

Article

Creation of an Experimental Series of Vaccine Against Infectious Necrotic Hepatitis of Sheep and Determination of its Sterility and Injury

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Abstract: This article presents the technology for producing a vaccine against infectious necrotic hepatitis in sheep and the results of scientific research on the development of a pilot series from the local epizootic strain J-22 isolated in 2022. It also presents the results of studies conducted to determine the sterility and safety of the prepared vaccine series.

Keywords: Infectious Necrotic Hepatitis, Sheep, Kitt-Tarossi, Pathogen, Cl.Novyi, Vaccine, Necrosis, Dose, Liver, Heart, Fluid

1. Introduction

Providing employment to the rural population, increasing the number of dehqan farms raising cattle and sheep, and providing our Republic with hides, meat, milk and other dairy and meat products are among the urgent problems of our time[1]. There are a number of factors that negatively affect the increase in the number of livestock and their productivity, and without eliminating them, achieving the above goals will create a number of difficulties[2].

Diseases caused by anaerobic pathogens cause significant damage to sheep reproduction and, at the same time, lead to their death. Infectious necrotic hepatitis of sheep also occupies a significant place among the diseases that cause serious economic damage to the income of families raising sheep[3]. This disease is widespread in many countries of the world with developed sheep farming, and the disease also occurs from time to time in our Republic[4].

The causative agent of the disease is a spore-forming, motile anaerobic bacterium that forms spores in the body of a dead animal and in the external environment. The causative agent of infectious necrotic hepatitis of sheep persists in nature for many years and cannot be destroyed[5]. The spores of the causative agent spread and are transmitted through feed, water, soil, and manure. The causative

agent, which enters the animal's body, enters the blood and through it spreads throughout the body, multiplying in places where it is poorly supplied with oxygen[6]. The pathogen secretes a strong toxin, which poisons the animal and disables all its defenses, and as a result, the animal cannot fight the disease. The disease is an acute infectious disease, lasting 8-12 hours, sometimes 1-2 days. Treatment of sick animals in most cases is ineffective and the animal dies. Prevention is the main measure in the fight against this disease. To prevent the disease, animals prone to the disease are vaccinated against the disease[7]. No biological vaccine is produced in our Republic to prevent the disease. Taking this problem into account, scientific research should be directed to the creation of a vaccine against infectious necrotic hepatitis of sheep, which is currently extremely urgent[8].

2. Materials and Methods

Experimental guinea pigs of the Laboratory of Immunology and Biotechnology of the Veterinary Research Institute, local epizootic strains of the causative agent of infectious necrotic hepatitis, Kitt-Tarosii nutrient media available in the laboratory served as the research object.[9]

To create an experimental series of vaccines against infectious necrotic hepatitis of sheep from local epizootic strains, two highly pathogenic local epizootic strains, J-22 and PY-23, were selected. Of these, strain J-22 was the production strain used in vaccine preparation, and strain PY-23 served as a control strain to test the immunogenicity of the vaccine. Before vaccine preparation, the production strain J-22 was inoculated into Kitt-Tarozzi nutrient medium in a test tube, and then onto a special agar.[10] From the pure culture grown on agar, 0.5-1 liter flasks were inoculated into Kitt-Tarozzi nutrient medium and placed in a thermostat at 37.5-38.5 0C for cultivation. After one day (24 hours), the culture medium was cleaned of liver fragments and 0.4% chemically pure formalin was added to it to inactivate the pathogen. The culture medium with formalin was kept in a thermostat at a temperature of 38-39 0C for 3 days, stirring 2-3 times a day. On the fourth day, 15% of a 3% sterile aluminum hydroxide solution was added to it to precipitate the pathogens in the medium, mixed thoroughly, and settled at room temperature for 2-3 days at 18-26 0C. The upper two-thirds of the settled culture medium was removed. The remaining liquid was heated in a water bath to a temperature of 37 0C, and 0.1% sterile agar-agar was added to it. [11]

The resulting vaccine was inoculated into appropriate nutrient media (GPK, GPA, Sabouraud agar and broth, GPJK-i.e. Kitt-Tarozzi nutrient medium), bacteriologically tested and determined to be sterile, then packaged in vials, closed with a rubber stopper, sealed with an aluminum cap and labeled.[12]

In this way, an experimental series of concentrated GOA formol vaccine against infectious necrotic hepatitis of sheep from the local epizootic strain J-22 was prepared.

To determine the sterility of the experimental series of packaged vaccines against infectious necrotic hepatitis of sheep, 4 vials of vaccine were isolated and 25 ml of vaccine was taken from each of them with a sterile syringe, poured into a clean sterile vial and mixed thoroughly. From the vaccine mixture in this vial, inoculations were inoculated onto GPK, GPA, Sabouraud agar and broth, and Kitt-Tarozzi nutrient media and placed in a thermostat at 37.5-38.5 0C for growth. The inoculations were kept in the thermostat for 10 days, and Sabouraud nutrient media for 30 days, and during this observation period, no microbes or fungi grew in the nutrient media, and it was concluded that the experimental series of the vaccine was sterile.[13] To check the safety of the vaccine, 1 ml of the vaccine mixture was injected subcutaneously into 2 places around the abdominal muscles of 2 guinea pigs weighing 350-450 grams. These guinea pigs were under constant observation for 10 days. Until the end of the observation period, no clinical signs of the disease were observed in the vaccinated guinea pigs and they remained healthy. The experimental series of the vaccine was considered harmless.[14]

3. Results and Discussion

As a result of the conducted experiments, an experimental series of a concentrated GOA formol vaccine against infectious necrotic hepatitis of sheep was successfully developed using the local epizootic strain J-22.

The sterility tests showed that no microbial or fungal growth was observed in any of the inoculated nutrient media throughout the entire observation period. This confirms that the prepared vaccine series meets sterility requirements.

The safety assessment demonstrated that guinea pigs injected with the vaccine did not exhibit any clinical signs of disease during the 10-day observation period. The animals remained healthy, indicating that the vaccine is non-toxic and safe for use.

These findings confirm the effectiveness of the selected production technology, including the use of formalin inactivation and aluminum hydroxide as an adjuvant. The obtained results are consistent with existing studies on the development of vaccines against anaerobic infections in livestock.

The use of a local epizootic strain (J-22) is particularly significant, as it enhances the relevance and potential effectiveness of the vaccine under regional conditions. This approach contributes to improving disease prevention strategies and reducing economic losses in sheep farming.

Overall, the developed vaccine demonstrates promising characteristics in terms of sterility, safety, and applicability, which supports its further testing and potential implementation in veterinary practice.[15]

5. Conclusion

Thus, an experimental series of a vaccine against infectious necrotic hepatitis of sheep from the local epizootic strain J-22, which is highly pathogenic for this disease, was created. The vaccine was tested using appropriate methods, and its sterility and harmlessness were determined when administered to guinea pigs.

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