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Prospect of Using *B. Anthracis* Exotoxin in the Design of Anti-Selective Emergency Preparations

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Abstract. The relevance of the study is conditioned upon the fact that outbreaks of anthrax are periodically recorded on the territory of Ukraine, not only in ruminants, but also in pigs, fur animals, dogs, and people. The purpose of the study is to investigate the protective properties of the experimental vaccines and the abacillary vaccine “Antracol” and to prove the immunogenic effect of the extracellular toxin from the *B. anthracis* K-79 Z strain. Cultures of vaccine strains of anthrax were used for the experiments: *B. anthracis* 55, *B. anthracis* SB, *B. anthracis* K-79 Z and the “Antracol” vaccine (experimental development). Microbiological, clinical-biological, and biotechnological research methods were used in the study. The protective effect was investigated on guinea pigs (*Cavia porcellus*). An acute experiment was performed with a virulent strain *B. anthracis* 92 Z. Exotoxin was obtained from the specified cultures. The titre of the exotoxin was found in the disk precipitation reaction. The highest result regarding exotoxin production was recorded in *B. anthracis* K-79 Z 1 : 128 with a total protein concentration of 0.19 mg/ml, while the exotoxin of *B. anthracis* strain 55 with a titre of 1 : 32 showed a high total protein concentration of 0.4 mg/ml. The effect of *B. anthracis* exotoxins on the body was investigated by administering them to laboratory animals in different titres of exotoxins, followed by infection with the pathogenic strain *B. anthracis* 92 Z. The exotoxin of the vaccine strain *B. anthracis* K-79 Z in a titre of 1 : 64-1 : 128 shows the best protective properties against the pathogenic strain. It was found that the vaccine strains of *B. anthracis* SB and *B. anthracis* K-79 Z have the same level of protection of laboratory animals during experimental infection, which is 60%, while the vaccine from the strain *B. anthracis* 34F₂ showed a level of protection of 20%. Based on the results of the study, it was found appropriate to use exotoxin *B. anthracis* in the development of prophylactic preparations against anthrax. The research results can be used by scientists and specialists in the field of veterinary medicine to develop new and improve the available vaccines for effective anthrax prevention

Keywords: anthrax, metabolites, preventive effect, abacillary vaccine “Antracol”

Introduction

The main problem of the study is anthrax, a zoonotic disease that affects humans, domestic, and wild animals. First of all, this is a disease of herbivores [1]. In economically developed countries, it is rare, thanks to the developed

system of total vaccination of susceptible livestock and prompt control of anthrax burial sites. Anthrax is a disease registered on all continents, as the infection is of natural origin and occurs in the farming communities of

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developing countries. Anthrax is still an endemic disease in some parts of the world, namely in the countries of Central Asia, in many regions of the Russian Federation, Africa (south of the Sahara Desert), Central and South America, especially in arid and tropical rural areas [2; 3]. According to statistics, the incidence of anthrax worldwide ranges from 20,000 to 100,000 cases per year. Therewith, about 63.8 million pastoralists and 1.1 billion pets are at risk [4].

The scientific literature [5-7] contains a lot of information about anthrax research. Presently, a critical area is the development and improvement of existing avirulent spore vaccines, as well as the study of reactogenicity of preparations and treatment protocols. Special attention is paid to studying the mechanism of action of anthrax exotoxins and their effect on the immune system of animals [6]. The spore formation of anthrax strains and the mechanism of action at the molecular and genetic level of composite vaccines during the development of infection are investigated [7]. Currently, the issue of creating a combined preparation against anthrax as a preventive and curative agent that can be used in foci of infection outbreaks stays unresolved [8].

Currently, only live spore anthrax vaccines are registered and used in Ukraine. They have many limitations: they are used for ruminants aged from 8 months and a satisfactory immune state. The production of such prophylactic preparations is also conditioned upon the risk to the health of people who work with the pathogen (anthrax belongs to microorganisms of the pathogenicity group II, it can lead to infection of service personnel) [9]. The abacillary vaccine will solve the problem of vaccination of all mammals. The design of such a preparation is safe for the environment and humans. Researchers of the "State Centre for Innovative Biotechnologies" conducted several experiments that yielded positive results on the creation and use of vaccines that contained the products of the anthrax pathogen's vital activity in the absence of spores of the bacillus itself [10-12].

Studies of spore-forming bacteria have shown their resistance to chemicals and elevated temperatures. This property can vary both in diverse types of microorganisms and in strains of the same species [13]. It was established that variable sporulation conditions, including the use of liquid or solid media, levels of nutrients and divalent cations, as well as pH and temperature of the sporulation medium, can affect the persistence properties of the resulting spores [14].

Contamination of the environment with virulent pathogens requires the creation of protection for animals and people in the shortest possible time. Modern vaccines do not meet these requirements because immunity is formed much longer (14-30 days) than the incubation period of the disease. Considering the danger of zoonanthropotic infection, there is a need for a quick and effective response to anthrax outbreaks. Thus, the creation of new and improvement of available means of anthrax prevention is a relevant and urgent need.

In the 20th century, anthrax outbreaks were recorded in most regions of Ukraine, which led to a tense epizootic situation. Before the development and introduction into production of the first Ukrainian vaccine "Live vaccine against animal anthrax from the K-79Z strain" (author academician

A.I. Zaviriukha), outbreaks of anthrax were registered annually, mainly among cattle (71.7%) [15]. Thus, in 1999-2009, the largest number of outbreaks (53.8-75%) was observed in the Chernivtsi, Vinnytsia, Kharkiv, Cherkasy, Luhansk, Mykolaiv, Rivne, Donetsk, Kirovohrad, and Kyiv regions of Ukraine [15]. In addition, animals infected with anthrax and the products of their forced slaughter infected and sickened people – 30 cases of the disease over 20 years in 9 regions of the country [15]. Outbreaks were registered mainly due to insufficient intensity and duration of specific immunity formed after vaccination with the vaccine "Live vaccine against anthrax from the STI strain" of Agrovet LLC, which was used until 1998 [9]. The intensive spread of anthrax on the territory of Ukraine was also facilitated by black earth soils, which are a suitable environment for the presence of *B. anthracis* spores [1; 9; 15].

The basis for the prevention and control of anthrax is vaccination of animals. Modern commercial anthrax vaccines are made from spores of vaccine strains. A distinctive feature of the biology of the anthrax pathogen is the production of extracellular toxin [16; 17].

The use of bacterial exotoxins has become an innovative approach in the treatment and prevention of many diseases of viral and bacterial aetiology. In Ukraine, spore vaccines from strains Sterne, K-79 Z, UA-07, SB are used for preventive and forced vaccinations of animals against anthrax. Despite the effectiveness of modern anti-selective vaccines, they need to be improved. An important criterion is an increase in the duration of immunity, an increase in the production of protective antigen by vaccine producing strains.

The purpose of this study is to investigate the protective properties of the experimental vaccines and the abacillary vaccine "Antracol" and to prove the immunogenic effect of the extracellular toxin from the *B. anthracis* K-79 Z strain, which is part of this vaccine preparation.

Literature Review

The main means of preventing and controlling anthrax is vaccination of animals, and in case of infection – use of antibiotics. The most effective against *B. anthracis* are penicillin, amoxicillin, levofloxacin, and ciprofloxacin. It is proved that anthrax is a toxigenic disease, in which the cessation of bacterial growth in the host/carrier body under the action of antibiotics does not mean a positive dynamic of the disease. If the toxins enter the host/carrier cell in sufficient quantities, they can show their pathogenic effects for up to 5 days [18-20]. Anthrax spores persist in soil or water for hundreds of years. Anthrax spores can withstand direct sunlight for 10 days. They can also be stored for years in animal corpses, wool, and salted meat. Anthrax spores germinate after entering the body through damaged areas of the skin (cutaneous anthrax) either by air (inhaled anthrax) or with food (gastrointestinal form of anthrax). Most spores germinate within 48 hours, but this pathway of pathogen development can occur up to 60 days. While the inhaled form of anthrax almost always results in a lethal outcome, the intestinal form is fatal in 25-60% of cases. Up to 20% of patients with the cutaneous form of anthrax die, and the remaining animals recover [21].

In Ukraine, spore vaccines from strains Sterne, K-79 Z, UA-07, SB are used for preventive and forced vaccinations of animals against anthrax [22; 23]. A distinctive

feature of the biology of the anthrax pathogen (field and vaccine strains) is the production of an extracellular toxin. This product of the metabolism of the vegetative form of bacilli consists of three fractions: a) protective, b) edematous, and c) lethal. Both field and vaccine strains of the anthrax pathogen can produce a toxin. *B. anthracis* has two major virulence factors: a poly- γ -D-glutamic acid capsule and a tripartite toxin [23-25]. The capsule plays an anti-phagocytic role, which allows bacteria to avoid absorption by macrophages. The tripartite toxin includes protective antigen (PA), lethal factor (LF) and edema factor (EF) [26]. These three proteins combine to form two toxins, a lethal toxin (LT, a combination of PA and LF) and an edematous toxin (ET, a combination of PA and EF). Toxins alter the signalling pathways of cells in a living organism to interfere with the innate immune response in the initial stages of infection and cause vascular collapse in the later stages of the disease. Toxins affect the innate and adaptive immune systems and the subsequent effects of the disease. Numerous studies prove that the virulence of the anthrax pathogen, which has lost the ability to form a capsule, decreases, while its immunogenic properties are preserved [27; 28]. Despite the effectiveness of modern vaccines, scientists are searching for the development of the most areactogenic preparations, namely the improvement of anthrax vaccines to reduce irritation and inflammation at the injection site. Similar reactions occur in case of an edematous and lethal factor [29-31]. Another important criterion is an increase in the duration of immunity, an increase in the production of protective antigen by vaccine producing strains. Such opportunities are provided by genetic engineering methods, since manipulation with the genes of plasmids pXO1 and pXO2 allows increasing the immunogenicity of anthrax vaccine strains by introducing added, foreign genetic fragments [32; 33]. Modern commercial anthrax vaccines are made from spores of the vaccine strains and are a spore suspension preserved in 30% glycerol with or without the addition of appropriate adjuvants. The reliability of such vaccines and the prolongation of post-vaccination immunity primarily depends on the immunobiological properties of the anthrax strain used for vaccine production. Almost all anthrax vaccines in use and under development are based on the production of the exotoxin PA as the main immunogen. The BioThrax vaccine in the US (formerly known as AVA) and the similar AVP vaccine in the UK are alum-adsorbed culture filtrates of vaccine strains of the anthrax pathogen that contain PA. Earlier studies have shown that exotoxin immunization of various animal models provides protection against anthrax infection [29-31]. A post-vaccination correlation of PA antibody titres has also been established [32; 33]. In new generations of vaccines, improvements are aimed at reducing toxicity, allergenic action, methods, and doses of administration and compliance with modern requirements for the quality of immunobiological preparations [34-36].

The creation of vaccines against anthrax based on mutant variants of PA exotoxin, which have lower toxicity and greater immunogenicity, is a promising area in the development and improvement of vaccines. Preclinical and clinical trials of vaccine candidates are being conducted [37-39]. Protein-based subunit vaccines are a safe alternative. They are less reactogenic, are characterized by

an immunogenic orientation, belong to purified bacterial preparations and, as a rule, do not cause side immunological effects during immunization. Nanolipoprotein particles (NLP) containing Toll-like receptor 4 agonist monophosphoryl lipid A (MPLA) were used as a platform for intranasal vaccination against *Bacillus anthracis*. Modified lipids made it possible to attach different antigens of spore proteins and toxins. Administration of combinations of constructs induced responses of several antigens, indicating the potential for a polyvalent vaccine. No off-target responses to the NLP framework protein were detected. Thus, the NLP platform has been proven to enhance humoral and mucosal responses to intranasal immunization. This indicates the promise of NLP as a flexible and reliable vaccine platform against *B. anthracis* [40].

Protein-based subunit vaccines are a safer alternative because they are less reactogenic, characterized by immunogenicity, belong to purified bacterial preparations and, as a rule, do not cause adverse immunological effects after immunization. Such preparations show a low allergenic effect and reduced toxicity to the body of laboratory animals. The limitations of physiological instability and low immunogenicity of such vaccines require an efficient delivery system to stimulate strong immune responses. Trials of an antigen delivery system based on the bacteriophage T4 capsid have been reported [40], with a high probability of eliciting both humoral and cellular immune responses without adjuvant. Such an anthrax vaccine, using a T4 capsid platform to display and deliver the protective antigen 83 kDa PA, a key component of the anthrax toxin, can be used as a rapid response preparation [40].

The US-licensed anthrax vaccine adsorbed (AVA) and UK anthrax vaccine precipitated (AVP) can generate a protective immune response but are associated with side effects. This refers to the reactogenicity of vaccines and the frequency of administration, age restrictions. Therefore, work on the investigation and improvement of preparations continues [41].

Production of anthrax vaccine (AVP) in the United Kingdom has focused on the production of PA from the *Bacillus anthracis* Sterne strain. Although it has been used for decades, some fundamental properties of AVP are understudied, including its exact composition and which proteins other than PA may contribute to protection. Recent studies have shown [42-44] that the effectiveness of AVP in humans may depend not only on PA. Firstly, there is strong evidence that LF plays a protective role, and computational predictions suggest that added proteins may be important in individuals with specific combinations of HLA alleles [43; 45; 46]. Secondly, despite the differences in the sequences of key antigenic proteins in different strains of *B. anthracis*, it is unlikely that they will affect the cross-strain protection provided by AVP [47-49].

Currently, scientific studies of prophylactic preparations against anthrax AV 7909 are being conducted as next-generation vaccines for post-exposure prophylaxis (PEP) [26]. AV 7909 consists of bulk drug substance adsorbed to anthrax vaccine (AVA, BioThrax®) with an adjuvant immunostimulatory oligodeoxynucleotide (ODN) compound, CPG 7909. The addition of CPG 7909 makes AV 7909 an acceptable next-generation vaccine for use in post-exposure prophylaxis. Immunization with AV 7909 induced a rapid protective response of toxin-neutralizing antibodies

in guinea pigs. The toxin-neutralizing antibody threshold associated with a 70% chance of survival for AV 7909 immunized animals was found to be significantly lower than that established for the licensed AVA vaccine [26; 35].

The use of bacterial exotoxins has become an innovative approach in the treatment and prevention of many diseases of viral and bacterial aetiology. Experimental preparations containing exotoxins demonstrate *in vivo* rapid growth of macrophages, and *in vitro* – high preventive activity and formation of immunity in laboratory animals. It has been proven that PA is a harmless subunit of the toxin that causes a protective immune response and constitutes the basis for all preventive measures against anthrax. Accordingly, anthrax protein vaccines licensed for human use include partially purified PA fortified with various adjuvants [44; 45].

Materials and Methods

The study was conducted at the State Scientific Institution (SSI) “State Center for Innovative Biotechnologies” during 2018-2021 under state budget topics: No. 0111U0006386 “Scientific research to develop methods of early prevention and treatment of infectious diseases common to humans and animals” and No. 0119U100900 “Exotoxin of the causative agent of anthrax. Characterization, identity between strains, an alternative to antibiotic therapy”.

The extracellular toxins of *B. anthracis* have become the basis for new experimental preparations, which are an alternative to the use of antibiotics in case of anthrax outbreaks and are not limited in applications.

The following vaccines against anthrax were used: *B. anthracis* 55 (FRCVM), *B. anthracis* SB (Sumy biofactory, Ukraine), *B. anthracis* K-79 Z (Ukraine, Kherson biofactory) and “Antracol”. An experimental series of bacterial-free (abacilar) vaccine “Antracol” was created at the State Scientific Institution “State Centre of Innovative Biotechnologies”. The inventors of this preparation are A.I. Zaviriukha, H.A. Zaviriukha. The main feature of “Antracol” is that the vaccine is abacillary, contains products of cultivation of the vaccine strain and differs in that the titre of anthrax protein in the products of cultivation of this strain is 1 : 2 – 1 : 512. The component of the vaccine is the extracellular toxin – the protective antigen of *B. anthracis* K-79 Z, deposited by the State Scientific Control Institute of Biotechnology and Strains of Microorganisms (State Scientific Control Institute of Biotechnology and Strains of Microorganisms) under No. 069. There are no analogues of this vaccine in Ukraine.

Vaccine preparations were manufactured per TUU 46.15.132.96 [50]. Exotoxins of vaccine strains *B. anthracis* 55, *B. anthracis* 34F₂, *B. anthracis* K-79 Z, virulent strain *B. anthracis* 92 Z were used.

Before conducting the experiment, the experimental cultures were tested for purity by inoculation on nutrient media: Nutrient Agar HIMEDIA, Nutrient Broth HIMEDIA, Meat-peptone broth (MPB), Meat-peptone agar (MPA).

Cultures were involved in the experiment, in which the number of microbial cells in 1 cm³ of culture liquid was as follows: *B. anthracis* K-79 Z – 30.5×10⁶ colony-forming units (CFU), for *B. anthracis* 55 – 114×10⁶ CFU, *B. anthracis* SB – 20.4×10⁶ CFU. The specified number of microorganisms was used to obtain exotoxins with different titres. Exotoxins of the specified strains were obtained by filtration using

a bacterial candle [51]. After figuring out the concentration of total protein in the exotoxins of *B. anthracis* vaccine strains, the following studies were conducted on guinea pigs to investigate the effect of metabolites of the anthrax pathogen on their bodies when infected with the virulent *B. anthracis* 92 Z strain. Experimental guinea pigs were injected with exotoxins of vaccine strains in different titres. Subsequently, the animals were infected with a virulent *B. anthracis* 92 Z strain. *Cavia porcellus* guinea pigs were used as laboratory animals (n = 80, m = 324.4 ± 2.30 g) and (n = 30, m = 334 ± 2.26 g).

The animals were given exotoxins of vaccine strains in different titres. Titres were identified by the Ascoli reaction or disc reaction-precipitation (DRP). From the vaccine strains *B. anthracis* 55, *B. anthracis* 34F₂, *B. anthracis* SB and K-79 Z, suspensions were prepared for experiments, which were checked for purity and typicality of growth, motility, and the number of microbial cells in 1 cm³ was determined. Glycerin was added to the spore suspension to obtain a final concentration of 30%. Each vaccine contained 10 million live spores in 1 cm³. Immunization was performed subcutaneously in a dose of 1 cm³ near the base of the tail. The “Antracol” vaccine is abacillary and contains the extracellular toxin of *B. anthracis* K-79 Z in a titre 1 : 2 – 1 : 256, a specific anthrax protein by the disc reaction-precipitation or Ascoli.

A virulent strain of *B. anthracis* 92 Z (SSI National Center for Innovative Biotechnologies) was used to infect laboratory animals. To compare it with vaccines used on the territory of Ukraine, employees conducted an experiment with infection of laboratory animals. Guinea pigs were vaccinated with the above-mentioned vaccines, and then, three days later, they were infected with a pathogenic strain of anthrax.

To investigate the immunogenic effect of experimental vaccines, animals were placed in six sections, 5 animals for each vaccine preparation, including control. The study was conducted on clinically healthy laboratory guinea pigs. Before starting the study, each animal was weighed, and groups of analogues were formed. The animals were placed in separate boxes for 6 days (quarantine), provided with a balanced diet and pathogen-free drinking water. In the room where the animals were located, the temperature was at 24°C.

Animals of the control group were injected subcutaneously with a 0.9% solution of sodium chloride in the amount of 1 cm³ around the base of the tail. A virulent strain of *B. anthracis* 92 Z (SSI National Centre for Innovative Biotechnologies) was used to infect laboratory animals. The dose of infection with this pathogen was 500,000 spores (LD₁₀₀). The strain was injected in the amount of 0.5 cm³ into the abdomen 72 hours after vaccination. The animals were observed for 10 days. Experiments on animals were conducted in the vivarium of the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences.

Experiments on laboratory animals were conducted according to the general principles of animal experiments, considering the requirements of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes” (Strasbourg, 1986) [52], “European Convention for the Protection of Domestic Animals” (2013) [53] and the Law of Ukraine “On the Protection of Animals from Cruelty” (2006) [54]. Statistical processing of experimental results was performed using the Microsoft Excel program.

Results and Discussion

According to the results of experimental studies, it was established that epizootic strains of *B. anthracis* do not produce enough exotoxin during cultivation in liquid nutrient media. Whereas the vaccine strains of anthrax produce a

toxin with a different titre, which is determined by the disc reaction-precipitation.

According to the results presented in Table 1, the total protein concentration in exotoxins of vaccine strains is not the same.

Table 1. Total exotoxin protein of vaccine strains *B. anthracis* K-79 Z, *B. anthracis* SB and *B. anthracis* 55

No.	Strain	Titre for the disc reaction-precipitation	Total protein concentration, mg/ml
1	<i>B. anthracis</i> K-79Z	1 : 32	0.39
2	<i>B. anthracis</i> K-79Z	1 : 64	0.20
3	<i>B. anthracis</i> K-79Z	1 : 128	0.19
4	<i>B. anthracis</i> SB	1 : 16	0.56
5	<i>B. anthracis</i> SB	1 : 32	0.40
6	<i>B. anthracis</i> 55	1 : 16	0.50
7	Nutrient broth	–	0.57

Note: The study was conducted in compliance with the rules of asepsis and antiseptics

As Table 1 shows, a lower total protein concentration is set at a titre 1 : 32. The exotoxin of *B. anthracis* 55 strain with a titre of 1 : 16 is characterized by a high concentration of total protein. The study of exotoxin *B. anthracis* K-79 Z showed that the concentration of the total protein decreases as the titre of the specific anthrax antigen increases. Its lowest content was found in the exotoxin of the

B. anthracis K-79 Z strain with a titre 1 : 128. This indicates the effectiveness of using this vaccine strain to produce the exotoxin with the highest titre.

Table 2 presents the results of experimental studies on the effect of anthrax pathogen metabolites on the body of guinea pigs during their infection with the virulent strain *B. anthracis* 92 Z.

Table 2. The effect of metabolites of the anthrax pathogen on the body of guinea pigs during their infection with the virulent *B. anthracis* 92 Z strain (n = 80, m = 324.4 ± 2.30 g)

Group No.	Number of animals, n	First and second immunization, volume	Exotoxin, titre	LD infection with <i>B. anthracis</i> 92Z	Died	Survived
1	5	1 cm ³ + 1 cm ³	0.9% NaCl	LD ₁₀₀ (500×10 ³ CFU)	5	–
2	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> K-79 Z, 1 : 32	LD ₁₀₀ (500×10 ³ CFU)	5	–
3	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> K-79 Z, 1 : 64	LD ₁₀₀ (500×10 ³ CFU)	5	–
4	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> K-79 Z, 1 : 128	LD ₅₀ (250×10 ³ CFU)	–	5
5	5	1 cm ³ + 1 cm ³	0.9% NaCl	LD ₅₀ (250×10 ³ CFU); 2 heads infected with LD ₂₅ (125×10 ³ CFU)	5	–
6	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> K-79 Z, 1 : 32	LD ₅₀ (250×10 ³ CFU);	5	–
7	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> K-79 Z, 1 : 64	LD ₅₀ (250×10 ³ CFU);	–	5
8	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> K-79 Z, 1 : 128	LD ₅₀ (250×10 ³ CFU);	–	5
9	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> 55, 1 : 16	LD ₅₀ (250×10 ³ CFU);	5	–
10	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> 34F ₂ , 1 : 16	LD ₅₀ (250×10 ³ CFU);	5	–
11	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> 34F ₂ , 1 : 16	LD ₁₀₀ (500×10 ³ CFU)	5	–
12	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> 55, 1 : 16	LD ₁₀₀ (500×10 ³ CFU)	5	–

Table 2, Continued

Group No.	Number of animals, n	First and second immunization, volume	Exotoxin, titre	LD infection with <i>B. anthracis</i> 92Z	Died	Survived
13	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> K-79 Z, 1 : 128	LD ₂₅ (125×10 ³ CFU)	–	5
14	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> K-79 Z, 1 : 64	LD ₂₅ (125×10 ³ CFU)	–	5
15	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> K-79 Z, 1 : 34	LD ₂₅ (125×10 ³ CFU)	5	–
16	5	1 cm ³ + 1 cm ³	<i>B. anthracis</i> 34F ₂ , 1 : 16	LD ₂₅ (125×10 ³ CFU)	5	–
Total	80				55	25

Note: Strains were cultured, and exotoxins were obtained in nutrient broth at 37.0°C for 48 hours

The results of experimental studies presented in Table 2 show that the exotoxin of the vaccine strain *B. anthracis* K-79 Z in a titre 1 : 64-1 : 128 has the best protective properties against the pathogenic *B. anthracis* 92 Z strain.

Vaccine strains of foreign origin (*B. anthracis* 34F₂

and *B. anthracis* 55) do not produce exotoxins in high titres according to the Ascoli reaction, and therefore they do not have sufficient immunogenic properties. The results of experimental studies on testing anthrax vaccines for the formation of immunity in animals are presented in Table 3.

Table 3. Results of testing anthrax vaccines for the formation of immunity in animals (n = 30, m = 334±2.26 g), M ± m.

Vaccine	Day										M±m	P ≥	
	Number of vaccinated animals that survived infection, %												
	1	2	3	4	5	6	7	8	9	10			
<i>B. anthracis</i> 55	5, 100%	5, 100%	5, 100%	1, 20%	1, 20%	0	0	0	0	0	0	2.1 ± 1.84	≥ 0.99
<i>B. anthracis</i> 34F ₂ Sterne	5, 100%	5, 100%	5, 100%	4, 80%	1, 20%	1, 20%	0	0	0	0	0	2.1 ± 1.84	≥ 0.96
<i>B. anthracis</i> SB	5, 100%	5, 100%	5, 100%	3, 60%	3, 60%	2, 50%	2, 50%	2, 50%	2, 50%	2, 50%	2, 50%	3.11 ± 1.09	≥ 0.66
<i>B. anthracis</i> K-79 Z	5, 100%	5, 100%	5, 100%	3, 60%	3, 60%	3, 60%	3, 60%	3, 60%	3, 60%	3, 60%	3, 60%	3.6 ± 0.73	≥ 0.47
Anthracosis	5, 100%	5, 100%	5, 100%	5, 100%	4, 80%	3, 60%	3, 60%	3, 60%	3, 60%	3, 60%	3, 60%	3.6 ± 0.73	≥ 0.47
Control	5, 100%	5, 100%	5, 100%	3, 60%	2, 50%	1, 20%	1, 20%	0	0	0	0	2.2 ± 1.6	

Note: 10 days before vaccination, animals should not be given antibiotics, they were kept in quarantine for 10 days before the start of the experiment

The results of experimental studies presented in Table 3 indicate that the vaccine from the *B. anthracis* 55 strain has the least immunogenic activity. Three days after infection (five days after immunization), only one guinea pig survived, i.e., 20% of the total number of animals involved in the experiment. On Day 6, all animals died.

The vaccine from the *B. anthracis* 34F₂ Sterne strain has an average level of immunogenic activity. Three days after infection of guinea pigs with the pathogenic strain, not a single animal died, the protection was 100%.

On Day 4, 1 animal died, protection was 80%. On Days 5 and 6, one guinea pig in each group survived, which accounted for 20% of the protection, and on Day 7, all experimental animals died (Table 3).

The vaccine strains *B. anthracis* SB and *B. anthracis* K-79 Z have almost the same level of protection of laboratory animals during experimental infection. Three days after infection of guinea pigs with the pathogenic strain, not a single animal died, the protection was 100%. On Day 5, 2 animals died in each group, which accounted for 60% of the protection. Starting from Day 6 and until the end of experimental studies, not a single guinea pig died. It can be assumed that these are genetically related strains.

The abacillary vaccine “Antracol”, made from the extracellular toxin of *B. anthracis* K-79 Z, protects infected guinea pigs during the first three days – 100%. On Day 10 after the introduction of the pathogenic strain, 60% of guinea pigs stayed alive in the experiment.

In the control group of animals injected with 0.9% normal saline (NaCl), 100% death of guinea pigs was recorded on Day 8 of experimental studies (Table 1).

Considering the world experience in the use of exotoxins of pathogenic microorganisms to combat infectious diseases of various aetiologies, the staff of the SSI State Centre of Innovative Biotechnologies developed the preparation “Antracol”, the basis of which is the exotoxin of *Bacillus anthracis*. The “Antracol” vaccine has demonstrated its advantages in the formation of immunity in experimental animals (guinea pigs). The “Antracol” vaccine included the life products of the virulent strain *E. coli* IVM-1, deposited in the State Scientific Control Institute of Biotechnology and Strains of Microorganisms, and the vaccine strain *B. anthracis* K-79 Z. The use of the “Antracol” vaccine is accompanied by the creation of antitoxic immunity against the causative agent of anthrax. The formation of immunity begins 3-4 hours after vaccination, due to the presence of a specific antigen (exotoxin) in the vaccine. The use of the “Antracol” vaccine to fight anthrax neutralizes the adverse impact of the disease on animal health and reduces economic losses. During an outbreak of anthrax in farms where the “Antracol” vaccine is used, the epizootic situation normalizes, and losses from the disease decrease. “Antracol” vaccine is an abacillary preparation for the rapid creation of anti-anthrax immunity in the foci of an anthrax outbreak, in field conditions when there is a threat of mass infection of animals, and for the immunization of animals for which the use of live spore vaccine is prohibited – exhausted, deep-bodied, in difficult working conditions, young animals up to 8 months old, etc. Vaccines against anthrax used in farms of Ukraine are live spore vaccines (Vaccine against animal anthrax from the strain “Sterne 34F₂”, Live spore vaccine against animal anthrax from the strain “SB”, Vaccine against animal anthrax from the strain *Bacillus anthracis* UA-07 “Antravak”, Vaccine against animal anthrax from strain K-79Z), is used to create active immunity against anthrax in farm animals. Immunity in animals occurs 10 days after vaccination, they are not used as emergency preparations.

Analysing world literary sources [42; 43], regarding the construction of an abacillary vaccine, it was concluded that prophylactic preparations based on *Bacillus anthracis* metabolite products are not manufactured in Ukraine.

According to the authors [43], *B. anthracis* “Sterne 34F₂” is a strain traditionally used to produce vaccines. The authors of the “AVA” vaccine registered allergic reactions in volunteers after its use. Scientists at the SSI State Centre of Innovative Biotechnologies have proven that it has a high concentration of total protein and does not produce exotoxin in high titres, unlike *B. anthracis* K-79 Z.

As a result of the conducted studies, it was established that the *B. anthracis* K-79 Z strain does not cause an allergic reaction in laboratory animals and provides their protection at a high titre (60%).

The main factor in the reactogenicity of modern anti-anthrax vaccines is the features of the vaccine strains used in the spore suspension with the addition of preservatives. Therefore, the search for both exotoxin producers and compositions of exotoxin compounds continues [37-39], both according to indicators of an allergic reaction and reduction

of post-vaccination consequences [34-36], also considering the duration of the protective effect and improving the compositions [27; 28]. Immunization of animals [29-31] shows a more efficacious influence of exotoxin as the main component of the preparation or the main ingredient of the composition [32; 33].

The “Antracol” vaccine does not contain a live spore culture of the anthrax vaccine strain and is composed of exotoxins without chemical preservatives that can cause post-vaccination complications. The study of exotoxins of vaccine strains and exotoxin as a component of the preparation is relevant and synergizes with the research of foreign scientists (USA, Great Britain, Belgium) [32; 33]. The search for the safest and most universal preparation against anthrax continues today around the world.

Conclusions

The protective function of the vaccine preparation against anthrax depends on the protective features of the vaccine strain included in its composition. The “Antracol” vaccine, which includes the extracellular toxin of *B. anthracis* K-79 Z in a titre 1 : 64 – 1 : 128, has increased protection against the pathogenic strain of *B. anthracis* 92 Z, which forms a capsule.

The lowest level of total protein was found in the exotoxin of the *B. anthracis* K-79 Z strain with a titre 1 : 128 – 0.19 mg/ml. While during the study of the exotoxin from *B. anthracis* SB, a lower concentration of total protein was noted at a titre 1 : 32 – 0.4 mg/ml, and *B. anthracis* 55 – 1 : 16 has a high concentration of total protein – 0.5 mg/ml. This characteristic of *B. anthracis* K-79 Z provides an advantage for the use of its metabolic products during the production of prophylactic preparations.

Vaccine strains *B. anthracis* 34F₂ Sterne and *B. anthracis* 55 do not produce exotoxins in high titres according to the Ascoli reaction and have an average level of immunogenic activity.

A comparison of vaccines, spore (traditional) with the abacillary vaccine “Antracol” showed that during infection of *B. anthracis* 92 Z animals that were vaccinated, 60% of protection was registered when using prophylactic preparations from strains of *B. anthracis* SB and *B. anthracis* K-79 Z, as well as “Antracol”.

Based on the study results, scientists at SSI “State Centre for Innovative Biotechnologies” can convincingly claim that the absence of spores in vaccines based on exotoxins allows solving the problems of vaccinating all types of mammals, including animals of different age groups and regardless of immune status. In the future, research on exotoxins of *B. anthracis* will be continued with the purpose of creating new anti-anthrax preparations. Continuation of the study of preparations based on exotoxins, with the purpose of improvement and implementation in the practice of veterinary medicine.

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Перспектива використання екзотоксину *B. anthracis* у конструюванні протисибіркових препаратів екстреної дії

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Анотація. Актуальність дослідження зумовлена тим, що на території України періодично реєструються спалахи сибірки, не тільки в жуйних тварин, а й в свиней, хутрових звірів, собак і людей. Мета роботи – провести дослідження захисних властивостей дослідних вакцин і абацільярної вакцини «Антракол» і довести імуногенну дію екстрацелюлярного токсину зі штаму *B. anthracis* K-79 Z. Для дослідів використовували культури вакцинних штамів антракса: *B. anthracis* 55, *B. anthracis* CB, *B. anthracis* K-79 Z і вакцину «Антракол» (експериментальна розробка). В роботі використовували мікробіологічні, клініко-біологічні та біотехнологічні методи досліджень. Захисну дію вивчали на мурчаках (*Cavia porcellus*). Проведено гострий дослід із вірулентним штамом *B. anthracis* 92 Z. Від зазначених культур отримали екзотоксин. Його титр визначали в реакції диск-преципітації. Найвищий результат щодо продукції екзотоксину зафіксовано у *B. anthracis* K-79 Z 1 : 128 за концентрації сумарного білка – 0,19 мг/мл, тоді як екзотоксин штаму *B. anthracis* 55 з титром 1 : 32 показав високу концентрацію сумарного білка – 0,4 мг/мл. Вплив екзотоксинів *B. anthracis* на організм вивчали шляхом їх введення лабораторним тваринам в різних титрах екзотоксинів із наступним зараженням патогенним штамом *B. anthracis* 92 Z. Екзотоксин вакцинного штаму *B. anthracis* K-79 Z в титрі 1 : 64 – 1 : 128 виявляє найкращі захисні властивості проти патогенного штаму. Встановлено, що вакцинні штами *B. anthracis* CB і *B. anthracis* K-79 Z володіють однаковим рівнем захисту лабораторних тварин за експериментального зараження, що становить 60 %, тоді як вакцина зі штаму *B. anthracis* 34F2 показала рівень захисту 20 %. За результатами проведених досліджень виявилось доцільним використовувати екзотоксин *B. anthracis* у розробці профілактичних препаратів щодо сибірки. Результати досліджень можуть бути використані науковцями і спеціалістами в галузі ветеринарної медицини у розробці нових та вдосконаленні наявних вакцин для ефективного профілактики сибірки *B. anthracis*

Ключові слова: сибірка, метаболіти, превентивна дія, абацільярна вакцина «Антракол»