

DETERMINATION OF THE OPTIMUM TEMPERATURE FAVORABLE FOR THE DEVELOPMENT OF SPORES OF THE *BACILLUS ANTHRACIS* VACCINE STRAIN

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Annotation. Animal husbandry is one of the main branches of agriculture in the Republic of Kazakhstan, in connection with which the primary task of the country's veterinary service is the prevention of infectious diseases of animals, primarily zoonanthropous. Anthrax of farm animals (the Kazakh name is topalan) is one of these diseases, and the fight against it has not only epizootological, but also epidemic, environmental, economic and great social significance.

There are data of domestic and foreign researchers devoted to the topic of cultivation of the anthrax pathogen, but they poorly show the influence of the temperature regime on the increase in the number of viable spores. This article reflects the results of experiments with VNIIVVM strain 55 to achieve the above goal. The studies were carried out using generally accepted methods in compliance with biological safety standards. It was found that when cultivating VNIIVVM strain 55 at $+32 \pm 1^{\circ}\text{C}$, for 96 hours, the highest sporadic rate was observed at the level of 91%. The use of the established cultivation regime will reduce the cost of vaccine production.

Keywords: bacterial mass, spores, temperature factor, topalan, vaccine strain.

Introduction. Despite the fact that more than 150 years have passed since the discovery of the Topalan pathogen, the fight against this epidemic remains very relevant to this day, as the ways of its complete eradication are one of the most urgent problems not only in our country, but also in other countries of the world. Topalan epidemic is a very contagious disease that affects the skin, intestines, lungs and runs septic. The causative agent is a bacillus that forms spores and capsules. Spores of Topalan are very stable in the external environment, they can persist in the soil for up to a hundred years or more.

The natural focus of the Topalan bacillus is the soil. The source of the epidemic is cattle, horses, camels, sheep and goats among domestic animals. Wild, carnivorous animals, laboratory animals, and humans are susceptible to Topalan. Sick animals excrete the pathogen through urine, feces, and other fluids. Humans become infected with topalan when keeping, slaughtering, butchering, and handling animal products (hides, wool, bristles). The outbreak can be transmitted through soil, in which case the spores penetrate through the affected skin. Topalan can be transmitted through poorly cooked meat and unboiled milk and by the aerogenic route.

According to the International Epizootic Bureau, UN FAO, and the International Health Organization, 5-6 cases of topalan epidemics are registered annually in all countries of the world. Annually, anthrax kills more than a million animals and sickens about 80 thousand people (Ipotenko N.G., 1980).

Among modern anti-epizootic measures, the most basic measure by countries of the world is immunization of animals against topalan. For this reason, improvement of the methods of obtaining the vaccine used for this purpose is considered a very urgent issue.

According to many scientists, the most important of the anti-epizootic measures during an epidemic is the vaccination of animals that have survived the epidemic.

Brauell F. A. [1], Koch R. [2], Pasteur L. [3], Tsenkovsky L. S. [4], Ginzburg N. N. [5], Kolesov S. G. [6], Bakulov I. A. [7], Gavrilov V. A. [8], Ipotenko N. G. [9], Manichev A. A. [10] and other scientists conducted research to study the pathogen, to improve control measures, and to produce vaccine.

As a result of numerous studies, VNIIVVM strain 55 without capsule was obtained from the All-Union Scientific Research Institute of Veterinary Virology and Microbiology in 1983. The authors of the strain were I. A. Bakulov, V. A. Gavrilov, and V. V. Seliverstov [11]. The vaccine developed from VNIIVVM strain 55, widely distributed in vivo, demonstrated the breadth of its immunogenic range for the vast majority of epizootically affected anthrax strains.

The most harmonious temperature of spore development in the report of Cherkassky B. L., Zhanuzakov N.N. [12] is within $+30+35^{\circ}\text{C}$. At $+30+37^{\circ}\text{C}$ sporadic development continues for 16 hours, at $+24^{\circ}\text{C}$ - 36 hours, and at $+18^{\circ}\text{C}$ - up to 50 hours. At temperatures below $+15^{\circ}\text{C}$ and above $+42^{\circ}\text{C}$, spore development does not occur. When growing cultures of the anthrax pathogen at $+42^{\circ}\text{C}$, they completely lose sporadic properties.

According to Pastukhova B. N. [13], spores of the anthrax pathogen develop at temperatures from $+12+15^{\circ}\text{C}$ to $+42.5^{\circ}\text{C}$ at a certain humidity. Spore development does not occur at temperatures below $+12^{\circ}\text{C}$ and above $+43^{\circ}\text{C}$.

The danger of anthrax is primarily associated with the severity of the course of the disease in humans and animals, high lethality, significant economic costs of treating patients and conducting anti-epidemic and anti-epizootic measures. Another aspect of this problem is the prolonged survival of the anthrax pathogen in spore form in the environment, which leads to the formation of persistent soil foci [14-20].

Turnbull P.C.B., Frawley D.A., Bull R.L. reports that estimating the true number of viable anthrax spores is complex. Optimal heat activation conditions vary with species, media and germinants. Published time/temperature combinations for *Bacillus anthracis* spores range from 60 degrees C for ≤ 90 min to boiling for 1 min. Results presented here indicate that temperatures are best kept to ≤ 70 degrees C and holding times need not exceed 15-30 min. Under conditions of 60 degrees C for 90 min, 62-23 degrees C for 15 min and 70 degrees C for 15 or 30 min, although the ratio of heated: unheated counts ranged from <1 to >1 , post-heating counts were less than their pre-heating counterparts on between 71% and 88% of occasions. A high probability was found of viable spore counts differing significantly from counts determined microscopically, with differences of almost 1 log possible. Viable counts were lower than microscopic counts in 15 of 18 tests [21].

The most basic component in vaccine development is the microbial mass obtained from the vaccine strain in special nutrient media. In this sense, the importance of improving the nutrient media in which the microbial mass can be obtained as much as possible on an industrial basis, without altering the properties of the vaccine strain necessary for the development of a vaccine drug.

The scientists note that the above data, along with other factors, influence the change in artificial nutrient media from capsule-type pathogen to spore-type, from high-yielding to low-yielding, and that they undergo culture and other changes in different nutrient media.

Recent studies have shown that the function of the capsule of the anthrax microbe in the pathogenesis of infection is not limited only to protection against phagocytosis.

The presence of an appropriate genetic apparatus and fine regulation of capsule formation allow the microbe, probably at different stages of development, to use the highly polymeric capsule

as an antiphagocytic component of pathogenicity, and the product of its depolymerization to inhibit other immune mechanisms. In addition, the interaction of the capsule with the lethal toxin of the anthrax microbe increases its toxic effect, and its effect on macrophages triggers inflammatory immune reactions. It appears that the use of enzymes that destroy the capsule of *B. anthracis* may be a means of anthrax therapy.

Particular attention in anthrax vaccine development has been given to obtaining its spore form. Although some components of the nutrient medium composition have favorable effects on spore development because of the multiplication limit and environmental conditions (pH) and temperature, the occurrence or alteration of these factors beyond the periphery promotes the transformation of spores into vegetative forms that are considered unnecessary for vaccine development.

It is therefore important to determine the temperature regime of the nutrient-containing medium that affects the development of spores used against anthrax and which are the basis of the vaccine.

Materials and methods. Materials used: *Bacillus anthracis* VNIIVVM strain 55, meat-peptone broth (MPB), meat-peptone liver broth soup (MPPB), meat-peptone agar (MPA), Sabouraud medium, autoclaves, biological microscope according to normative and technical documents, thermostat with heating temperature $37 \pm 1^\circ\text{C}$, vials with capacity of 100 ml; pipettes with capacity of 0,1; 1,0; 2,0; 5,0; 10,0 cm³ according to GOST 29230; Petri dish according to GOST 25336; glass tubes according to GOST 25336; physiological sterile solution pH 7,0-7,2; agar meat-peptone according to GOST 29112; distilled water according to GOST 6709.

To obtain a vegetative culture of anthrax pathogen VNIIVVM strain 55 was sown into meat-peptone soup medium and grown in the thermostat at $+37^\circ\text{C}$ for 18-20 hours. The concentration of microbial cells in vegetative cultures of the anthrax pathogen was determined according to the optimal model of the L.A. Tarasevich Institute of GISC.

To obtain a spore culture, the vegetative culture was grown in meat-peptone agar at $+28$ - $+37^\circ\text{C}$ in the thermostat for 12-120 hours. Every 12, 24, 36, 48, 60, 72, 81, 96, 108 and 120 hours, samples were taken from each culture grown under different temperature regimes, and the number of viable spores was determined according to the standard method.

Results. To study the dependence of the sporadic phenomenon of *Bacillus anthracis* VNIIVVM strain 55 on the temperature factor, the dynamics of spore accumulation in different temperature regimes of cultivation was tested.

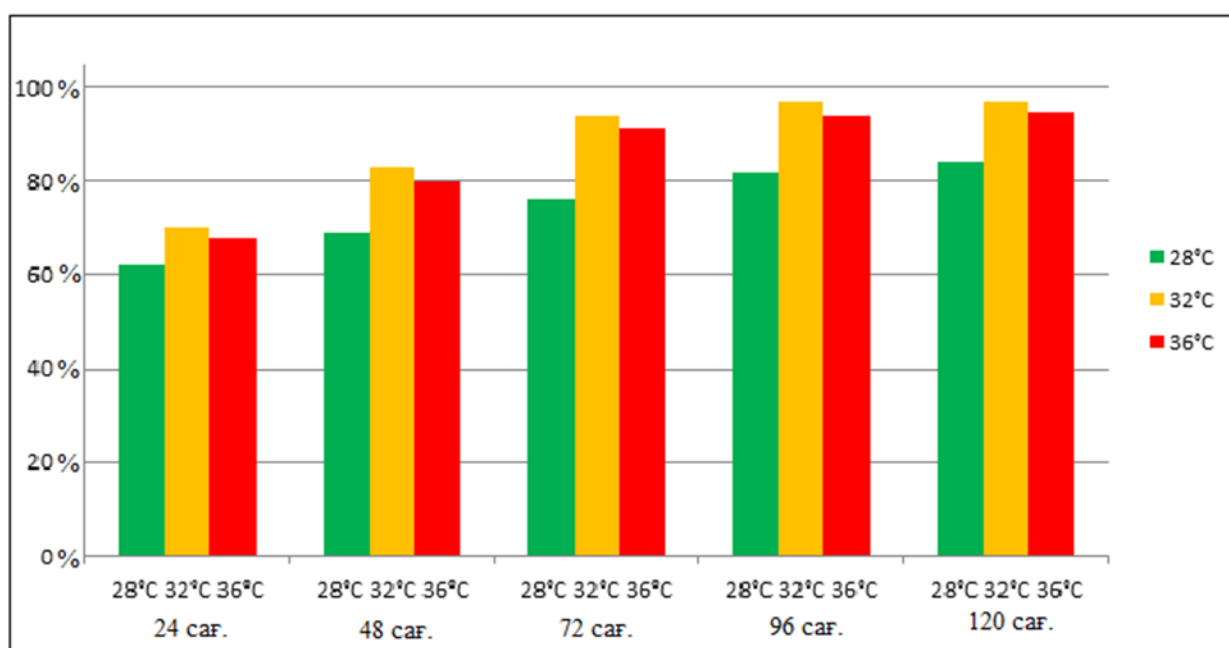
For this purpose 9 Petri dishes were extracted, poured into them meat-peptone agar nutrient medium and tested for neutralization by keeping them in the thermostat at $+37^\circ\text{C}$ for three days. *Bacillus anthracis* VNIIVVM strain 55 was inoculated into the nutrient medium on which neutralization was checked and the grown cultures were divided into three groups.

The cultures of the first group were left to determine the level of sporadicity in a thermostat at $+28 \pm 1^\circ\text{C}$, the cultures of the second group were left in a thermostat at $+32 \pm 1^\circ\text{C}$, and the cultures of the third group were left in a thermostat at $+36 \pm 1^\circ\text{C}$ to determine the level of sporadicity.

From each culture grown under different temperature regimes, samples were taken every 12, 24, 36, 48, 60, 72, 81, 96, 108 and 120 hours, in which the number of viable spores was determined according to the standard method.

For ease of analysis and summarization, the percentage of the number of life-prone spores in the total number of spores detected was determined. The results of the experiment are presented in Diagram 1.

As can be seen from the diagram, when cultivating *Bacillus anthracis* VNIIVVM strain 55 in different temperature regimes, the highest sporadicity rate (91%) was found when cultures were grown in a thermostat with a temperature of $+32 \pm 1^\circ\text{C}$. When the cultures were grown at $+36 \pm 1^\circ\text{C}$, the rate was slightly lower at 87%.



Note: car-hours

Diagram 1 – Dependence of sporadics of vaccine strain 55VNIIVVIM on temperature regime

The lowest rate was determined when shoots were grown in thermostat with temperature $+28\pm 1^{\circ}\text{C}$, it did not exceed 68 percent. The highest sporadicity rate was found when shoots were grown for 96 hours (4 days). When crops were grown for 5 days (120 h) and 6 days (144 h), no increase in sporadicity rate was found in samples taken on the respective days. Although the stable temperature regime for pathogen growth is $+36\pm 1^{\circ}\text{C}$, the high maximum sporadicity rate at $+32\pm 1^{\circ}\text{C}$ can be explained as follows. Considering that the sporadicity of the pathogen is its adaptation to an environment unfavorable for growth, it can be assumed that in this temperature regime the phenomenon of sporadicity was at a high level, because the temperature regime of $+32\pm 1^{\circ}\text{C}$ is a relatively unfavorable environment for the growth of VNIIVVIM strain 55 compared to the temperature regime of $+36\pm 1^{\circ}\text{C}$.

Based on the results of this study, to obtain the maximum number of spores from cultures of VNIIVVIM strain 55, it is recommended to grow this strain in a mode with a temperature of $+32\pm 1^{\circ}\text{C}$.

The maximum level of spore content when growing in the thermostat of VNIIVVIM strain 55 with temperature $+32\pm 1^{\circ}\text{C}$ was determined after 96 hours (4 days). Extending the germination period of cultures for 120 hours (5 days) and 144 hours (6 days) did not have a favorable effect on increasing the level of spore formation. Therefore, incubation of cultures at $+32\pm 1^{\circ}\text{C}$ should be carried out not more than 96 hours (4 days) in order to reduce the time of vaccine development technology from the indicated strain.

Since at cultivation of vaccine strains in the temperature regime of $+32\pm 1^{\circ}\text{C}$ the content of sporadicity exceeds 90 percent, the need to add turpentine solution used to increase spore formation is eliminated. As a consequence, the vaccine under development will not contain turpentine, which adversely affects vaccinated animals, and it will be possible to simplify the technology of vaccine development.

In order to develop vaccines in large quantities industrially, the strain cultures required for the vaccine are grown not in thermostats but in special thermal rooms. The size of thermal rooms is several tens of times larger than that of thermostats and is usually 25-30 m². Maintaining the temperature in thermal rooms for up to 4 days at our recommended temperature of $+32\pm 1^{\circ}\text{C}$ rather

than $+36\pm 1^{\circ}\text{C}$ to obtain spores of the vaccine strain will save energy. Saving electricity helps to reduce the cost of the vaccine.

Discussion. In our experiments, we identified the optimal temperature factor affecting the bacterial mass correction of the vaccine strain as a group among many factors.

Sporulation is a developmental process undertaken by members of the *Bacillus* genus in response to unfavorable or nutrient-deplete growth conditions. The spore form of the bacterium is metabolically inactive, resistant to environmental stresses, and can survive until conditions are favorable for germination into a vegetative cell. Sporulation is energy exhaustive and is considered a last resort for survival. The sporulation pathway has been well-studied in the archetype *Bacillus* species, *Bacillus subtilis*. Nutrient deprivation is sensed by a multi-component signal transduction phosphorelay ultimately resulting in phosphorylation of the master response regulator Spo0A, and a commitment to sporulation. Orthologs of the signal transduction phosphorelay are found in members of the *Bacillus cereus* group, including the anthrax-causing bacterium *Bacillus anthracis*, enabling these species to sporulate in a manner similar to that of *B. subtilis*.

When conditions are not conducive to growth and multiplication of the vegetative forms of *B. anthracis*, they start to form spores. Sporulation requires the presence of free oxygen. In the natural situation, this means the vegetative cycles occur within the low oxygen environment of the infected host and, within the host, the organism is exclusively in the vegetative form. Once outside the host, sporulation commences upon exposure to the air and the spore forms are essentially the exclusive phase in the environment.

It is very largely through the uptake of spores from the environment that anthrax is contracted. Within the infected host the spores germinate to produce the vegetative forms which multiply, eventually killing the host. A proportion of the bacilli released by the dying or dead animal into the environment (usually soil under the carcass) sporulate, ready to be taken up by another animal. This uptake by the next host may happen at any time, from less than one hour to many decades later.

It is noted in the specialized scientific literature that the virulence and pathogenic effect of the anthrax pathogen is related to its capsule, exotoxin and aggressin, while immunogenicity depends on its sporogenicity. For this reason, its spore-bearing forms are used to develop an anthrax vaccine. According to N.G. Ipatenko [9], the role of spores of the anthrax pathogen is to preserve the species of bacilli in an environment unfavorable for their existence. Taking into account these conditions, in our experiments we considered the influence of the temperature factor on obtaining the maximum possible number of its spore forms for the development of a production-based vaccine from VNIIVVM strain 55.

In the reports of Ginzburg N.N. [5], Kolesov S.G. [6], Ipatenko N.G. [9], Cherkassky V.L. [12] it is noted that the group pathogen can grow and form spores within $+12+42^{\circ}\text{C}$. As you can see, the growth range of the pathogen spores is very wide—from $+12+42^{\circ}\text{C}$ to $+30^{\circ}\text{C}$. In the specialized literature there is no data on at what temperature within $+12+42^{\circ}\text{C}$ the sporadica grows well and accumulates as many spores as possible. Well, there are no contradictions of different levels in the existing data either.

Kolesov S.G. [6] states that spores of the thrush pathogen grown at $+37^{\circ}\text{C}$, sown into nutrient medium of meat-peptone agar, begin to grow in 1-2 hours, with 2-hour growth usually consisting only of vegetative species. The same phenomenon lasts up to 7 hours when lyophilic dehydrated spores are grown.

In the studies of Ginzburg N.N. [5] it is indicated that the most compatible temperature of sporadics of the pathogen topalan is $+35+37^{\circ}\text{C}$.

The data presented by Ipatenko N.G. [9] on the development of spores of the topalan pathogen, although similar to those of B.L. Cherkassky [12], have significant differences. He believes that the development of spores, along with the temperature factor, is influenced by the

peculiarities of the strain used. Development of spores ends in 1-2 hours at $+30$ – $+37^{\circ}\text{C}$, in $+24^{\circ}\text{C}$ - 16 hours, and in $+18^{\circ}\text{C}$ - up to 70 hours.

The above data indicate that the rate of sporadicity of a vaccine strain depends largely on the temperature of its cultivation. Therefore, the determination of the most stable temperature regime, at which the sporadicity of the vaccine strain occurs at the highest possible level, is of great importance in the technology of developing a high-quality vaccine.

On the basis of the conducted experiments it was established that when cultivating *Bacillus anthracis* VNIIVVM strain 55 in different temperature regimes, the highest indicator of sporadicity level (91%) was found when growing cultures in the thermostat with temperature $+32\pm 1^{\circ}\text{C}$.

Conclusion. The anthrax pathogen is a large anthrax bacillus (*Bacillus anthracis*), 6-10 μm long and 1-2 μm wide, with ends cut off at right angles, immobile, Gram-positive, forms spores and a capsule, facultative aerobe.

Vegetative forms are found in sick or dead people and animals. The bacterium has capsular and somatic antigens. The capsule distinguishes the anthrax bacterium from other members of the genus *Bacillus*; it provides the microorganism with protection from phagocytes. Vegetative forms quickly die without access to air, are sensitive to boiling (at a temperature of 75 – 80°C they die in 1 minute), chlorine and mercury-containing disinfectants.

Spores are resistant to poor environmental conditions and can persist for years on dried or treated hides and soil. Autoclaved (110°C), they die after only 40 minutes.

The dependence of the sporadic phenomenon of *Bacillus anthracis* VNIIVVM strain 55 on the temperature factor was studied, for which the dynamics of spore accumulation in different temperature regimes of cultivation was tested. For this purpose, the culture of *Bacillus anthracis* VNIIVVM strain 55 was introduced into the nutrient medium, where sterility was checked, and the grown cultures were divided into three groups.

The cultures of the first group were left to determine the level of sporadicity in a thermostat at $+28\pm 10^{\circ}\text{C}$, the cultures of the second group were left in a thermostat at $+32\pm 1^{\circ}\text{C}$ and the cultures of the third group were left in a thermostat at $+36\pm 1^{\circ}\text{C}$ to determine the level of sporadicity.

In conclusion, it was found that the maximum sporadicity rate (91%) was registered when the culture of VNIIVVM strain 55 was grown in thermostat with temperature $+32\pm 1^{\circ}\text{C}$, compared to other modes of cultivation. When the culture was grown at $+36\pm 1^{\circ}\text{C}$, this index was slightly lower and amounted to 87 percent. The lowest indicator was determined when growing shoots in thermostat with temperature $+28\pm 1^{\circ}\text{C}$, its size did not exceed 68 percent. Taking into account that sporadicity of the pathogen is its adaptation to an unfavorable environment for growth, it can be assumed that in this temperature regime the phenomenon of sporadicity was at a high level, since the temperature regime $+32\pm 1^{\circ}\text{C}$ is a relatively unfavorable environment for the growth of VNIIVVM strain 55 compared to the temperature regime $+36\pm 1^{\circ}\text{C}$.

Based on the results of this study, to obtain the maximum number of spores from the growth of VNIIVVM strain 55, it is recommended to grow sprouts of this strain in a temperature regime with a temperature of $+32\pm 1^{\circ}\text{C}$. The size of thermal rooms is several tens of times larger than that of thermostats and is usually 25-30 m^2 . Maintaining the temperature in thermal rooms for up to 4 days at our recommended temperature of $+32\pm 1^{\circ}\text{C}$ rather than $+36\pm 1^{\circ}\text{C}$ to obtain spores of the vaccine strain will save energy. Saving electricity helps to reduce the cost of the vaccine.

References:

- [1] **Brauell, F.A.** Versuche und Untersuchungen betreffend den Milrbrand des Menschen und der Tiere // Virchows Arch. Pathol. Anat. Und physiol. – 1857. – Bd. 11. – 132 p.
- [2] **Koch, P.** Cohn's Beitrage zur Pflauzen. – 1876. – V.2. – P. 276-292
- [3] **Pasteur, L., Chamberland Ch. Ed. Roux E.C.R.** Acad. Sci. – 1881. – V. 92. – P. 429-521.
- [4] **Ценковский, Л.С.** Отчет о прививках антракса в больших размерах / Сб. Херсонского земства, 1886. – В.19. – Т. 6. – С.6-12.

- [5] **Гинсбург, Н.Н.** Сибирская язва. – Москва, 1975. – С. 25-51.
- [6] **Колесов, С.Г.,** Михайлов Н.А., Преснов И.Н. Изготовление и применение сибиреязвенных вакцин // Труды ГНКИ ветеринарных препаратов. – Москва, 1961. – Т.9. – С. 159-165.
- [7] **Бакулов, И.А.,** Гаврилов В.А. Оценка эффективности 10-летнего применения вакцины против сибирской язвы из штамма 55 ВНИИВВиМ // Ветеринария, 1994. – №8. – С. 11-15.
- [8] **Гаврилов, В.А.** Иммуногенетические аспекты разработки противосибиреязвенных вакцин // Ветеринария, 1989. – № 10. – С. 27-29.
- [9] **Ипатенко, Н.Г.** Опыт профилактики сибирской язвы сельскохозяйственных животных в России // Ветеринария, 1995. – № 5. – С. 82-87.
- [10] **Маничев, А.А.** Препараты для специфической профилактики, лечения и диагностики сибирской язвы сельскохозяйственных животных: автореферат дисс. доктора вет. наук. — Москва, 1994. – 46 с.
- [11] АС. 1809555 А1, РФ. Способ изготовления вакцины против сибирской язвы животных. Бакулов И.А., Гаврилов В.А., Селиверстов В.В.; опубл. 26.12.83 РК.
- [12] **Черкасский, Б.Л.,** Жанузаков Н.Ж. Сибирская язва. – Алма-Ата: Қайнар, 1980. – С. 4-11.
- [13] **Пастухова, Б.Н.** Сибирская язва. – Москва, 1962. – 146 с.
- [14] **Шаров, Д.А.,** Лещенко А.А., Коваленко Е.А., Боровской Д.В., Крупин В.В., Багин С.В., Мохов Д.А., Лазыкин А.Г., Косенков И.В., Мошков А.Н. Разработка технологии приготовления сухой питательной среды для производства сибиреязвенной вакцины // Биопрепараты. Профилактика, диагностика, лечение, 2022. – Т. 22. – № 2. – С. 187-195.
- [15] **Задорина, И.И.** Антигенная и молекулярно-генетическая оценка стабильности вакцинного сибиреязвенного штамма ланге после длительного хранения: автореферат кандидатской диссертации. – Казань, 2020. – 25 с.
- [16] **Немировская, Т.И.,** Касина И.В., Александрова Н.В., Алексеева С.А. Анализ современных регуляторных требований к качеству живых бактериальных вакцин и перспективы их усовершенствования // Биопрепараты. Профилактика, диагностика, лечение, 2024. – 24 (4). – С. 456-466.
- [17] **Дятлов, И.А.** Современные технологические платформы для разработки вакцин против опасных бактериальных инфекций (часть II) // Бактериология, 2022. – 7 (4). – С. 5-9.
- [18] **Алексеева, С.А.,** Касина И.В., Немировская Т.И. Сравнительный анализ результатов испытаний вакцины сибиреязвенной живой по показателю «Подлинность» иммунофлуоресцентным и иммунохроматографическим методами // Биопрепараты. Профилактика, диагностика, лечение, 2024. – 24 (3). – С. 348-56.
- [19] **Касина, И.В.,** Алексеева С.А., Немировская Т.И. Теоретическое и экспериментальное обоснование перспективных методов экспертизы качества вакцины сибиреязвенной живой // Биопрепараты. Профилактика, диагностика, лечение, 2020. – 20 (4). – С. 277-84.
- [20] **Медуницын, Н.В.,** Катлинский А.В., Ворслов Л.О. Вакцинология: монография. М.: Практическая медицина, 2022. – 480 с.
- [21] **Turnbull, P.C.B.,** Frawley D.A., Bull R.L. Heat activation/shock temperatures for *Bacillus anthracis* spores and the issue of spore plate counts versus true numbers of spores // Journal of Microbiological Methods, 2006. – 68(2). – P. 353-357.

References:

- [1] **Brauell, F.A.** Verzuhe ud Untersuchunqen betreffend den Milrbrand des Menschen und der Tiere // Virchows Arch. Pathol. Anot. Und physol. – 1857. – Bd. 11. – 132 p.
- [2] **Koch, P.** Cohn's Beitrage zur Pflauzen. – 1876. – V. 2. – P. 276-292
- [3] **Pasteur, L.,** Chamberlond, Ch. Ed. Roux, E.C.R. Acad. Sci. – 1881. – V. 92. – P. 429-521.
- [4] **Tsenkovsky, L.S.** Otchet o privivkah antraksa v bol'shih razmerah // Sb. Hersonskogo zemstva, 1886. – V. 19. – T. 6. – S.6-12. [in Russian]
- [5] **Ginsburg, N.N.** Sibirskaya jazva. – Moskva, 1975. – S. 25-51. [in Russian]
- [6] **Kolesov, S.G.,** Mikhailov N.A., Presnov I.N. Izgotovlenie i primeneniye sibirejazvennyh vaksin // Trudy GNKI veterinarnykh preparatov. – Moskva, 1961. – T.9. – S. 159-165. [in Russian]

- [7] **Bakulov, I.A.**, Gavrilov V.A. Otsenka effektivnosti 10-letnego primeneniya vaktsin protiv sibirskoi jazvy iz shtamma 55 VNII VViM // Veterinaria, 1994. – №8. – S. 11-15. [in Russian]
- [8] **Gavrilov, V.A.** Immunogeneticheskie aspekty razrabotki protiv sibirezavennykh vaktsin // Veterinaria, 1989. – № 10. – S. 27-29. [in Russian]
- [9] **Ipatenko, N.G.** Opyt profilaktiki sibirskoi jazvy sel'skhozjastvennykh zhivotnykh v Rossii // Veterinaria, 1995. – № 5. – S. 82-87. [in Russian]
- [10] **Manichev, A.A.** Preparaty dlja spetsificheskoi profilaktiki, licheniya i diagnostiki sibirskoi jazvy sel'skhozjastvennykh zhivotnykh // Avtoreferat diss. doktora vet. nauk. – Moskva, 1994. – 46 s. [in Russian]
- [11] AS. 1809555 A1, RF. Sposob izgotovleniya vaktsiny protiv sibirskoi jazvy zhivotnykh. Bakulov, I.A., Gavrilov, V.A., Seliverstov, V.V.; opubl. 26.12.83 RK. [in Russian]
- [12] **Cherkassky, B.L.**, Zhanuzakov N.Zh. Sibirskaya jazva. – Alma-Ata: Kainar, 1980. – S. 4-11. [in Russian]
- [13] **Pastukhova, B.N.** Sibirskaya jazva. – Moskva, 1962. – 146 s. [in Russian]
- [14] **Sharov, D.A.**, Leshchenko A.A., Kovalenko E.A., Borovskoy D.V., Krupin V.V., Bagin S.V., Mokhov D.A., Lazykin A.G., Kosenkov I.V., Moshkov A.N. Razrabotka tehnologii prigotovleniya suhoi pitatel'noi sredy dlja proizvodstva sibirezavennoi vaktsiny // Biopreparaty. Profilaktika, diagnostika, lechenie, 2022. – T. 22. – № 2. – S. 187-195. [in Russian]
- [15] **Zadorina, I.I.** Antigennaja i molekuljarno-geneticheskaja otsenka stabil'nosti vactsinnogo sibirezavennogo shtamma lange posle dlitel'nogo hranenija. Avtoreferat kandidatskoj dissertatsii. – Kazan', 2020. – 25 s. [in Russian]
- [16] **Nemirovskaya, T.I.**, Kasina I.V., Aleksandrova N.V., Alekseeva S.A. Analiz sovremennykh reguljatornykh trebovanii k kachestvu zhivykh bakterial'nykh vaktsin i perspektivy ih usovershenstvovaniya // Biopreparaty. Profilaktika, diagnostika, lechenie, 2024. – 24 (4). – С. 456-466. [in Russian]
- [17] **Dyatlov, I.A.** Sovremennije tehnologicheskie platformy dlja razrabotki vaktsin protiv opasnykh bakterial'nykh infektsii (chast' II) // Bakteriologija, 2022. – 7 (4). S. 5-9. [in Russian]
- [18] **Alekseeva, S.A.**, Kasina I.V., Nemirovskaya T.I. Sravnitel'nyi analiz rezul'tatov ispytanii vaktsiny sibirezavennoi zhivoi po pokazatel'iu «Podlinnost'» immunofluorescentnym i immunohromatograficheskim metodami // Biopreparaty. Profilaktika, diagnostika, lechenie, 2024. – 24 (3). – S. 348-56. [in Russian]
- [19] **Kasina, I.V.**, Alekseeva S.A., Nemirovskaya T.I. Teoreticheskoe i eksperimental'noe obosnovanie perspektivnykh metodov ekspertizy kachestva vaktsiny sibirezavennoi zhivoi // Biopreparaty. Profilaktika, diagnostika, lechenie, 2020. – 20 (4). – S. 277-84. [in Russian]
- [20] **Medunitsyn, N.V.**, Katlinsky A.V., Vorslov L.O. Vaktsinologija: monografija. M.: Prakticheskaja meditsina, 2022. – 480 s. [in Russian]
- [21] **Turnbull, P.C.B.**, Frawley D.A., Bull R.L. Heat activation/shock temperatures for Bacillus anthracis spores and the issue of spore plate counts versus true numbers of spores // Journal of Microbiological Methods, 2006. – 68(2). – P. 353-357.

ТОПАЛАҢ ІНДЕТІ ВАКЦИНАЛЫҚ ШТАММЫНЫҢ СПОРА ДАМУЫНА ҚОЛАЙЛЫ ӘСЕР ЕТЕТІН ОПТИМАЛДІ ТЕМПЕРАТУРАНЫ АНЫҚТАУ

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Аңдатпа. Мал шаруашылығы Қазақстан Республикасы ауыл шаруашылығының негізгі салаларының бірі болып табылады, сондықтан еліміздің ветеринария қызметінің негізгі міндеті

жануарлардың жұқпалы ауруларының, ең алдымен зооантропоноздардың алдын алу болып табылады.

Ауыл шаруашылығы жануарларының сібір жарасы (қазақша атауы – топалаң) осы аурулардың бірі болып табылады және онымен күресудің тек эпизоотологиялық ғана емес, эпидемиялық, экологиялық, экономикалық және әлеуметтік үлкен маңызы бар. Споралы өсіндіні алу үшін вегетативті культураны ет пептонды агарда $+28-+37^{\circ}\text{C}$ температурада термостатта 12-120 сағат бойы өсірдік.

Әр 12, 24, 36, 48, 60, 72, 81, 96, 108 және 120 сағат сайын әртүрлі температурада өсірілген әр өсіндіден үлгілер алынып, стандартты әдістермен өміршең споралар саны анықталды.

VNIIVViM 55 штаммы термостатта $+32\pm 1^{\circ}\text{C}$ температурада басқа өсіру режимдерімен салыстырғанда культивацияланғанда спорадияның максималды деңгейі (91%) тіркелді.

Тірек сөздер: бактериялық масса, вакциналық штамм, споралар, температуралық фактор, топалаң.

ОПРЕДЕЛЕНИЕ ОПТИМАЛЬНОЙ ТЕМПЕРАТУРЫ, БЛАГОПРИЯТНО ВЛИЯЮЩЕЙ НА РАЗВИТИЕ СПОР ВАКЦИННЫХ ШТАММОВ СИБИРСКОЙ ЯЗВЫ

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Аннотация. Животноводство – одна из основных отраслей сельского хозяйства Республики Казахстан, в связи с чем первостепенной задачей ветеринарной службы страны является профилактика инфекционных болезней животных, в первую очередь зооантропонозов. Сибирская язва сельскохозяйственных животных (казахское название - топалаң) относится к числу таких болезней, и борьба с ней имеет не только эпизоотологическое, но и эпидемическое, экологическое, экономическое и большое социальное значение. Для получения культуры спор вегетативную культуру выращивали на мясопептонном агаре при температуре $+28-+37^{\circ}\text{C}$ в термостате в течение 12-120 часов. Через каждые 12, 24, 36, 48, 60, 72, 81, 96, 108 и 120 часов из каждой культуры, выращенной при различных температурных режимах, отбирали пробы и определяли количество жизнеспособных спор по стандартной методике.

Максимальный показатель уровня спорадичности (91%) зарегистрирован при культивировании штамма ВНИИВВиМ 55 в термостате при температуре $+32\pm 1^{\circ}\text{C}$, по сравнению с другими режимами культивирования.

Ключевые слова: бактериальная масса, вакцинный штамм, споры, температурный фактор, топалаң.