

Immunogenic characteristics of *Avibacterium paragallinarum* (serogroup B) isolates and strains

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SUMMARY

Infectious disease of chickens caused by the bacterium *Avibacterium paragallinarum* remains one of the urgent problems of the poultry industry, as evidenced by numerous reports of recurrent infectious coryza outbreaks in chickens around the world. Bacteriological tests performed in 2014–2019 demonstrated that the disease caused by *Avibacterium paragallinarum* (serogroup B) is endemic in the Russian Federation. The paper presents the results of tests for immunogenic properties of antigens of 13 infectious coryza isolates recovered from the pathological material delivered to the FGBI "ARRIAH" from poultry farms of the Russian Federation and the Republic of Belarus. For this, samples of the vaccine containing formalin-inactivated *Avibacterium paragallinarum* cells and an oil adjuvant were prepared. The poultry was immunized followed by challenge with homologous and heterologous isolates. The degree of manifestation of the disease clinical signs was assessed according to the method proposed by V. E. Soriano. The vaccine sample based on the antigen of the ApB08 isolate induced an insufficient immune response in poultry when infected with the ApB04 and ApB12 isolates. Conversely, a high level of animal protection was demonstrated when infected with the ApB08 isolate. ApB04, ApB08 and ApB12 isolates were comprehensively studied, identified as the most promising for production of vaccines against infectious coryza in chickens, and deposited in the FGBI "ARRIAH" Microorganism Strain Collection under numbers 1116, 5111 and 1818, respectively. Also, a comparative assessment of potency of the experimental vaccine and two commercial products against infectious coryza, including antigens of strains No. 1116, 5111 and 1818, was performed. The experimental vaccine demonstrated maximum protection against infection with *Avibacterium paragallinarum* homologous strains.

Key words: infectious coryza, isolates, strains, vaccine, challenge infection, *Avibacterium paragallinarum*.

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Иммуногенные свойства изолятов и штаммов *Avibacterium paragallinarum* серогруппы B

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РЕЗЮМЕ

Инфекционное заболевание кур, вызываемое бактерией *Avibacterium paragallinarum*, остается одной из актуальных проблем птицеводческой отрасли, о чем говорят многочисленные сообщения о периодических вспышках инфекционного ринита кур в разных странах мира. Проведенные с 2014 по 2019 г.

Бактериологические исследования показали, что Российская Федерация эндемична по данному заболеванию, вызываемому *Avibacterium paragallinarum* серогруппы В. Представлены результаты исследования по изучению иммуногенных свойств антигенов 13 изолятов возбудителя инфекционного ринита кур, выделенных из патологического материала, доставленного в ФГБУ «ВНИИЗЖ» с птицефабрик Российской Федерации и Республики Беларусь. Для этого готовили образцы вакцины, содержащей в своем составе инактивированные формалином клетки *Avibacterium paragallinarum* и масляный адъювант. Птиц иммунизировали с последующим контрольным заражением гомологичными и гетерологичными изолятами. Степень проявления клинических признаков заболевания оценивали по методике, предложенной V. E. Soriano. Образец вакцины на основе антигена изолята АрВ08 индуцировал недостаточный иммунный ответ у птиц при инфицировании изолятами АрВ04 и АрВ12. В свою очередь, при заражении изолятом АрВ08 был показан высокий уровень защиты животных. Изоляты АрВ04, АрВ08 и АрВ12 были всесторонне изучены, определены как наиболее перспективные для производства вакцины против инфекционного ринита кур и депонированы в Государственную коллекцию штаммов микроорганизмов ФГБУ «ВНИИЗЖ» под номерами 1116, 5111 и 1818 соответственно. Также была проведена сравнительная оценка иммуногенной активности экспериментальной вакцины против инфекционного ринита кур, включающей антигены штаммов № 1116, 5111 и 1818, с двумя коммерческими препаратами. Экспериментальный препарат показал максимальный процент защиты птиц при заражении гомологичными штаммами *Avibacterium paragallinarum*.

Ключевые слова: инфекционный ринит кур, изоляты, штаммы, вакцина, контрольное заражение, *Avibacterium paragallinarum*.

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INTRODUCTION

The agent of infectious coryza in chickens is the representative of the *Pasteurellaceae* family, *Avibacterium paragallinarum* bacteria, known as *Haemophilus paragallinarum*. The disease can occur on its own and it can manifest itself in the form of associations with other infectious diseases [1–4].

Numerous reports of recurrent infectious coryza outbreaks in different countries of the world indicate the urgency of the disease problem. Bacteriological studies carried out at the FGBI "ARRIAH" from 2014 to 2019 showed that the Russian Federation is endemic for infectious coryza caused by *Avibacterium paragallinarum* (serogroup B).

Economic losses in the event of infection can be significant, primarily due to poor growth and decrease in egg production in hens at the productivity peak up to 40% within 2–3 weeks [5, 6]. The disease is characterized by facial swelling and serous-fibrinous inflammation of the infraorbital sinuses, air sacs and the mucous membrane of the upper respiratory tract. With an associative course, the duration and severity of the disease may increase. Improper microclimate in the poultry house can provoke the occurrence of the infection, which subsequently leads to an increase in the mortality of birds up to 20–40% [7].

Diseased birds are the source of the infection. The pathogen can circulate in the flock for a long time, this is especially true for small farms containing birds of different ages. When a disease occurs on such farms, it is very difficult to get rid of or control the pathogen. The pathogen is transmitted aerogenically and with drinking water. The incubation period is from one to ten days with the duration of the disease from 14 to 21 days.

One of the most effective measures to combat infectious coryza in chickens is specific prophylaxis. Vaccination reduces dependence on the constant use of antibacterial drugs, which in turn provides cost savings and also pre-

vents problems associated with antibiotic resistance of microorganisms or antibiotic residues in poultry products. However, the main obstacle in manufacturing an effective inactivated vaccine is the selection of commercial pathogen strains. Herewith, it should be borne in mind that the antigenic structure of *A. paragallinarum* is complex and diverse, and the induced immune response in chickens is serotype-specific, with partial cross-protection between individual serotypes.

Serological typing of *A. paragallinarum* is most often carried out according to two interrelated schemes. The three serogroups of the pathogen – A, B, C, are determined by the agglutination test according to the Page's scheme, and nine serotypes (A1–A4, B-1 and C1–C4) by hemagglutination inhibition assay according to K. Kume's scheme [8, 9].

Currently, a number of foreign drugs for specific prevention of infectious coryza in chickens have been registered in the Russian Federation, which differ in composition, production technology, scheme of use and effectiveness. Most of them provide protection against the most common serotypes of the pathogen, which is their undoubted advantage. The versatility of the drugs is due to the presence of cross-protection between serotypes within serogroup A, as well as between individual serogroup C serotypes. The situation with the choice of a serogroup B strain is much more complicated. According to the scheme proposed by K. Kume, there is only one serotype in serogroup B (B-1), however, as far as immunogenicity is concerned only partial cross-protection is observed between the individual strains/isolates [10–12].

In recent years, outbreaks caused by *A. paragallinarum* serotype B-1 have become more frequent in Europe and Asia, which sometimes has been concurrent with the use of commercial vaccines against infectious coryza [5]. At the same time, the reason for the weak cross-protection

between different strains of serotype B-1 has not yet been established. According to a number of researchers, this serotype is widespread in Argentina, Brazil, China, Ecuador, Egypt, Indonesia, Mexico, Peru, the Philippines, South Africa, Spain, the United States of America, and Zimbabwe [6, 11].

Since different strains of serotype B-1 provide only partial cross-protection with each other, it can be assumed that an effective vaccine can be made from only a few strains isolated in a specific geographic region where this serotype is endemic. In addition, commercial vaccines containing antigens of several variants of serotype B-1 strains may also be effective.

Weak cross-protection between different strains/isolates of serogroup B is possibly associated with their high virulence and the leading role of various pathogenic factors [3, 13].

When testing pathological material from birds delivered in the period from 2014 to 2018 to the FGBI "ARRIAH" from various regions of the Russian Federation and the Republic of Belarus, 13 isolates of the infectious coryza pathogen were recovered using bacteriological methods. Analysis of the pathogen's serological profile showed the prevalence of *A. paragallinarum*, serogroup B circulation [3].

In 2016, at two large poultry farms of the Russian Federation, infected with infectious coryza and using a commercial trivalent emulsion vaccine against infectious coryza, during serological typing of isolates recovered from the material derived from sick poultry, it was established that they belonged to serogroup B. This fact raises doubts about the versatility of the vaccine used in our country. Therefore, researches aimed at finding relevant strains of *A. paragallinarum*, serogroup B, will contribute to the creation of a competitive home-made vaccine.

The purpose of this research was to study the immunogenic properties of isolates and strains of *A. paragallinarum*, serogroup B, recovered in the territory of the Russian Federation and the Republic of Belarus, and the possibility of their use in the development of vaccines.

MATERIALS AND METHODS

Pathogen isolates. We used 13 isolates (ApB01–ApB13) of *A. paragallinarum*, serogroup B, recovered at the FGBI "ARRIAH" from material derived from chickens from poultry farms of the Russian Federation and the Republic of Belarus [3]. The origin of the isolates is shown in Table 1.

Isolate cultivation. Columbia agar and broth (Becton Dickinson and Co., USA) containing the following growth factors: 20 µg/ml nicotinamide adenine dinucleotide (NAD, AppliChem, Germany) and 5% horse blood serum were used as a growth medium for culturing *A. paragallinarum*. Cultivation of the bacteria in an agar medium was carried out at 37 °C for 24 h under conditions of an increased content of carbon dioxide, in a liquid nutrient medium – at 37 °C for 18 h under conditions of a normal atmosphere in an orbital shaker-incubator at 150 rpm.

Antigen preparation. Inactivation of *A. paragallinarum* isolates was performed using formaldehyde solution at 37 °C for 48 h, and the final formalin concentration was 0.2% by volume. Then the cells were centrifuged at 3,000 g for 20 min at 4 °C, the sediment was resuspended in sterile phosphate buffered saline with pH 7.4 to a concentration of 100 units (10^{10} m. c./cm³) according to the optical turbidity standard. The obtained antigens were stored at a temperature of (2–8) °C [3].

Experimental poultry. Hisex Brown hens 10 weeks of age were used in the testing. The birds were transported from a farm free of infectious coryza. In all the testings in the experimental and control groups there were ten birds each.

Table 1
***A. paragallinarum* isolate origin**

Таблица 1
Происхождение изолятов *A. paragallinarum*

Isolate	The agent isolated from	The year of isolation	Region
ApB01	Infraorbital sinuses	2014	Kostroma Oblast
ApB02	Conjunctival sac	2014	Moscow Oblast
ApB03	Infraorbital sinuses	2015	Moscow Oblast
ApB04	Infraorbital sinuses	2015	Republic of Belarus
ApB05	Infraorbital sinuses	2016	Republic of Tatarstan
ApB06	Lungs	2016	Vladimir Oblast
ApB07	Infraorbital sinuses	2016	Yaroslavl Oblast
ApB08	Infraorbital sinuses	2016	Orenburg Oblast
ApB09	Infraorbital sinuses	2017	Ulyanovsk Oblast
ApB10	Infraorbital sinuses	2017	Moscow Oblast
ApB11	Infraorbital sinuses	2017	Vladimir Oblast
ApB12	Infraorbital sinuses	2018	Republic of Mordovia
ApB13	Infraorbital sinuses	2018	Yaroslavl Oblast

All experiments on the tested poultry were carried out in strict accordance with the interstate standards for laboratory animal management and care, GOST 33216-2014 and GOST 33215-2014, adopted by the Interstate Council for Standardization, Metrology and Certification, as well as in accordance with the requirements of Directive 2010/63/EU of the European Parliament and the Council of the European Union as of 22.09.2010 on the protection of animals used for scientific purposes.

Preparation and use of the vaccine samples. The antigens of 13 *A. paragallinarum* isolates were used to prepare experimental mono- and multistrain vaccine samples. The antigen suspensions were mixed with Montanide ISA 70 VG oil adjuvant (Seppic, France) at a ratio of 30:70 by weight using a Silverson L4RT laboratory homogenizer. The concentration of each antigen in the inoculation dose of 0.5 cm³ was 5×10⁸ m. c. according to the optical turbidity standard. The preparations were injected twice with an interval of 21 days subcutaneously, in the region of the middle third of the neck from the dorsal side in the caudal direction.

Two commercial vaccines registered in the Russian Federation were used as a comparative control.

Determination of the vaccine immunogenic characteristics. The immunogenic characteristics of antigens were determined by the method of vaccination and subsequent challenge of immunized birds with homologous and heterologous isolates. Chickens were infected intranasally with daily broth cultures of isolates at a dose of 0.5 cm³ containing 10⁸ CFU of the pathogen.

Results of the infection. The degree of manifestation of the disease clinical signs was assessed according to the method proposed by V. E. Soriano [14].

The severity of the upper respiratory tract lesions in an infected bird was assessed using a point-based system:

- 0 – no clinical signs;
- 1 – weak discharge from the nasal passages and/or slight swelling of the infraorbital sinus area;
- 2 – moderate discharge from the nasal passages and/or moderate swelling of the infraorbital sinus area;
- 3 – severe nasal discharge and/or severe swelling of the infraorbital sinuses;
- 4 – severe nasal discharge and severe swelling of the infraorbital sinuses, rattling.

Clinical signs were monitored daily for each bird. Seven days after infection, the points were calculated for each group, and divided by the total number of the infected birds.

RESULTS AND DISCUSSION

The isolates of *A. paragallinarum* ApB01–ApB13 used in the research were recovered in the period from 2014 to 2018 from birds with respiratory pathology aged 38 to 211 days from farms of Vladimir, Kostroma, Moscow, Orenburg, Ulyanovsk and Yaroslavl oblasts, the Republics of Mordovia and Tatarstan, one isolate was recovered from poultry from the Republic of Belarus.

Isolates ApB04 and ApB08 were recovered during an outbreak of infectious coryza from chickens in 1–2 months following the use of commercial vaccines containing antigens of serogroups A, B and C.

In most cases, clinical signs in the diseased chickens were of the same type and were characterized by swelling of the infraorbital sinuses and conjunctival sacs, sometimes watery discharge from the nasal openings was observed. Some birds demonstrated mouth breathing with rattling due to blockage of the nasal passages. Most of

A. paragallinarum isolates were recovered from egg layers. The infraorbital sinuses were the main site of pathogen localization. In 24 h of incubation on the agar medium, the culture formed colonies of round and convex shape, with smooth edges and a smooth surface, gray, 0.5–1.0 mm in diameter (S-shape). A characteristic feature of 24-hour cultures was fluorescence of colonies in oblique light, which indicated the presence of a capsule in the bacteria.

In previous researches, we studied the virulent properties of *A. paragallinarum*, serogroup B, isolates for chickens. When the birds were infected with various isolates of *A. paragallinarum*, the duration of the disease periods was observed to be the same. In sick chickens, similar clinical signs manifested by rhinitis, sinusitis and conjunctivitis were observed [1].

When studying the immunogenic properties of the isolates, a trial immunization ($n = 1$) of chickens with a sample of the vaccine with the ApB08 antigen was performed, followed by infection with homologous and heterologous isolates. The reason for choosing this isolate was that, when cultivated on a nutrient medium, it accumulated to high concentrations, while maintaining a stable hemagglutinating activity and a high level of virulence.

The results of infection of birds immunized with a vaccine sample containing the antigen of the ApB08 isolate are presented in Table 2.

The experiment showed that immunization with the vaccine sample containing the antigen of the ApB08 isolate provided protection of birds at a level of at least 80% when infected with homologous and heterologous isolates, except for ApB04 and ApB12. When the birds of the control groups were infected, an incidence of at least 80% was observed with the development of symptoms typical for infectious coryza. The first clinical signs in birds were observed 24–48 h after infection. Clinically, the disease was manifested by watery discharge from the nasal openings and slight one- or two-sided swelling of the infraorbital sinuses. In some cases, clinical signs were limited to the specified above symptoms. In some birds, the exudate gradually became cloudy and acquired a slimy consistency, as a result of which the nasal openings were obstructed, and the bird began to breathe through the mouth. In most of the infected birds, the disease was accompanied by considerable swelling of the infraorbital sinuses and conjunctival sacs, while in the diseased chickens depression, drowsiness and poor feed intake were observed. Sometimes, due to obstruction of the nasolacrimal duct, exudate penetrated into the oral cavity through the palatal cleft. Feathers in the area of the neck and wings were contaminated with the discharged exudate. Some birds developed unilateral or bilateral catarrhal conjunctivitis, subsequently fibrin was observed in the exudate, the eyelids swelled, and the palpebral fissure narrowed. When the infection was localized in the deeper parts of the respiratory tract, in some individuals, breathing was accompanied by rattling. The most severe symptoms of the disease were observed in birds in groups infected with isolates ApB03, ApB04, ApB08, ApB09, ApB12, and ApB13.

To confirm the revealed differences in the protective characteristics of the antigens ApB04, ApB08, and ApB12, an additional experiment on immunization was carried out, followed by challenge with homologous and heterologous isolates (Table 3).

When the immunized poultry were infected with homologous isolates, the percentage of protection was at least 86.7 ± 13.1%, and when infected with heterologous

Table 2
Immunogenic characteristics of ApB08 antigen when infecting chickens with the homologous and heterologous isolates ($n = 1$)

Таблица 2
Иммуногенные свойства антигена ApB08 при заражении кур гомологичным и гетерологичными изолятами ($n = 1$)

Isolate \ Group	Vaccine with the antigen of ApB08 isolate		Control	
	% P	S	% D	S
ApB01	100	0	100	1.8
ApB02	100	0	80	1.8
ApB03	80	0.8	100	2.2
ApB04	20	2.8	100	3.0
ApB05	100	0	100	1.6
ApB06	100	0	100	1.8
ApB07	80	0.6	90	1.2
ApB08	100	0	100	2.2
ApB09	80	0.8	100	2.4
ApB10	100	0	100	1.8
ApB11	100	0	100	1.6
ApB12	0	3.0	100	3.2
ApB13	80	0.8	100	2.4

% P – protection rate observed at the infection (процент защиты, наблюдаемый при заражении);

% D – percent of the diseased animals at the infection (процент заболевших животных при заражении);

S – severity of the manifested clinical signs (тяжесть проявления клинических признаков) (по V. E. Soriano [12]).

isolates, it did not exceed $26.7 \pm 13.1\%$ ($p \leq 0.05$). As a result of the tests performed, it was found that in terms of immunogenic activity, samples with isolates ApB04, ApB08 and ApB12 differ significantly from each other and therefore, are promising for vaccine production. The isolates were comprehensively studied and deposited in the State Microorganism Strain Collection of the FGBI “ARRIAH” as strains No. 1116, 5111, 1818, respectively.

At the next stage of the research, we carried out comparative tests of an experimental monovalent vaccine pro-

duced by the FGBI “ARRIAH”, including antigens of strains No. 1116, 5111, 1818, and two commercial polyvalent vaccines (Table 4).

The results of the tests performed demonstrated that both commercial vaccines induce an insufficient immune response in birds when infected with strains No. 1116 and 1818.

In addition to determining the immunogenicity of the tested vaccines, we evaluated the clinical profile of the vaccinated and non-vaccinated birds, which included

Table 3
Immunogenic characteristics of ApB04, ApB08, ApB12 antigens at the control challenge with the homologous and heterologous isolates ($n = 3$)

Таблица 3
Иммуногенные свойства антигенов ApB04, ApB08, ApB12 при контрольном заражении гомологичными и гетерологичными изолятами ($n = 3$)

Group	Isolate antigen	% protection in birds challenged with the isolate		
		ApB04	ApB08	ApB12
Immunized	ApB04	93.3 ± 13.1	20.0	26.7 ± 13.1
	ApB08	6.7 ± 13.1	86.7 ± 13.1	6.7 ± 13.1
	ApB12	13.3 ± 13.1	20.0	93.3 ± 13.1
Naive	–	0	0	6.7 ± 13.1

Table 4
Comparative assessment of potency of the commercial polyvalent vaccines and the experimental monovalent vaccine after infection with *A. paragallinarum* strains No. 1116, 5111, 1818 ($n = 3$)

Таблица 4
Сравнительная оценка иммуногенности коммерческих поливалентных вакцин и экспериментальной моновалентной вакцины после заражения штаммами № 1116, 5111, 1818 *A. paragallinarum* ($n = 3$)

Vaccine	% protection in birds challenged with the strain		
	No. 1116	No. 5111	No. 1818
Commercial vaccine 1	46.6 ± 13.1	86.6 ± 6.5	53.3 ± 17.3
Commercial vaccine 2	40.0 ± 11.3	90.0 ± 11.3	53.3 ± 13.1
FGBI "ARRIAH" vaccine	96.6 ± 6.5	100	90.0 ± 11.3
Control	0	0	0

determining the severity and duration of the disease when infected with *A. paragallinarum* strains No. 1116, 5111 and 1818 (Fig. 1–3).

As can be seen from Figure 1, after the challenge of vaccinated and non-vaccinated birds with strain No. 1116, the disease incubation period was less than 24 hours. The maximum development of the disease clinical signs was observed in 48 hours after infection. According to a number of researchers [8, 9, 11], in case of intranasal infection of birds, the incubation period is 24–48 h, in case of contact between the diseased and healthy poultry kept in cages – three days, and in case of aerogenic pathogen transmission – up to six days. On the third day, a decrease in the severity of disease symptoms was observed in poultry of the control group and immunized with commercial vaccines, and in the group of birds immunized with the FGBI "ARRIAH" vaccine based on the homologous strain, complete reco-

very was observed. The duration of the disease in poultry of the control group was seven days, and in chickens immunized with commercial vaccines – five days.

As can be seen in Figure 2, the disease incubation period in birds of the control group was less than 24 hours, and the maximum development of the disease clinical signs was observed 48 hours after infection, with the average point 3.1. The FGBI "ARRIAH" vaccine provided proper protection of birds when challenged with strain No. 5111. Both commercial vaccines also showed a high degree of protection when infected with strain No. 5111, which indicates the presence of cross-immunity between the strains.

As can be seen from Figure 3, the results of infection with strain No. 1818 are quite similar to those for strain No. 5111. The most pronounced disease clinical signs in birds of the control group were observed 48 hours after infection, with an average point of 3.8. In the majority

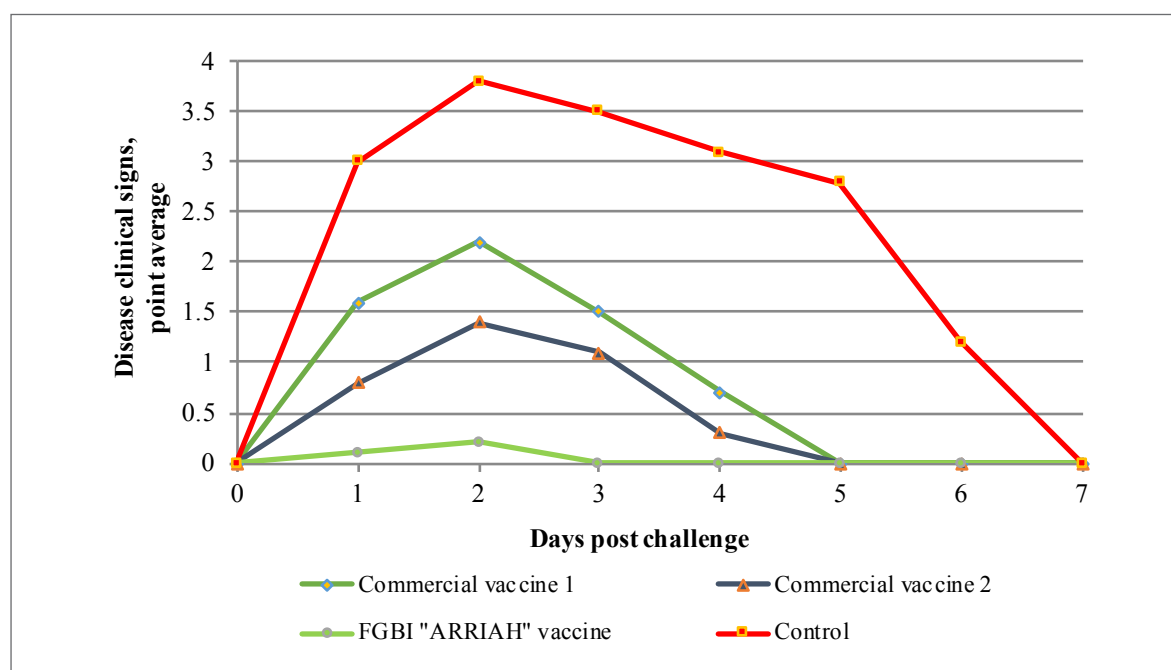


Fig. 1. Clinical profiles of vaccinated and non-vaccinated poultry infected with *A. paragallinarum* strain No. 1116

Рис. 1. Клинические профили вакцинированных и невакцинированных птиц при заражении штаммом № 1116 *A. paragallinarum*

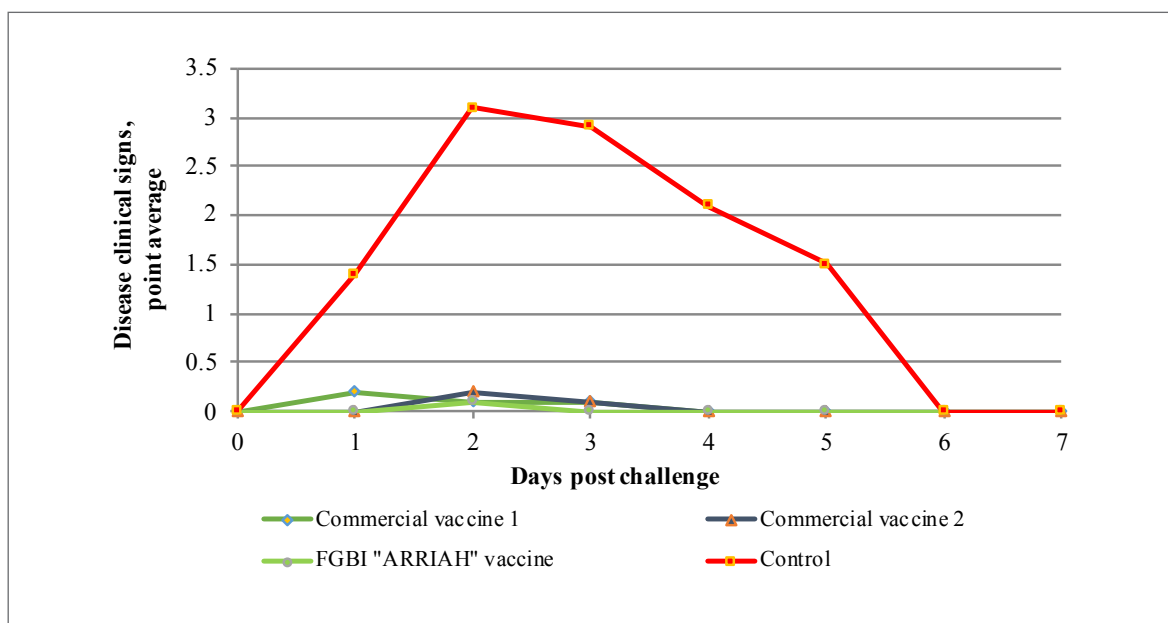


Fig. 2. Clinical profiles of vaccinated and non-vaccinated poultry infected with *A. paragallinarum* strain No. 5111

Рис. 2. Клинические профили вакцинированных и невакцинированных птиц при заражении штаммом № 5111 *A. paragallinarum*

of diseased birds, clinical signs were limited to mild, moderate or severe swelling of the infraorbital sinuses and conjunctival sacs; in sick chickens, depression, drowsiness and poor feed intake were observed. On the third day post infection, a decrease in the severity of disease symptoms was observed in birds of the control and experimental groups. The duration of the disease in birds of the control group was seven days, in chickens immunized with the commercial vaccines – four and five days, respectively, and

in birds vaccinated with the FGBI "ARRIAH" vaccine – three days. According to a number of researchers [7, 8, 11], the duration of the disease in chickens in natural conditions is usually 2–3 weeks, and in case of an experimental infection – 5–7 days.

Partial cross-protection between the vaccine strain and field isolates of *A. paragallinarum*, serogroup B, does not provide a positive effect of poultry immunization [10–13]. Therefore, as it is like that there is no cross-protective

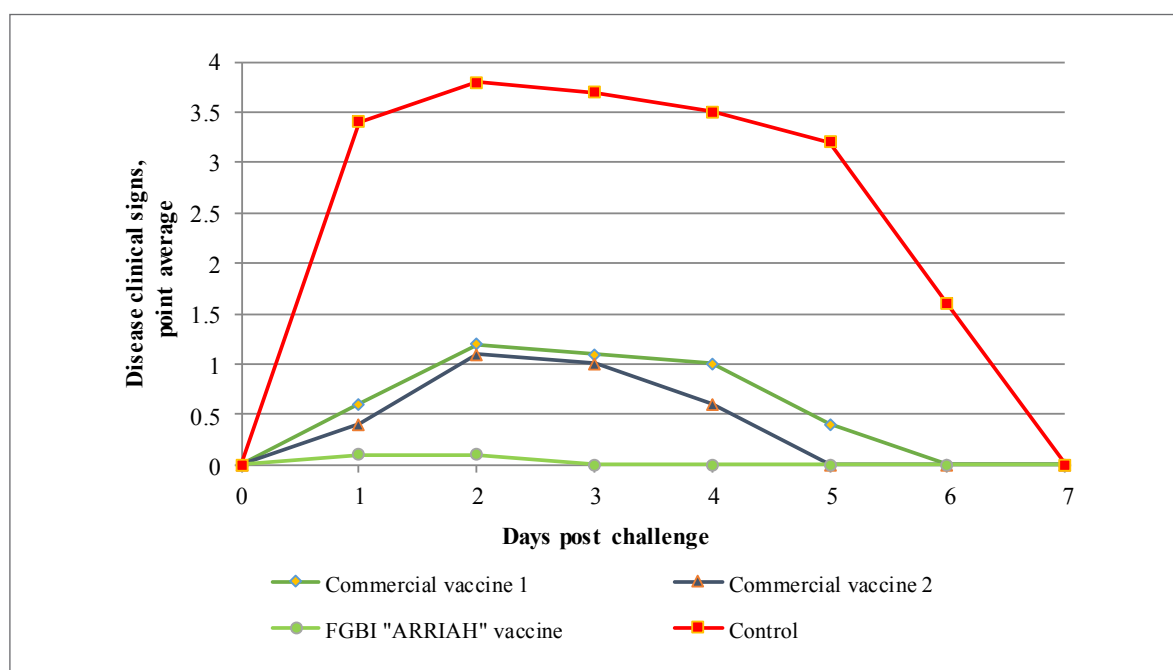


Fig. 3. Clinical profiles of vaccinated and non-vaccinated poultry infected with *A. paragallinarum* strain No. 1818

Рис. 3. Клинические профили вакцинированных и невакцинированных птиц при заражении штаммом № 1818 *A. paragallinarum*

mechanism between strains of *A. paragallinarum*, serogroup B, vaccines containing the maximum possible set of pathogen strains relevant to the region of application are most preferred. In addition, the experience of many countries shows that the most effective in this situation is the use of autogenous vaccines, which are quite effective against any circulating serotype of the pathogen [11].

CONCLUSION

The study of the immunogenic characteristics of antigens of 13 isolates of *A. paragallinarum*, serogroup B, recovered from the pathological material from chickens, delivered to the FGBI "ARRIAH" from poultry farms of the Russian Federation and the Republic of Belarus, showed that vaccine samples based on the antigens of the isolates ApB04, ApB08 and ApB12 induced an insufficient immune response in the poultry in case of cross-infection with heterologous isolates. The percentage of protection was $26.7 \pm 13.1\%$. Conversely, during infection with homologous isolates, a high level of protection was demonstrated – $86.7 \pm 13.1\%$ ($p \leq 0.05$). Isolates ApB04, ApB08 and ApB12 were comprehensively studied, identified as the most promising for the production of vaccines against infectious coryza of chickens, and deposited in the Microorganism Strain Collection of the FGBI "ARRIAH" under numbers 1116, 5111 and 1818, respectively.

The comparative assessment of the immunogenicity of the experimental vaccine against infectious coryza, containing antigens of strains No. 1116, 5111 and 1818, and two commercial preparations, demonstrated that protection induced in birds immunized with the FGBI "ARRIAH" vaccine based on the homologous strains of *A. paragallinarum* was $\geq 90.0 \pm 11.3\%$, and commercial biological products – $46.6 \pm 13.1\%$ and $53.3 \pm 17.3\%$, respectively.

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