" that produce adhesive antigens K99 and A20, respectively, which were isolated from the material of calves died from gastrointestinal diseases. After studying their cultural, morphological and biological properties, the isolated bacteria were deposited in the State collection of pathogenic bacteria of Risk Groups II–IV in FGBNU "FCTRB-VNIVI".

C. perfringens industrial strains were grown on meat–liver–casein medium in a 10-liter reactor for 6–8 hours at a temperature of 37–38°C until the number of microbial cells reached at least 4 billion/ml. The toxicity of the grown culture was tested in white mice. To prepare the vaccine, culture suspensions containing no less than 6000 DLM/ml were used.

To obtain E. coli bacterial mass, beef-extract agar (for "PZ-3" strain that synthesizes adhesive antigens A20) and the Mink medium (for "KV-1"strain that synthesizes adhesive antigens K99) were used. The strains were grown in 1.5-liter frosted flasks at a temperature of 37–38°C. The grown colonies of E. coli cultures were washed with a 0.85% sterile sodium chloride solution24 hours later, then we prepared a suspension at concentrations of 100–120 billion m.c/ml by optical turbidity standard of GISK named after K. A. Tarasevich.

The inactivation of bacterial mass in both cases was performed with formalin.

To obtain E. coli toxoid, each strain was inoculated separately into the reactor with Hottinger's broth, grown for 5–7 days at a temperature of 37–38°C. The concentration of TL-, TC-toxins was determined in the reaction of diffusion precipitation. The titer of TL-, TC-toxins in the culture fluid was at least 1:4 to 1:8.

Experimental samples of the combined vaccine against anaerobic enterotoxemia and escherichiosis in calves were prepared in the following ratio of components in 1 liter of the vaccine:

- suspensions of microbial cells of strains No. 28, No. 392 and No. 213 strains of C perfringens in the culture medium at concentrations of $3.5 \cdot 10^{12}$ to $4.0 \cdot 10^{12}$ см$^3$––150.0 ml of the suspension of each strain respectively;
- suspensions of microbial cells of KV-1 and PZ-3 strains of E. coli, in physiological solution at concentrations of $100 \cdot 10^{12}$––$120 \cdot 10^{12}$ см$^3$–– 30.0 ml of the suspension of each strain;
- aluminum hydroxide, 6% –––100 ml;
- formalin 4 ml.

The volume of the obtained suspension of bacteria, aluminum hydroxide, and formaldehyde was adjusted to 1 liter by adding TC and TL-toxoids of E. coli KV-1 and PZ-3 strains in the ratio 1:1 in the culture medium with a titer in the RDP 1:8–1:16.

The test on the harmlessness of the vaccine was performed in 10 white mice with a live weight of 16–18 g, 0.5 ml. of the vaccine was administered subcutaneously. The vaccine was considered to be harmless if the mice remained alive and clinically healthy for 10 days after the vaccination.

The test on the the immunogenic activity of the vaccine was performed in 3 rabbits; the preparation was administered intramuscularly at a dose of 4 ml twice with an interval of 15 days. Twenty days after the second injection, the titer of antitoxin antibodies was determined in the serum of each rabbit in the neutralization of C. perfringens toxin in white mice. The vaccine was considered to be active against enterotoxemia if the serum of immunized rabbits protected at least two of the three experimental mice while all the mice in the control group died. Immunogenic activity of the vaccine to E. coli was tested in 40 white mice of 16–18 g. weight. The vaccine was administered subcutaneously into twenty experimental mice twice with an interval of 10 days at a dose of 0.3 ml; twenty control mice were not vaccinated. 15 days after the second immunization, a sub titer lethal dose of two control strains of E. coli (KV-1 and PG-3) was administered intraperitoneally to animals using ten vaccinated and ten unvaccinated animals for each E. coli strain. The vaccine was considered to be active against E. coli if at least seven of the ten vaccinated animals stayed alive and not less than 8 non-vaccinated white mice died.


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Immunogenicity test of prepared vaccine samples was conducted on the farms with a high incidence of anaerobic enterotoxemia and escherichiosis in calves. The vaccine was administered subcutaneously into down-calving cows at a dose of 10 ml 50–60 days before calving twice with an interval of 14 days. Calves from these cows were vaccinated at the age of 18–20 days at a dose of 3 ml twice with an interval of 14 days.

Hyperimmune serum was obtained from study bulls aged 18– 24 months with a live weight of not less than 350–400 kg by their hyperimmunization with inactivated antigens of C. perfringens of Serotypes A, C, D and E. coli (K99 and A20). While hyperimmunized bulls, C. perfringens antigens were administered subcutaneously at increasing doses four times with an interval of 14 days into one side of the neck, and E. coli antigens were also administered subcutaneously four times into the other side of the neck. The total blood taking from stud bulls was performed if in the serum of their blood there were specific antibodies to C. perfringens in the titer not less than 1:12800 in ELISA, to E. coli – not less than 1:1600 in AR, to E. coli thermostable and thermolabile toxoids– not less than 1:8 in RDP.

For preventive purposes, hyperimmune serum was administered intramuscularly into newborn calves at a dose of 2 ml, for therapeutic purposes –at a dose of 2.5 ml /1 kg of body weight. In the case of severe disease, the serum was administered again at the same doses 1–3 days later. The daily therapeutic dose of the serum was divided into 2 doses and administered with an interval of 6 hours.

The effectiveness of the vaccine and therapeutic and preventive hyperimmune serum was assessed by comparing the morbidity, recovery and survival level of calves before and after the application of the biopreparations. The therapeutic effectiveness of hyperimmune serum was a determined in comparison with the state of control calves that were treated with antibiotics (gentamicin sulfate and streptomycin).

**Table 1** **The content of specific antibodies in the serum of vaccinated cows (M±m, n = 5, $log_2$)**

| Antibodies to antigens | The period of the study | | |
|---|---|---|---|
| | before vaccination | 14 days after the first vaccination | 14 days after the second vaccination |
| C. perfringens Type A | 2.64±0.35 | 11.24±0.27 | 13.44±0.22 |
| C. perfringens Type C | - | 9.840±0.42 | 13.24±0.27 |
| C. perfringens TypeD | - | 10.04±0.27 | 13.04±0.27 |
| E. coli K 99 | 1.5±0.42 | 7.52±0.22 | 8.72±0.27 |
| E. coli A20 | - | 7.32±0.50 | 9.12±0.22 |

Note–specific antibodies in the blood serum of cows before vaccination indicates the presence of the infections in livestock.

## RESULTS AND DISCUSSION

Laboratory research on prepared samples of the combined vaccine and hyperimmune therapeutic and preventive serum against anaerobic enterotoxemia and escherichiosis in calves showed that they are all sterile, harmless, possess high immunogenic and therapeutic activity. Antitoxic antibodies in the serum of the vaccinated rabbits in the reaction of neutralization in white mice prevented 80–90% of the white mice after they were infected with 2 titrated lethal doses of C. perfringens and E .coli.

The study of the effectiveness of the combined vaccine against anaerobic enterotoxemia and escherichiosis in calves was carried out on three farms where cases of combined anaerobic enterotoxemia and escherichiosis in calves were registered.

Most researchers agree that to develop colostral immunity in newborn calves,it is necessary to vaccinate pregnant cows and heifers 50–60 days before calving so that calves can receive ready-made –antibodies with colostrum. It is always necessary to consider vaccination as a forced procedure; it should be performed only if there are epizootic indicators for it.

To this end, we prepared experimental samples of the combined vaccine. First, administering it into a limited number of pregnant cows it was found that the vaccine is harmless for pregnant cows, does not cause complications and induces the formation of specific antibodies in their blood against the used antigens.

The test results are shown in Tables 1 and 2.

The data presented in Tables 1 and 2 indicate that the body of cows after vaccination creates specific antibodies in high titers to the antigens in the vaccine that protect newborn calves from gastrointestinal diseases, particularly, in the case of combined anaerobic enterotoxemia and escherichiosis. So, after administering the vaccine, the incidence of gastrointestinal diseases in newborn calves on the farms with a high incidence of these infections decreased from 91.0% to 19.7%, i.e. by 4.6 times, the survival of the calves increased from 77.8% to 94.4%, i.e. by 16.6%.

The assessment of the therapeutic and preventive effectiveness of hyperimmune serum was performed on two farms with a high incidence of anaerobic enterotoxemia and escherichiosis in calves. The results are presented in Tables 3 and 4.

The data in Tables 3 and 4 show that the hyperimmune serum possesses pronounced therapeutic and preventive properties. In the group of calves treated with preventive hyperimmune serum, 29.6% of the animals fell ill, and 5.7% of the animals died, whereas in the control group ––82.7% and

**Table 2** Indicators of the effectiveness of the combined vaccine

| Farm | Total number of calves | Fell ill | | Died | | Stayed alive% |
|---|---|---|---|---|---|---|
| | | number | % | number | % | |
| **before administering combined vaccine** | | | | | | |
| №1 | 1041 | 887 | 85.2 | 197 | 18.9 | 81.1 |
| №2 | 1986 | 1901 | 95.7 | 473 | 23.8 | 76.2 |
| №3 | 324 | 262 | 80.8 | 74 | 22.9 | 77.1 |
| Total | 3351 | 3050 | 91.0 | 744 | 22.2 | 77.8 |
| **after administering combined vaccine** | | | | | | |
| №1 | 1221 | 212 | 17.4 | 47 | 3.8 | 96.1 |
| №2 | 1608 | 352 | 21.9 | 114 | 7.1 | 92.9 |
| №3 | 268 | 48 | 17.9 | 13 | 4.8 | 95.2 |
| Total | 3097 | 612 | 19.7 | 174 | 5.6 | 94.4 |

**Table 3** Research data on preventive effectiveness of hyperimmune serum

| Farm | Animal group | Number of animals | Fell ill | | Died | |
|---|---|---|---|---|---|---|
| | | | number | % | number | % |
| No. 4 | experimental | 85 | 22 | 25.9 | 2 | 2.35 |
| | control | 63 | 51 | 81.0 | 9 | 14.3 |
| No. 5 | experimental | 128 | 41 | 32.0 | 9 | 7.03 |
| | control | 139 | 116 | 83.5 | 26 | 18.3 |
| Total | experimental | 213 | 63 | 29.6 | 11 | 5.7 |
| | control | 202 | 167 | 82.7 | 35 | 17.3 |

**Table 4** Research data on therapeutic effectiveness of hyperimmune serum

| Farm | Animal group | Number of animals | Fell ill | | Died | |
|---|---|---|---|---|---|---|
| | | | number | % | number | % |
| No. 4 | experimental | 47 | 45 | 95.7 | 2 | 4.3 |
| | control | 25 | 22 | 88.0 | 3 | 12.0 |
| No. 5 | experimental | 68 | 62 | 91.2 | 6 | 8.8 |
| | control | 67 | 51 | 76.2 | 16 | 23.8 |
| Total | experimental | 115 | 107 | 93.0 | 8 | 7.0 |
| | control | 92 | 73 | 79.4 | 19 | 20.6 |

17.3%, respectively. About 94.3% of the calves in the experimental group stayed alive, in the control group––82.7%. The application of the serum for therapeutic purposes allowed us to cure 93.0% of the sick calves, whereas in the control group where antibiotics were used for therapeutic purposes only 79.4% of the calves stayed alive that is 13.6% lower than in the experimental group.

Based on the results of laboratory and on-farm experiments on the combined vaccine and hyperimmune therapeutic and preventive serum normative documents regulating their production, control and application were developed, the invention patents of the Russian Federation No. 2428202 and No. 2523389 were granted.

## CONCLUSION

The combined vaccine and hyperimmune therapeutic and preventive serum against anaerobic enterotoxemia and escherichiosis in calves were developed and tested. It was established that the combined vaccine possesses high antigenic and immunogenic activity. Its application for preventive purposes on farms with a high incidence of anaerobic enterotoxemia and escherichiosis reduces the cases of these diseases in calves by 4.6 times, thereby increasing their survival by 16.6%. The hyperimmune serum against anaerobic enterotoxemia and escherichiosis in calves possesses pronounced therapeutic and preventive properties. The application of the serum for therapeutic purposes allowed us to cure 93.0% of the sick calves that is 13.6% more than in the group of the calves which were treated with antibiotics. Based on the results of laboratory and on-farm experiments on testing the developed biological preparations normative documents regulating their production and application were developed and approved by the Scientific Council of the FGBI "Federal Centre for Animal Health" and the Rossel khoznadzor with the aim to implement them in veterinary practice.

The novelty of the developed therapeutic and preventive preparations was confirmed by the invention patents of the Russian Federation No. 2428202 and No. 2523389.

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