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The Technology for the Production of the Vaccine for Pseudomonosis of Pigs

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Abstract

The paper reflects the results of the development and special features of the technological process for the production of an inactivated vaccine for pseudomonosis of pigs. We isolated from the biological material from diseased and fallen pigs strains of the blue pus bacillus – Pseudomonas aeruginosa. Their cultural and morphological properties were determined, typing of serological properties was made, these strains were deposited in the All - Russian collection of microorganisms and transferred to the enterprise of the biological industry of the Russian Federation for the industrial production of a new biological preparation. A scheme for the cultivation and inactivation of Pseudomonas aeruginosa has been developed, the optimal ratio of Pseudomonas aeruginosa antigens of the serological groups 01, 03, 04, 06, 011, 019 and constituents in the vaccine manufacturing process has been determined.

Keywords: pigs, Pseudomonas aeruginosa, technology, vaccine.

INTRODUCTION

Infectious diseases of pigs and young pigs are one of the main problems of pig farming. A significant place among them occupy infections caused by blue pus bacillus – Pseudomonas aeruginosa, manifested by severe clinical course and high mortality rate of young pigs, postpartum diseases of adult animals and represent a significant problem not only for veterinary, but also for humanitarian medicine [1-7].

In connection with this, the development of biological preparations for prevention is one of the promising areas of veterinary science, since mutations and variability of bacteria, including the causative agent of Pseudomonas aeruginosa, constantly occur during the evolution process [8-10]. In the Russian Federation, the vaccine, with the goal of pseudomonas preventing in pig farming, was not produced by the Russian biological industry.

MATERIALS AND METHODS

For laboratory studies, we selected the diagnostic material from clinically ill and fallen pigs on the pig farms and complexes of agricultural enterprises in the Krasnodar Territory. The study of tinctorial, morphological, cultural, biochemical and serological properties of Pseudomonosis aeruginosa strains was carried out in the veterinary laboratories of the Krasnodar Territory, Krasnodar Research Institute for Veterinary Medicine, FGUP Armavir biofactory, strain control in FGBU VGNKI (Moscow, Russia) in the period from 2003 to 2013. Bacteriological studies were conducted in accordance with the Methodological Guidelines for Laboratory Research on Pseudomonosis of Animals and Birds approved by the Chief Veterinary Department of the Ministry of Agriculture of the USSR № 432-3 dated 14.11.1988; the biochemical properties of the strains were determined using the test kits of the NEFERMtest systems of the Lachema company of the Czech Republic, antigenic properties – using sets of receptor pseudomonas sera of the State Research Institute for Standardization and Control of Medical Biological Preparations named after L.A. Tarasevich, Moscow, Russia. Mattress flasks, 20-30-liter glass containers, industrial biological reactors for the cultivation of microorganisms and reactors with adjuvant-alumina hydrate, nutrient media for the cultivation of microorganisms of meat infusion broth (MIB), meat infusion agar (MIA), liver meat infusion agar (LMIA), Saburo medium, nutrient media obtained from the hydrolysis of meat, the author of which is R. Hottinger - broth and semi-liquid Hottinger agar, semi-automatic bottling lines, capping and bottle marking were used in the technological process. Stability of experimental batches of vaccine was made in accordance with STO 00482849-0060-2014, sterility in accordance with GOST 28085-89, acceptance of the vaccine series in accordance with STO 00482849-0051-2011, GOST 9142, GOST 14192. As antigens were strains of Pseudomonas aeruginosa of serological groups: 01, 03, 04, 06, 011, 019.

RESULTS AND DISCUSSION

Production of the vaccine was carried out according to the technological scheme, including the main technological processes: preparation of nutrient medium and production strains, preparation of matrix broods, cultivation of microorganisms, preparation of vaccine series, bottling of the vaccine in bottles, capping of bottles, control, labeling and marking of finished products and directing it to the warehouse, Figure 1. For cultivation of the production and control strains, Hottinger broth was used, the cultures were stored on the semi-liquid Hottinger agar and in a lyophilized state in the laboratory of the Armavir biofactory. In the future, the cultures of the production strains were used to make matrix broods:

- they were dispersed in Petri dishes on meat infusion agar (MIA) and grown for 18 hours at a temperature of (37 ± 0.5) °C, viewed under a microscope with magnification of 16-56 times, 3-5 typical "S" form colonies were selected, plated again on semi-liquid MIA, meat infusion broth (MIB) and slanting MIA and grown for 16 hours (37 ± 0.5) °C;
- grown in semi-liquid MIA cultures were packaged in Pasteur pipettes or ampoules and sealed. Sealed pipettes, ampoules (Production Seed) were sent for storage according to regulatory documentation for strains at a temperature from 2°C to 8°C. In the following, one pipette was used for each matrix brood.

To obtain the matrix brood of Pseudomonas aeruginosa in bottles of 0.5 cm^3, the cultures from semi-liquid agar were inoculated into 200 cm^3 bottles containing 80-100 cm^3 of

Hottinger broth. At the same time, the culture was inoculated into MIA and MIB tubes and cultured for 10-14 hours at (37 ± 1) ºC. The grown cultures were tested for growth purity by smear microscopy of Gram stain, for specificity with monoreceptor sera, biochemical and enzymatic properties using a test system. When a pseudomonous culture of production strains was produced in bottles, the culture to be tested was inoculated from a vial at the rate of 20-30 cm^3 into a 10 liter bottle with 4-5 dm^3 of Hottinger broth or a 20 liter bottle containing 10-12 liters of Hottinger broth was grown for 10-12 hours at (37 ± 1) ºC and used as a matrix brood. The matrix brood before inoculation into the reactor was checked for macroscopic growth cleanliness and smear microscopy of Gram stain and inoculation on media: MIA, MIB, Endo, or Ploskirev.

The cultivation of Pseudomonas aeruginosa was carried out by inoculation of the matrix culture into the reactor in an amount of 5-10% to the volume of the nutrient medium. Cultures were grown for10-12 hours (37 ± 1) ºC with continuous aeration. After every 2 hours of cultivation, samples were taken from the reactor to determine the concentration, purity of the culture and pH. To improve the growth of microbes and reduce the pH when it is increased it to 7.6-7.8, a sterile 40% glucose solution was added every 2 hours. The growing was stopped at the end of the exponential growth phase. At the end of the culture, the concentration of microbial bodies, the pH and the culture growth purity were determined by microscopy of Gram stained smears, inoculation on a differential medium, a grown culture with monoreceptor pseudomonas sera was typed. The bacterial mass obtained in the reactor was adjusted to a concentration of 6 billion/cm^3 by sterile saline solution.

The culture brood of Pseudomonas aeruginosa containing 6 billion/cm of microbial cells was inactivated by technical formalin in accordance with GOST 1625-75 with formaldehyde content of at least 36.0% at a final concentration of 0.4%, 6% gel of alumina hydrate was used as an adjuvant. The culture was inactivated for 15-16 days at a temperature of (37-38) ºC. Completeness of inactivation was determined by the method of inoculation into nutrient media: (MIB, MIA, LMIB (liver meat infusion broth) and Saburo slanting agar).

The vaccine was packaged with a constantly switched stirrer in sterile glass bottles of 100 cm^3 ± 3% or 200 cm^3 ± 3%, sealed with sterile stoppers, sealed with aluminum caps on the semiautomatic filling machine at the bottling, capping and labeling bottles line (MM 35, ST 36, LN 37). The label was affixed to the bottle with the text in accordance with STO 00482849-0051-2011.

The ready vaccine was tested for sterility by the method of inoculation with 0.1-0.2 cm^3 in two tubes with MIA, MIB, LMIB, Saburo agar from 5 vials with the vaccine. The inoculations were kept in a thermostat at a temperature (37 ± 0.5) ° C for 2 days. Then, we carried out the transfer from liquid media to two test tubes with MIB, MIA, LMIB. Sterility control of primary inoculation was carried out for 14 days, secondary - 12 days. The media in the test tubes remained sterile. The vaccine was tested for harmlessness on 5 white mice weighing 18-19 g by the method of intraperitoneal injection of 0.3 cm^3 of the preparation. The vaccine was considered harmless with the survival rate of 100% of animals during 10 days of observation. Acceptance (verification) of each vaccine series was carried out by the Department of Biological and Technological Control in accordance with STO 00482849-0051-2011. The vaccine in the manufacturing organization, in trade organizations and at the consumer was recommended to store in a dry dark place in boxes of factory packaging or shipping containers at a temperature of 2° C to 15° C. The expiration date of the vaccine is within 12 months.

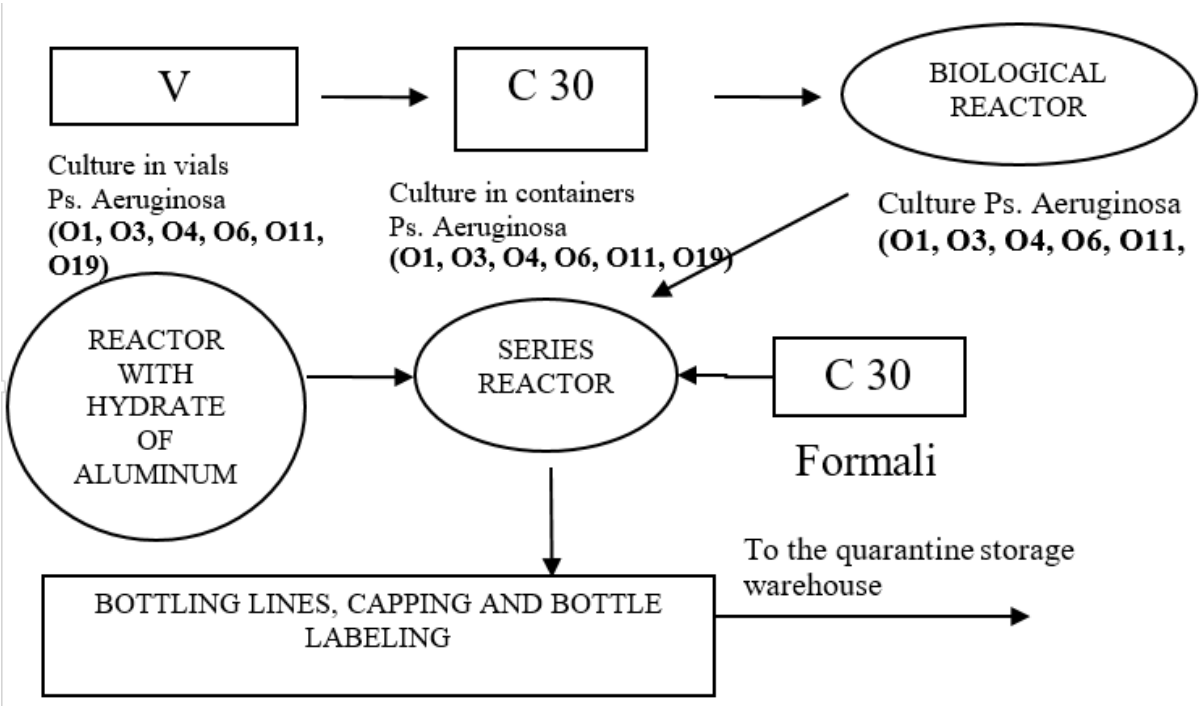

Figure 1 – Scheme of the technological process of manufacturing the vaccine

CONCLUSIONS

As a result, we have worked out the technology of industrial production of inactivated vaccine for pseudomonosis of pigs. The regulatory documentation for its manufacture, control and use has been developed and approved, and pilot-industrial vaccine series have been produced.

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