DEVELOPMENT OF A METHOD FOR MANUFACTURING AND OBTAINING HYPERIMMUNE SERUM AGAINST STREPTOCOCCOSIS IN FARM ANIMALS

ANNOTATION

In the livestock farms of our country, infectious diseases are becoming more and more common in their etiology, the dominant role belongs to the opportunistic microflora. Streptococcus also belongs to this group of microorganisms [1].

Streptococcosis is an infectious disease of young farm animals, characterized by severe septic phenomena, inflammation of the respiratory system, gastrointestinal tract and joints [2, 5, 7].

All types of young animals are susceptible to streptococcosis, but calves, lambs and foals get sick more often, starting from the first days of life, up to 3-4 months. A significant source of the pathogen are sick animals. The disease begins with a respiratory rate, the appearance of breath sounds and wheezing, and cough associated with the development of pneumonia. The first signs of the disease appear in foals at the age of 1.5 to 4 weeks. Clinical manifestations differ depending on the location and extent of metastatic abscesses. Mortality from complications reaches 10%. Factors contributing and predisposing to
the emergence and development of this disease are inadequate balanced nutrition, violation of the rules for the care and maintenance of calves [3, 4].

The basis for the prevention of streptococcosis should be a complex of veterinary and sanitary measures and good nutrition. On dysfunctional farms, it is impossible to keep sick and recovered cows with newborns, as well as drink their colostrum and milk [6]. In this regard, it is very important for veterinary medicine to investigate infectious diseases of cattle, as well as to develop effective means for protecting animals, improving measures related to the prevention and elimination of this disease [7, 8]. This work presents data on the isolation of a field isolate of bovine streptococcosis, an indication and identification of a bacterial culture. Bacterial mass obtained and prepared antigen for development hyperimmune serum. The scheme of immunization and obtaining hyperimmune serum on laboratory and industrial animals has been worked out. Carried out laboratory and industrial tests of hyperimmune serum. The work was carried out within the framework of the budget program 267 "Improving the availability of knowledge and scientific research", subprogram 101 "Program-targeted financing of scientific research and events" on the basis of KazSRVII LLP.

Key words: streptococcosis, animals, antigen, blood serum, hyperimmunization, efficiency, drug.

Introduction. Animal husbandry is an important branch of the national economy. However, the successful development of animal husbandry hinders the emergence and spread of infectious diseases in farms. These diseases cause pronounced economic damage, which consists of a case, a decrease in the productivity of animals and monetary costs for therapeutic and preventive measures [1].

Streptococcosis of farm animals remains an insufficiently studied infection, as a result of which huge damage is caused to animal husbandry. On farms up to 75% of calves fall ill with streptococcosis, with a fatal outcome up to 65% [2].

For prevention, it is important to comply with zoohygienic, veterinary and sanitary standards for the care and maintenance of pregnant animals and their offspring. Avoiding contact of young animals with cows with mastitis or drinking milk from these cows [3, 5].

The maintenance and feeding of young animals are systematically controlled, current disinfection is carried out [6].

Reducing the loss of animals from infectious diseases, among which streptococcosis has increased significantly in recent years, is one of the urgent problems of veterinary science and practice [1, 7].

One of the acute problems of modern animal husbandry is massive respiratory diseases of calves, causing significant economic damage [8, 9, 11].

About equipment of the population of our country with veterinarily harmless and cheap food of animal origin is an important task, the implementation of which is impossible without improving the quality of animal welfare, increasing their performance and reduce the cost of the product [10, 13, 15].

The main goal of research is to develop a method for obtaining hyperimmune serum with streptococcosis in animals. The research results were achieved by immunization of donor animals by subcutaneous and intramuscular administration of streptococcosis antigen in increasing doses, with an interval of 7 days. As a result of the research, a method has been developed for the manufacture and production of hyperimmune serum for the prevention and treatment of streptococcosis in animals [12, 16].

One of the common infectious disease among animals on the territory of the Republic of Kazakhstan is streptococcosis. This disease causes not only great economic damage to animal husbandry, but also has social significance, since it is not uncommon for a person to get sick [14, 17].

Streptococcosis is often found in organs and tissues in young animals and adult animals, including those without clinical manifestations, which indicates its general distribution. In connection with this situation, there is a need for a comprehensive a comprehensive study of streptococcosis, both in terms of individual microorganisms and their properties, and general epizootological features characteristic of this disease [18].

To combat this infectious disease, it is necessary to develop effective methods and means. This requires a range of activities aimed at preventing the occurrence and spread of streptococcosis [19, 20].

Materials and methods research. The aim of our research was to obtain bacterial mass and isolation of antigen to obtain hyperimmune serum blood from healthy cattle hyperimmunized according to a special scheme and its use in therapeutic and preventive measures for young cattle.

The work was done in the laboratory bacteriology, experimental studies on animals were carried out in the vivarium of the Institute and farms of the Almaty region.
30 heads of calves aged from 1 to 3 months were examined at the Kerbulak transhumance site of Bayserke-Agro LLP. With clinical signs of streptococcosis such as cough, shortness of breath, purulent discharge from the nasal sinuses, reduced appetite, body temperature was 40-41 ° C - 5 animals were identified. The observation period was 10 days, and one head fell. A total of 7 samples were taken.

In the laboratory, the test material was seeded on nutrient media. To determine the properties characteristic of the causative agent of streptococcosis, the isolated culture of the microorganism was inoculated, which was carried out from the pathological material into enriched with 10% inactivated horse serum and glucose up to 0.2% of the final concentration of meat-peptone broth (MPB), as well as on a dense medium, in Petri dishes enriched with 5% defibrinated sheep blood and glucose up to 0.2% of the final concentration of meat-peptone agar (MPA). After that, the media with cultures were incubated in a thermostat at a temperature of 37°C for 18-20 hours.

In enriched MPA, culture growth is observed, characterized by diffuse turbidity of the nutrient medium used. On dense nutrient media - on cups Petri dishes containing MPA enriched with blood showed growth of small, smooth the edges of the colonies, slightly cloudy, having the appearance of dew characteristic of the pathogen of streptococcosis.

Determination of the pathogenic properties of pure cultures of streptococci was carried out on white mice weighing 14-16 g. Only freshly isolated cultures were used for infection streptococci 18-20-hour growth in glucose-serum broth.

A daily culture of streptococci was administered intraperitoneally to three white mice at a dose of 0.5 cm³ containing 1000 microbial cells. When infected with a pathogenic culture white mice died, as a rule, in 2-3 days. The experimental animals were observed for 5 days.

A culture is considered pathogenic if at least two white mice die within 72 hours. From the spinal cord, blood of the heart, liver and spleen, each dead mouse was made inoculations on glucose-blood agar and glucose-serum broth to isolate the original culture.

The indication and identification of the culture of streptococcus was determined, the enzymatic properties of the studied had slight differences. It was characterized by the fermentation of glucose, sucrose, mannitol, sorbitol, maltose, with the formation of acid and without gas evolution. Weakly fermented xylose. Did not ferment lactose, arabinose, raffinose. Didn't curdle milk. Didn't liquefy the gelatin. Formed indole, hydrogen sulfide, reduced nitrates.

Obtaining hyperimmune serum for the treatment of streptococcosis of farm animals, which includes immunization of donor animals with an antigen.

A method for preparing an antigen for obtaining serum in the treatment of streptococcosis in farm animals involves growing a culture of streptococcus on a solid nutrient medium for 18-20 hours. In a thermostat at a temperature of 37 ° C, followed by washing it from the surface with saline, the resulting bacterial mass is heated in a water bath at 80°C for 1 hour with constant stirring. To isolate the antigen, the bacterial mass was homogenized at 5000 rpm for 15 min, then the resulting of homogenizate was sonicated on an ultrasonic apparatus in parameters with a wave frequency of 22 kHz and an intensity of 100 W/cm² (As a rule, ultrasonic vibrations lead bacterial cells to death, while this suspension does not loses its immunogenic and antigenic properties).

The resulting disintegrate was centrifuged at 4000-5000 rpm for 20-30 minutes. The supernatant containing 5 ml of protein per 1.0 ml in the amount of 200.0 cm² was used as an antigen and mixed with an oil adjuvant in a 50/50 ratio. Subsequently, 50.0 cm³ of the obtained preparation was used for immunization.

To obtain hyperimmune serum, oxen-producers (cattle) weighing 250-300 kg were used. in the amount of 2 heads.

Animal hyperimmunization method is presented in Table 1.

Table 1 – Data on hyperimmunization of producing oxen (cattle)

<table>
<thead>
<tr>
<th>dose of antigen</th>
<th>interval between injections</th>
<th>method of antigen administration</th>
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<tbody>
<tr>
<td>3.0</td>
<td>7</td>
<td>in the right pre--scapular and left inguinal lymph nodes</td>
</tr>
<tr>
<td>5.0</td>
<td>7</td>
<td>subcutaneously</td>
</tr>
<tr>
<td>10.0</td>
<td>7</td>
<td>intramuscularly</td>
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The hyperimmunization cycle lasts 21 days. On the 30th day after the introduction of antigens, a test blood collection from the jugular vein is performed.

The resulting blood serum from each producer is examined for the presence of streptococcosis antibodies in the diffuse precipitation reaction (RDP). With the accumulation of antibody titer in RDP 1:8–1:16, blood is taken to obtain serum.

For testing experimental series of hyperimmune serum two groups were created 10 calves at the age of one month weighing 35-40 kg.

With preventive purpose Serum was administered to newborn calves intramuscularly at a dose of 2 cm³ per 1 kg of body weight. For therapeutic purposes, serum was administered intramuscularly at a dose of 2.5 cm³ per 1 kg of body weight. In severe cases of the disease, the serum was administered again after 1-3 days in the same doses. The daily therapeutic dose of serum was administered in 2 doses with an interval of 6 hours.

Serum test results from which it follows that hyperimmune serum has pronounced therapeutic and prophylactic properties. For example, in the group of calves treated with hyperimmune serum, only 29.6% fell ill, 5.7% of calves died, while in the control (intact) group these figures were 82.7% and 17.3%, respectively. At the same time, the safety of calves in the experimental group was 94.3% versus 82.7% in the control group. The use of serum in therapeutic doses made it possible to cure 93.0% of sick calves.

Positive test results of hyperimmune serum showed that hyperimmune serum obtained by the proposed method is harmless, a drug with a pronounced therapeutic and prophylactic effect against bovine streptococcosis.

Results and its discussion. The developed hyperimmune serum against streptococcosis of farm animals is intended for therapy and prophylactic immunization of farm animals susceptible to this infection.

In appearance, hyperimmune serum is a transparent, slightly opalescent liquid of a light yellow color, sometimes with a reddish tint, a liquid with a slight protein precipitate at the bottom of the vial, which, when shaken, easily breaks into a uniform suspension.

Checking for sterility, activity and harmlessness.

The serum was tested for sterility according to GOST 28085-2013 "Biological medicinal products for veterinary use". Sterility control methods. Sterility control was carried out according to the generally accepted method by seeding from the preparation on nutrient media MPA, MPB with glucose, MPPB under oil, on Sabouraud agar or Chapek's medium to exclude fungal microflora.

In accordance with the current recipe prepared pMPB and MPA nutrient media were poured into test tubes of 5-6 cm³, MPPB under vaseline oil - 10-12 cm³ into vials/ampoules, closed with cotton-gauze stoppers and sterilized in an autoclave at a temperature of 121±1ºС for 20 minutes. After sterilization, the MPA was mowed and only dried nutrient medium was used for work. Test tubes and vials/ampoules with MPPB under vaseline oil were reduced before use, for this they were placed in a water bath, the water in which was brought to a boil and boiled for 20 minutes in order to remove the air dissolved in the medium.

Conducting a test. Cultures were made from each hyperimmune serum sample. 0.2-0.3 cm³ in MPB, MPA and 0.5-1.0 cm³ each in MPB under vaseline oil, Sabouraud agar. Three test tubes and two cups with a nutrient medium were used for inoculation. After 3 days of incubation, inoculations from MPPB under vaseline oil were reseeded onto a similar nutrient medium.

Test tubes and cups with inoculations on all media were kept in a thermostat at a temperature of (37±1)ºC within 10 days, and reseeding - 7 days at a temperature of (37±1) ºC. During the specified time, the crops were examined daily for purity of growth.

Results processing: In all nutrient media there should be no growth of extraneous bacterial and fungal microflora. Activity each series of prepared serum is controlled on 3 rabbits weighing 2.0-2.5 kg, which are injected subcutaneously at a dose of 2 cm³ twice with an interval of 14 days. 14 days after the last immunization, rabbits are bled from the ear vein and the serum is examined for the presence of specific antibodies to antigens of streptococcus bacteria in the RDP (Diffusion precipitation reaction). The activity of hyperimmune serum is determined in titers and ranges from 1:4 to 1:8.

Results processing: Serum was considered active if there were specific antibodies to antigens of streptococcus bacteria in DPR (Diffusion precipitation reaction).

Estimate harmlessness of serum was carried out according to GOST 31926-2013 "Medicines for veterinary use". Methods for determining harmlessness. For this purpose, 5 white mice with a live weight
of 18-20 g were used, which were injected subcutaneously with hyperimmune serum at a dose of 0.5 cm³. After the introduction of serum, daily clinical monitoring of the state of mice was carried out during a 10-day observation. During this time, all experimental mice remained healthy, without signs of illness and intoxication.

From each series, hyperimmune serum was tested for white mice weighing 18-20 g (pregnant women are not taken into experience)

Conducting the test:

For the test, a total serum sample was prepared from 5 vials. From each vial, 20-25 cm³ of serum was taken into a sterile vial, mixed and injected subcutaneously into five white mice weighing 18-20 g, 0.5 cm³ each. The indicated doses of serum should not cause disease and death of animals within 10 days of observation.

Results processing:

Serum was considered harmless if all animals remained alive after administration of the drug.

Conclusion. As a result of the research, a method has been developed for the manufacture and production of hyperimmune serum for the treatment and prevention of streptococcosis in cattle. It has been established that subcutaneous administration of hyperimmune serum for prophylactic purposes prevents their incidence in the critical age period (from 1 to 3 months) after birth, thereby causing a decrease in the death of animals by almost 7 times. The drug is harmless, active. The novelty and originality of the method for obtaining hyperimmune blood serum against bovine streptococcosis are protected by the RK patent № 7153.

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ТУЙІН

Еліміздің мал шаруашылықтарында жұқпалы аурулардың таралуының басты себебі шартты-потогенді микрофлоралар болып табылады. Стрептококкәр да осы микроорганизмдер тобына жатады [1].

Стрептококкоз (стрептококкоз) - ауылдық жауарлар төлдерінің аса септикалық, тыныс алу мүшелерінің, асқазан-ішек жолдарының және буындардың қабынуымен сипатталатын жұқпалы ауру [2, 5, 7].

Жас малдың барлық түрлері стрептококк ауруына шалдық, бірақ бұзаулар, қозылдар, құлындар өмірінің алғашқы күндерінен бастап, 3-4 айға дейін жиі ауырады. Қоздырушының басты көзі - ауру жануарлар болып табылады. Ауру тыныс алу жіп-ілейімен, тыныс дыбыстары мен сырылдардың пайда болуымен, пневмонияның дамуына байланысты жөтелден басталады.

Аурудың алғашқы белгілері құлындарда 1,5-4 апталық жаста байқалады. Метастатикалық абсцесстердің орналасуы мен дәрежесі бойынша клиникалық мәліметтер ерекшеленеді.

Асқыңуынан болатын өлім 10% жетеді [3, 4].

Бұл аурулардың пайда болуы мен дамуына ықпал ететін және бейімділік факторлары төнгөрімді азықтаңдымдың жетікіліксіздігі, бұзаулардың күтің-багу ережелерінің бұзылысы болып табылады. Стрептококкоздің алдын ала ауру жылды жүргізіледі, сондай-ақ олардың уызы мен сүтін ішуге болмайды. Осы туралы ветеринария және гипериммунді сарысуды алуға антиген дайындалды. Зертханалық және өндірістік құрылыстарды иммундау және гипериммунді сарысуды алу схемасы әзірленді.

РЕЗЮМЕ

В животноводческих хозяйствах нашей страны все более и более становятся распространены инфекционные болезни в их этиологии главенствующая роль принадлежит условно-патогенной микрофлоре. К данной группе микроорганизмов относят и стрептококк [1].

Стрептококкоз (streptococcosis) - инфекционное заболевание молодняка сельскохозяйственных животных, характеризующееся тяжелыми септическими явлениями, воспалением органов дыхания, желудочно-кишечного тракта и суставов [2, 5, 7].

Все виды молодняка животных восприимчивы к стрептококкозу, но телята, ягнята и жеребята болеют чаще, начиная с первых дней жизни, до 3-4 месяцев. Существенным источником возбудителя являются больные животные. Заболевание начинается с частоты дыхания, появления дыхательных шумов и хрипов, а также кашля, связанного с развитием пневмонии. Первые признаки заболевания появляются у жеребят в возрасте от 1,5 до 4 недель. Клинические проявления различаются в зависимости от локализации и степени метастатических абсцессов. Смертность от осложнений достигает 10% [3, 4].

Факторами, способствующими и предрасполагающими к возникновению и развитию данного заболевания, являются недостаточное сбалансированное питание, нарушение правил ухода и содержания телят. Основой профилактики стрептококкоза должен стать комплекс ветеринарно-санитарных мероприятий и полноценное питание. На неблагополучных фермах нельзя держать больных и переболевших коров вместе с новорожденными, а также выпивать их молозиво и молоко.

В связи с этим очень важно для ветеринарии исследовать инфекционные заболевания крупного рогатого скота, а также разрабатывать эффективные средства для защиты животных, улучшение мероприятий, связанных с профилактикой и устранением данной болезни. В данной работе приведены данные о выделении полевого изолята стрептококкоза КРС, проведена индикация и идентификация бактериальной культуры. Получена бактериальная масса и приготовлен антиген для разработки гипериммунной сыворотки. Отработана схема иммунизации и получения гипериммунной сыворотки на лабораторных и производственных животных. Проведены лабораторные и производственные испытания гипериммунной сыворотки. Работа проведена в рамках бюджетной программы 267 «Повышение доступности знаний и научных исследований» подпрограмме 101 «Программно-целевое финансирование научных исследований и мероприятий» на базе ТОО «КазНИВИ».