Development and testing of a vaccine against infectious atrophic rhinitis and pasteurellosis in pigs

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Abstract. This work presents the results of the development and testing of a domestic vaccine against infectious atrophic rhinitis and pasteurellosis in pigs caused by Bordetella bronchiseptica and Pasteurella multocida type A and D. A drug was designed with the following antigenic composition: B. bronchiseptica (strain B-1341) - 6 * 10⁹ m.c. / dose; antigen of P. multocida type A (strain B-1303) - 3 * 10⁹ m.c. / dose; antigen of P. multocida type D (strain B-1308) - 3 * 10⁹ m. c. / dose. A 3% solution of Aluminum hydroxide in a proportion of 10% was used as an adjuvant. One immunizing dose is 2 cm³. In the course of the study, it was found that the survival of piglets obtained from vaccinated sows is 97.16%, which is comparable with the same indicator in the group of animals immunized with the reference drug - 96.96%. Despite the fact that the average weight of piglets in the experimental group on the 45th day of life was 130 g less than of those in the control group, by the 180th day of life, the experimental piglets were 4.33 kg heavier than the animals in the control group. The average daily gain in the experimental group was 33 g more than the one in the control group. Thus, the selected antigenic composition for the inactivated vaccine is optimal, and is capable of providing high immunogenic and protective activity, both in a vivarium and in an industrial pig-breeding enterprise.

1 Introduction

Respiratory diseases in pigs are one of the leading causes of death and culling in the pig industry worldwide [1]. The complex of respiratory infections of pigs is represented by viral and bacterial pathologies occurring in the form of monoinfection or associations, while their course may be complicated by certain predisposing factors that reduce the body's natural resistance to the pathogen. One of the clear examples of such a disease is infectious atrophic rhinitis in pigs caused by Bordetella bronchiseptica and Pasteurella multocida [2]. The indicated microorganisms can be both primary etiological agents and secondary ones. In addition, microorganisms of both types may not have any specific etiological role, since there are isolates that do not have pathogenic properties (unable to cause disease) [3].

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The infectious atrophic rhinitis (IAR) in pigs is a widespread disease characterized by deformation, twisting or shortening of the snout as a result of atrophy of its bony structures. According to generally accepted data, toxigenic strains of Bordetella bronchiseptica and Pasteurella multocida type D are involved in the development of the disease [4]. Clinical signs of IAR vary. Thus, in a mild course of the disease, there are fever, cough, sneezing, tearing, lymphadenitis, while in severe cases there is pneumonia accompanied by shortness of breath, cyanosis and death. B. bronchiseptica also causes primary pneumonia in newborn piglets and secondary pneumonia in adult pigs [5].

Pasteurellosis is a contagious disease of most species of animals and birds caused by bacteria of the genus Pasteurella. In addition to damage to joints, mammary glands, organs of vision and pneumonia, pasteurellosis can also manifest itself in the form of IAR in pigs. Pasteurellosis in pigs in the pulmonary form is usually caused by Pasteurella multocida type A [6].

When studying the epizootic situation of pig breeding enterprises infamous for respiratory diseases in various regions of the Russian Federation, it was found that the incidence of Bordetella bronchiseptica isolation from the lungs and upper respiratory tract is in the range of 5-30%. Along with bordetella, Pasteurella multocida is found in 15-40% of cases (as of 2020). Due to the wide spread of these pathogens in the territory of the Russian Federation, as well as due to the development of antibiotic resistance, the most effective and appropriate way to combat the indicated pathologies in pigs is their specific prevention, that is, vaccination [7, 8, 9, 10].

That is why the purpose of this work was to develop a safe and effective vaccine aimed at specific prevention of infectious atrophic rhinitis and pasteurellosis in pigs.

2 Materials and methods

The study was carried out in the Federal State Budgetary Scientific Institution "Federal Scientific Centre Russian Research Institute of Experimental Veterinary Medicine named after K.I. Scriabin and Y.R. Kovalenko of the Russian Academy of Sciences", within the framework of the state assignment FGUG-2022-0007 “To develop a comprehensive system for predicting and combating the resistance of microorganisms of zoonotic, anthroponotic and sapronous origin, through the creation of new modern methods and test systems, immunobiological agents, including bacteriophages and immunoglobulins, antimicrobial and antymycotic drugs, preservatives and disinfectants for the diagnosis, prevention and treatment of infectious diseases of farm animals, the iteration of which in the agro-industrial, the manufacturing and processing sector of the Russian Federation will prevent and control the spread of drug resistance, increase the export potential of the country, ensure food security and independence”.

The work used industrial strains of microorganisms: Bordetellabronchiseptica B-1341; Pasteurella multocida B-1303 type A; Pasteurella multocida B-1308 type D, which have previously been confirmed to have high antigenic, immunogenic and protective activity. In addition, control cultures were used to assess the immunogenic properties of the drug: Bordetella bronchiseptica B-1343; Pasteurella multocida №1231 type A; Pasteurella multocida №T-80 type D. All the mentioned bacterial strains are certified and deposited in the All-Russian collection of pathogenic and vaccine strains of microorganisms-pathogens of infectious diseases of animals of the Federal State Budgetary Scientific Institution FSC VIEV RAS.

Obtaining antigens Bordetella bronchiseptica and Pasteurella multocida was carried out by submerged cultivation of each industrial strain separately, using brain heart broth (Becton Dickinson, USA), in a BIOSTAT-A bioreactor (SartoriusAG, Germany) under aerobic conditions at 37 ° C. During cultivation, the pH of the medium was maintained at 7.2-7.8.
The culture was also enriched with a 40% glucose solution (OOO NPP “Agrofarm”, Russia), in the proportion of 10 ml of glucose per 1 liter of nutrient medium once an hour, depending on the intensity of culture growth. The pH level during cultivation was maintained by adding a 10% alkali solution (NaOH). The cultivation time was about 20 hours.

Inactivation was carried out with formalin containing 37% formaldehyde (OOO “ErgoProduction”, Russia), adding 0.3% of the volume of the culture liquid. The inactivation process was carried out for 72 hours at a temperature of 21-22 °C.

Antigen concentration was carried out by centrifugation on MPW-380R equipment (MPW Med. Instruments, Poland) for 1 hour at 3000 r/m, RCF - 1861.

The concentration of the suspensions was determined using turbidity standards - a set of the OIS of the turbidity of bacterial suspensions of the SCPM of the FSBI "Scientific centre of medical production expertise" of the Ministry of Health of the Russian Federation.

The completeness of antigen inactivation was assessed by the absence of viable cells in the bacterial mass by inoculating samples on growth-supporting nutrient media in accordance with GOST 28085-2013 “Biological medicinal products for veterinary use. Method of bacteriological control of sterility ". The completeness of antigen inactivation was also assessed by the harmlessness of the inactivated bacterial mass to laboratory mice weighing 16-18 g. with intraperitoneal administration of 0.5 cm³ of antigen diluted to a concentration of 3 billion m. c./cm³. All antigens used to compose the three series of vaccines were sterile and did not cause death of laboratory animals within 14 days of observation.

Thus, using the obtained antigens, an experimental series of vaccine was developed with the following composition: B. bronchiseptica antigen (strain B-1341) - 6 * 10⁹ m.c. / dose; antigen of P. multocida type A (strain B-1303) - 3 * 10⁹ m. c. / dose; antigen of P. multocida type D (strain B-1308) - 3 * 10⁹ m. c. / dose; saline solution - as a diluents of antigens; aluminum hydroxide at a concentration of 3% in a proportion of 10%; 10% solution of merthiolate at the proportion of 1: 10000 - as a preservative; 10% sodium hydroxide solution - to adjust the pH of the vaccine batch at 7.6.

Since the used concentrations of bacterial antigens were determined as optimal in previous studies, in this work we assessed the harmlessness of the drug, its immunogenic activity using laboratory animals, as well as protective efficacy in the conditions of a pig-breeding enterprise.

The safety of the drug was assessed on laboratory mice (n = 5) weighing 16-18 g. with intramuscular administration of 0.5 cm³ of the vaccine. The duration of observation of the animals was 14 days.

To study the immunogenic activity of each pasteurellosis and bordetellosis components of the vaccine, we used 2 groups of white mice weighing 16-18 g (control and experimental) for each component, 10 animals each group. The mice of the experimental group were vaccinated intramuscularly twice with an interval of 14 days with a dose of 0.5 cm³. Fourteen days after the second vaccination, animals of both groups were subjected to intraperitoneal infection with the corresponding virulent strains of cultures (punchers) at a dose of 5 LD₅₀, precisely: BordetellabronchisepticaB-1343; Pasteurella multocida №1231 type A; Pasteurella multocida № T-80 type D. The vaccine was considered immunogenic if the survival rate of the animals in the experimental groups after infection was at least 80%, while the mortality rate in the control groups was at least 80%.

Clinical trials of the protective efficacy of the vaccine were carried out in the Tyumen region, at a pig-breeding enterprise, which is infamous for bordetellosis.

For the experiment, two groups of gestating sows were formed, which were vaccinated twice with an interval of 14 days 6 weeks before farrowing. The efficacy of the vaccination was assessed by the survival rate of the piglets obtained from sows vaccinated with an experimental vaccine and a reference drug (the vaccine of the company MSD Animal
Health), average daily gain and average live weight of piglets when they were transferred for slaughter at 180 days of age.

3 Results and discussion

When studying the safety of the drug in laboratory animals, it was found that the vaccine does not cause death of white mice or provoke any local and / or systemic reactions in them.

These results allowed us to move on to the next stage of the study, precisely, the assessment of the immunogenic activity. The obtained results are shown in table 1.

Table 1. The results of the assessment of the immunogenic activity of the experimental inactivated vaccine against IAR.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Experimental group №1 (infected by the strain Bordetella bronchiseptica B-1343)</th>
<th>Experimental group №2 (infected by the strain Pasteurella multocida №1231)</th>
<th>Experimental group №3 (infected by the strain Pasteurella multocida №1231)</th>
<th>Control group №1 (infected by the strain Bordetella bronchiseptica B-1343)</th>
<th>Control group №2 (infected by the strain Pasteurella multocida №1231)</th>
<th>Control group №3 (infected by the strain Pasteurella multocida №80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival due to the series №1, Aluminum hydroxide (all/died)</td>
<td>10/0</td>
<td>10/0</td>
<td>10/0</td>
<td>10/10</td>
<td>10/9</td>
<td>10/10</td>
</tr>
</tbody>
</table>

When carrying out this study, the results were obtained showing that the survival rate of the animals of the experimental groups was 100%, while 90-100% of the animals of the control groups died within 1-2 days after infection, which indicates a high level of immunogenic activity of the vaccine.

The results of assessment of the protective efficacy of the vaccine in pigs are shown in table 2.

Table 2. The results of the assessment of the efficacy of an experimental inactivated vaccine against IAR in pigs.

<table>
<thead>
<tr>
<th>№</th>
<th>Number of sows</th>
<th>Number of piglets obtained</th>
<th>Number of piglets before slaughter</th>
<th>Survival, %</th>
<th>Average weight of a 45-day-old piglet, kg</th>
<th>Average weight of a 180-day-old piglet, kg</th>
<th>Average daily gain, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experim</td>
<td>114</td>
<td>1596</td>
<td>1551</td>
<td>97.16</td>
<td>14.44</td>
<td>134.25</td>
<td>0.887</td>
</tr>
<tr>
<td>Control</td>
<td>246</td>
<td>3619</td>
<td>3508</td>
<td>96.96</td>
<td>14.57</td>
<td>129.92</td>
<td>0.854</td>
</tr>
</tbody>
</table>

The results indicate that the use of the experimental vaccine provides 97.16% survival of piglets, which is comparable with the same indicator in the groups of animals immunized with the reference drug - 96.96%. It should be noted that the average weight of a piglet in the experimental group on the 45th day of life was 130 g less than one in the control group; however, by the 180th day of life, the experimental piglets were 4.33 kg heavier than the animals in the control group. The average daily gain in the experimental group was 33 g more than in the control one.
During the research in the experimental and control groups of sows there were no recorded cases of death, disease, abortion or birth of piglets with congenital malformations, which indicates the safety of the experimental vaccine.

Thus, the results of the studies indicate that the selected antigenic composition for the inactivated vaccine against atrophic rhinitis and pasteurellosis in pigs is optimal, and is capable of providing high immunogenic and protective activity of the drug, both in a vivarium and in an industrial pig-breeding complex.

The study demonstrated the possibility of effectively combating IAR and pasteurellosis in pigs using domestic developments, which fully corresponds to the strategy of combating antibiotic resistance and the principles of import substitution.

Acknowledgement

Work is done within the approved state task and the plan of researches to Federal State Budget Institution «Federal Scientific Centre VIEV» (FSC VIEV) for 2022-2024 without attraction of additional sources of financing.

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