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HEMAGGLUTINATION AND ANTIGENIC PROPERTIES OF *AVIBACTERIUM PARAGALLINARUM* ISOLATES RECOVERED IN THE RUSSIAN FEDERATION AND REPUBLIC OF BELARUS

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SUMMARY

The paper demonstrates examination results of antigenic properties of *Avibacterium paragallinarum* reference strain and ten isolates thereof recovered in the Russian Federation and Republic of Belarus. Nine isolates and the reference strain demonstrated type L hemagglutinin (thermoliable, trypsin-sensitive, active against fresh and glutaraldehyde-treated RBC). One isolate was marked by type HI hemagglutinin (thermoliable, trypsin-resistant, active only against glutaraldehyde-treated RBC). The reference strain antigen proved to be inagglutinable by homologous antiserum, and unable to agglutinate RBCs due to hyaluronic acid in the capsular substance. Serological and hemagglutination non-reactivity was removed through the cell treatment with hyaluronidase. The agglutination test demonstrated that seven out of ten tested isolates belonged to the same serological group; herewith, the proportion of the bilateral antigenic relatedness amounted to ≥78.4%. HI results demonstrated ≥92.6% antigenic relatedness of the tested isolates being indicative of the fact that they belonged not only to the same serological group but also to the same serotype. No serological relatedness was identified between the tested isolates and reference strain of serogroup A both using agglutination test (≤23.6%) and HI (≤12.2%). Polymerase chain reaction demonstrated that all the isolates recovered in the Russian Federation and Republic of Belarus in 2014–2016 belonged to serogroup B.

Key words: chicken infectious coryza (Haemophilus infection), isolates, agglutinins, hemagglutinins, antigenic relatedness, *Avibacterium paragallinarum*.

INTRODUCTION

Under modern poultry production conditions, involving intensive raising techniques, high stocking density, quick change of technological processes and frequent vaccination, poultry are exposed to different stressful factors, compromising bird immunity. This increases the risks of different diseases including infectious coryza.

Infectious coryza (Haemophilus infection) is an acute infectious disease of chickens, caused by *Avibacterium paragallinarum* bacteria, previously referred to as *Haemophilus paragallinarum* [4, 15]. The disease involves catarrhal inflammation of nasal mucosa, conjunctiva and nasal passages, as well as subcutaneous head swelling and, rarely, pneumonia. Infectious coryza is reported all over the world and can cause significant economic losses, resulting from egg drop down to 40% for 2–3 weeks, especially during the production peak, less weight gain in broilers and stunted growth of chicks. Up to 10% mortality of young birds can be observed. Usually mortality is low; the majority of birds die if they are concurrently affected by infectious bronchitis, laryngotracheitis or respiratory mycoplasmosis [10, 12].

Antigenic structure of *A. paragallinarum* is complex and diverse. K. Hinz was the first to study the agent antigenic properties in detail against its hemagglutinating activity. It was established that serotype A encapsulated cells are able to agglutinate chicken erythrocytes. Later, using special bacterial treatment techniques, hemagglutinating ability was revealed for serotype C cultures. Based on electronic microscopy data, hemagglutinins concentrate on cell wall membrane; they are heterogenic in their serological and physical and chemical properties. The following types of hemagglutinins are distinguished: HS – heat stabile, trypsin resistant haemagglutinin; HL – a heat labile, trypsin resistant haemagglutinin; L – a heat sensitive, trypsin sensitive haemagglutinin, resistant to hyaluronidase and active against fresh, glutaric dialdehyde-treated chicken RBCs. HS and HL hemagglutinins are only active against non-treated fresh chicken RBCs. The agent antigenic structure is closely related to the type of colonies grown on agar: fluorescent colony bacteria contain type L specific antigen, hemagglutinin and are protective; non-fluorescent colonies comprise cells, deprived of the properties above.

Currently serological and molecular and genetic techniques are used to type the infectious coryza agent.

The first *A. paragallinarum* typing scheme was developed by L. Page using plate agglutination test to recognize the three serotypes – A, B, and C. It has been presumed for a long time that serotype B is not a distinct serotype, but an intermediary between serotypes A and C, which have lost their type-specific antigen [5, 19]. Subsequent tests demonstrated that serotype B is separate and does not have anything in common with serotypes A and C [13]. Based on the results of multiple tests of infectious coryza isolates it was established that serotypes A and B are predominant in Germany, A, B and C serotypes circulate in Spain; A and C are common in Australia, South Africa and Indonesia, and serotype A in Malaysia. The shortcoming of this scheme is the fact that about one third of all isolates cannot be typed, perhaps due to the lack of agglutinogen.

The second typing scheme was developed by Kume based on hemagglutination inhibition test and recognized seven serotypes (HA-1-HA-7), organized into three serogroups (I, II, III) [10]. The advantage of this scheme is that the majority of isolates, not typeable by agglutination test, were typed using HI test. However, the Kume scheme was not widely used due to technical difficulties in test procedure.

To make *A. paragallinarum* classification against antigenic properties more convenient P. Blackall combined Page and Kume test results and proposed a new scheme, involving 3 serogroups based on agglutinin and 9 serovars based on hemagglutinin. Currently serogroup A in the modified scheme comprises 4 serovars (A-1, A-2, A-3 и A-4), serogroup B contains one serovar (B-1), and serogroup C contains 4 serovars (C-1, C-2, C-3 и C-4) [6]. According to test results, the agent serovar profile in different countries is diverse with notable variations. For example in Australia serovars A-4, C-2, C-4 are prevalent, A-1, A-2, C-2, C-3 dominate in Africa, A-3 in Brazil, A-1, A-2, B-1, C-2 prevail in Germany, C-3 in Israel, A-1, A-2, B-1, C-2 in Mexico, A-1, B-1, C-2 in the USA, and A-1, C-1 in Japan [9].

The third agent serotyping technique is based on monoclonal antibody enzyme-linked immunosorbent assay [11]. Despite several reports about sufficient specificity and sensitivity of this technique, it has not found a wide use due to a high variability potential of *A. paragallinarum* and need in broad monoclonal antibody panels.

The fourth serotyping scheme is based on detection of heat stabile antigens by agar gel precipitation test. The shortcoming of this test was that it was not possible to correlate the 6 serovars, detected using this scheme, with the agent major immunotypes, that's why this scheme did not become popular too [5].

There is also a serum bactericidal test, which makes possible differentiation between serological groups A and C [18].

Recently infectious coryza molecular diagnostics techniques, based on polymerase chain reaction (PCR), have become widely used. These techniques have a number of advantages compared to conventional serological methods. PCR is characterized by rapidness and high specificity, excluding false positive results; it can be performed using samples from dead poultry, does not require any special storage or transportation conditions. Real-time PCRs are being developed, enabling high sensitivity-detection of IC agent genome [14]. Sakamoto et al. used multiplex PCR for effective differentiation of *A. paragallinarum* into large genetic groups, coinciding with serogroups [1].

Numerous reports about recent outbreaks of infectious coryza in different countries make this disease problem actual for avian respiratory pathology. Notwithstanding the progress in agent properties research and vaccine development, the global situation remains rather complicated; the disease covers larger areas, thus leading to significant economic losses [8]. Many aspects related to the study of antigenic properties of infectious coryza agent isolates, circulating in the Russian Federation and Republic of Belarus, are understudied, and therefore it makes the choice of strains for the development of domestic prevention tools much more complicated.

The aim of this work was to study hemagglutinating activity and serotyping of *A. paragallinarum* isolates, recovered in the Russian Federation and Republic of Belarus in 2014–2016.

MATERIALS AND METHODS

Strains and isolates. Reference strain No. 29545, serovar A-1 from American type culture collection and 10 *A. paragallinarum* isolates, recovered from chickens with respiratory signs from farms of the Russian Federation and Republic of Belarus. The isolates were stored freeze-dried at 2–4 °C or frozen at –50 °C.

Strain and isolate culture. Columbia agar and Columbia broth (Becton Dickinson and Co., USA) with addition of 20 µg/ml NAD (AppliChem, Germany) and 5% horse serum were used for culturing the strain and isolates. Bacteria were grown in agar medium for 24 hours at 37 °C under elevated CO_2. The strain and isolates were cultured in a liquid nutrient medium using orbital incubator shaker at 150 rpm for 18 hours at 37 °C in a standard atmosphere.

Antigen preparation. Bacteria were inactivated with final formalin concentration of 0.3% by volume at 37 °C for 48 hours. Then cells were sedimented by centrifugation at 3,000 g for 20 min at 4 °C, and the sediment was resuspended in pH 7.2 phosphate buffered saline up to concentration of 100 units (10^{10} m.c./cm^3) against turbidity standard. Final concentration thimerosal of 100 µg/cm^3 was added to the antigen as a preservative. The prepared antigens were stored at 4 °C.

A. paragallinarum isolate antigens were treated with hyaluronidase and trypsin. 100 units of hyaluronidase were added to 1.0 cm^3 of antigen and incubated in a thermostat at 37 °C for 2 hours. 0.25% final concentration of 10% crystalline trypsin solution was added to another part of the suspension and incubated at 37 °C for 30 min. After that, antigens were centrifuged at 3,000 g for 20 min. Supernatant was then removed and antigens were resuspended in initial volumes of phosphate buffered saline. Final concentration thimerosal of 100 µg/cm^3 was added to the antigens as a preservative.

Preparation of hyperimmune rabbit sera for A. paragallinarum isolate serotyping. Rabbit antisera were prepared according to A. M. Thornton and P. J. Blackall [20]. 2.0–2.5 kg rabbits were used. The first dose of 1.0 cm^3 antigen was mixed with Freund's adjuvant (Sigma). 10 days later, six consecutive intravenous antigen adjuvant-free injections (0.5; 1.0; 2.0; 3.0; 4.0 and 4.0 cm^3) were made every three days. Antigen concentration was 10^9 m.c./cm^3. 7 days post the last injection rabbits were bled; the prepared sera were kept frozen at –20 °C.

Agglutination test. Agglutination test (AT) was performed on polystyrene plates. Two-fold serum dilutions

from 1:50 to 1:3200 were made according to a conventional procedure [10].

Hemagglutination test (HAT). 0.4 cm^3 of two-fold antigen dilutions were prepared in round-well plates using phosphate buffered saline, containing 0.1% bovine albumen and 0.001% gelatin starting from 1:2 to 1:2048. Than 0.4 cm^3 of 1% RBC suspension was added to each well. Than the plate was shaken to mix the contents and left at room temperature for 1 hour (until erythrocytes settled in a control).

The results were interpreted using four-cross scoring system. The highest antigen dilution demonstrating clear RBC agglutination (+++ or ++++) was taken as the antigen titre. In parallel absence of RBC spontaneous agglutination was tested (negative control). Difference in isolate hemagglutinating activity was determined at 95% confidence level ($p > 0.05$).

Micro-hemagglutination inhibition test. Micro-hemagglutination inhibition test (micro HI test) was carried out in a conventional way [10]. The test comprised the following procedures: preparation of glutaric dialdehyde-treated suspended chicken RBC, determination of antigen hemagglutinating titre by HI test and antigen working dose, test run.

To remove hemagglutination non-specific inhibitors, the tested sera were inactivated by heating at 56 °C for 30 minutes before testing. 25 µl two-fold serum dilutions from 1:40 to 1:2560 were prepared in pH 7.2 phosphate buffered saline, containing 0.1% of bovine serum albumin and 0.001% of gelatin. 25 µl of antigen working dose (8 HAU) were added to each dilution. The mixture was shaken and left for an hour at (20±2) °C, after that 50 µl of 1% chicken RBC suspension was added to each well. The mixture was shaken again and left for one hour at (20±2) °C (until RBC sedimented in the control), then the results were read. In case specific antibodies were present in the serum, the RBC agglutination was inhibited. End-point dilution causing complete heamagglutination inhibition was taken as the serum titre.

Determination of reciprocal antigenic relationship. Degree of antigenic relationship between *A. paragallinarum* strain and isolates in HA and HI tests was estimated using the following formula by J. Archetti, F. L. Horsfall [3] and expressed as a percentage:

$$r_1 = \frac{\text{Titre } S_{stand}/Ag_{test}}{\text{Titre } S_{stand}/Ag_{test}}; \; r_2 = \frac{\text{Titre } S_{test}/Ag_{stand}}{\text{Titre } S_{test}/Ag_{test}}; \; R\% = 100\sqrt{r_1 r_2},$$

in which titre S_{stand}/Ag_{test} is rabbit serum titre against reference strain/isolate, containing the antigen of tested strain/isolate;

titre S_{stand}/Ag_{stand} is rabbit serum titre against reference strain/isolate, containing the antigen of reference strain/isolate;

titre S_{test}/Ag_{stand} is rabbit serum titre against tested strain/isolate, containing the antigen of reference strain/isolate;

titre S_{test}/Ag_{test} is rabbit serum titre against tested strain/isolate, containing the antigen of tested strain/isolate;

r_1 and r_2 are one-sided antigenic relationship values;

$R\%$ is strain/isolates reciprocal antigenic relationship value.

Serotyping of isolates by PCR. PCR included three stages: DNA extraction from the agent cells; amplification of *A. paragallinarum* genome specific fragment; detection and analysis of PCR products by electrophoresis. Four primers, proposed by R. Sakamoto et al. were used [16]. A forward primer, common for amplification of *A. paragallinarum* genome of three serogroups, and three reverse primers, each specific for a certain serogroup. 800, 1,000–1,100 and 1,500–1,600 b. p. cDNA fragments (amplicones) were considered to be positive results. Herewith 800 b. p. cDNA fragments suggested *A. paragallinarum* serogroup A genome; 1,000–1,100 b. p. fragment was indicative of Serogroup B and 1,500–1,600 b. p. fragment of Serogroup C. In case cDNA fragments were not detected or they did not correspond to the given values, the results were considered negative.

RESULTS AND DISCUSSION

A. paragallinarum studied isolates were detected during 2014–2016 from pathological material of 38–211 day-old poultry from farms of Vladimir, Moscow, Kostroma, Yaroslavl, Orenburg and Ulyanovsk Oblasts, and from the Republics of Mordovia and Tatarstan. Besides, one isolate was recovered from poultry from the Republic of Belarus. Short description of isolate origin is given in Table 1.

Table 1
Origin of infectious coryza isolates

Isolate	Virus localization site	Age, days	Poultry type	Region
1	Infraorbital sinuses	53	Egg-laying	Kostroma Oblast
2	Conjunctival sac	68	Egg-laying	Moscow Oblast
3	Infraorbital sinuses	190	Egg-laying	Orenburg Oblast
4	Infraorbital sinuses	38	Meat	Moscow Oblast
5	Infraorbital sinuses	211	Egg-laying	Republic of Belarus
6	Lungs	170	Egg-laying	Vladimir Oblast
7	Infraorbital sinuses	76	Egg-laying	Republic of Tatarstan
8	Infraorbital sinuses	164	Egg-laying	Yaroslavl Oblast
9	Infraorbital sinuses	114	Egg-laying	Republic of Mordovia
10	Infraorbital sinuses	80	Egg-laying	Ulyanovsk Oblast

Fig. 1. Swollen infraorbital area and nasal discharges caused by infectious coryza

Clinical signs in chickens usually manifested as aqueous nasal discharges. Sometimes swollen infraorbital sinuses and conjunctival sacs (Fig. 1). Some chickens demonstrated mouth breathing with rales due to blocked nasal passages.

The majority of isolates were recovered from egg-laying poultry. The virus was predominantly localized in infraorbital sinuses. A number of publications show that the infectious coryza agent is mostly isolated from the contents of infraorbital sinuses [2, 7, 21].

The previous publication reflects the study results of *A. paragallinarum* isolate virulence for chickens [2]. 9 isolates out of 10 turned to be pathogenic for birds. When infected with different *A. paragallinarum* isolates chickens demonstrated similar duration of the disease courses. Infected birds showed similar clinical signs, involving rhinitis, sinusitis and conjunctivitis. Notwithstanding the same type disease dynamics in infected birds, the isolate virulence turned out to be different. Isolate No. 6 proved to be apathogenic for chickens, but Isolate No. 8 caused 100% morbidity in chickens. 21 days post experimental infection the agent was successfully re-isolated from the contents of infraorbital sinuses of most birds irrespective of the clinical signs presence and disease severity. The obtained data suggested *A. paragallinarum* persistence on upper respiratory tract mucosa due to the presence of adhesins (hemagglutinins).

The agent reference strain and isolates were diverse in their hemagglutinating activities. At the starting stage of the study, the relatedness of isolates to serogroup C was excluded, because for RBC agglutination no additional ultrasound treatment is needed. Nine *A. paragallinarum* isolates out of ten demonstrated hemagguitinating activity against fresh chicken RBCs (Table 2).

When using glutaraldehyde treated RBCs, hemagglutination titre was 2–4 times higher, than when using fresh RBCs ($p < 0.05$). After treatment of different isolate antigens with hyaluronidase, their hemagglutinating activity did not change ($p > 0.05$), which is indicative of hyaluronic acid absence in the capsular material. When the antigens were treated with trypsin, hemagglutinating activity of all isolates, but Isolate No. 6, disappeared.

Serogroup A Reference strain No. 29545 native antigen possessed a low hemagglutinating activity against both fresh and glutaraldehyde treated RBCs. When antigen was treated with hyaluronidase hemagglutinating activity titre became 64 times higher ($p > 0.05$). Other researchers also report presence of hyaluronic acid in the capsular material of some strains and serogroup A isolates [17]. According to some authors, strains containing hyaluronic acid are highly virulent.

The results presented in Table 3 suggest absence of antigenic relationship between the reference strains and tested isolates. The studied isolates were homologous, except for isolates 6, 7 and 10. Herewith there was no reciprocity in antigenic relationship between antigen No. 6 and serum No. 3 and serum No. 6 and antigen No. 3.

Reference strain *A. paragallinarum* native antigen No. 29545 turned out to be inagglutinable in homologous

Table 2
Hemagglutinating activity of native and enzyme-treated *A. paragallinarum* antigens

Isolate, strain	No.	Native antigen		Hyaluronidase-treated antigen		Trypsine-treated antigen	
		Fresh RBCs	Glutaraldehyde treated RBCs	Fresh RBCs	Glutaraldehyde treated RBCs	Fresh RBCs	Glutaraldehyde treated RBCs
Isolate	1	1:64	1:512	1:64	1:512	1:64	<1:2
	2	1:64	1:128	1:128	1:256	1:64	<1:2
	3	1:64	1:128	1:64	1:128	1:64	<1:2
	4	1:256	1:512	1:256	1:512	1:256	<1:2
	5	1:256	1:1024	1:256	1:1024	1:256	<1:2
	6	<1:2	1:32	<1:2	1:32	<1:2	1:32
	7	1:128	1:512	1:128	1:512	1:128	<1:2
	8	1:64	1:256	1:64	1:256	1:64	<1:2
	9	1:32	1:256	1:32	1:256	1:32	<1:2
	10	1:16	1:64	1:16	1:64	1:16	<1:2
Strain	29545	1:4	1:8	1:256	1:512	1:4	<1:2

Table 3
Antigenic relationship between *A. paragallinarum* strain and isolates by agglutination test
n = 3

Antigen		Reciprocal relationship, *R*% (mean value)										
		Antigen specific serum										
		1	2	3	4	5	6	7	8	9	10	29545
Isolate	1	**100**	96.8	82.4	86.4	92.6	88.4	86.8	98.6	94.2	100	4.2
	2	94.2	**100**	82.4	100	82.8	94.2	100	88.4	96.8	78.8	23.6
	3	86.8	82.4	**100**	96.4	90.2	84.6	94.2	90.3	92.4	92.8	12.6
	4	82.4	98.4	92.6	**100**	86.4	100	98.4	92.8	100	86.2	2.8
	5	92.6	86.4	83.6	78.4	**100**	87.4	90.2	91.8	94.7	100	4.8
	6	80.7	84.8	62.4	100	96.6	**100**	87.4	84.3	84.6	94.6	11.8
	7	100	90.2	93.5	10.64	94.2	86.4	**100**	56.2	94.8	88.2	4.2
	8	86.4	82.8	94.2	100	92.8	78.8	96.0	**100**	82.8	96.8	11.8
	9	92.4	100	78.8	82.8	86.8	80.4	100	90.4	**100**	98.4	8.6
	10	100	94.8	80.4	43.6	100	86.2	96.4	84.2	100	**100**	10.2
Strain	29545	3.0	17.6	8.2	4.3	2.1	8.7	6.4	11.8	7.4	8.7	**100**

antiserum. Antigen serological inactivity disappeared when it was treated with hyaluronidase.

The obtained results show that the *A. paragallinarum* tested isolates belong to serogroup A.

Table 4 data indicate that all tested isolates were homologous against hemagglutinin. The minimum value of reciprocal relationship was 78.4%. The data obtained suggest that all tested isolates belong not only to one serogroup, but to one serovar.

Homogeneity of recovered isolates was confirmed by serotyping multiplex PCR (Fig. 2).

As a result of PCR product analysis it was established that amplicones of all tested isolates were 1000–1100 b. p. long, and this correlated with *A. paragallinarum* serogroup B amplicone length, measured by PCR. Thus all recovered isolates belonged to a genetically separate serogroup B, supported by serotyping results.

Fig. 2 Electropherogram of multiplex PCR products when testing A paragallinarum recovered isolates and strain

M – DNA fragment length marker;
A – amplicone of strain No. 29545 sample;
1 – amplicone of isolate No. 1 sample ;
2 – amplicone of isolate No. 2 sample;
3 – amplicone of isolate No. 3 sample;
4 – amplicone of isolate No. 4 sample;
5 – amplicone of isolate No. 5 sample;
6 – amplicone of isolate No. 6 sample;
7 – amplicone of isolate No. 7 sample;
8 – amplicone of isolate No. 8 sample;
9 – amplicone of isolate No. 9 sample;
10 – amplicone of isolate No. 10 sample.

CONCLUSION

Heat labile trypsin sensitive type L hemagglutinin, active against fresh and glutaraldehyde – treated chicken RBCs was found in 9 *A. paragallinarum* isolates. One isolate was characterized by HL hemagglutinin: heat labile, trypsin resistant and active only against glutaraldehyde-treated chicken RBCs. *A. paragallinarum* reference strain No. 29545 antigen turned out to be inagglutinable in homologous antiserum and unable to agglutinate fresh and glutaraldehyde-treated chicken RBCs. Antigen serological and hemagglutinating inactivity disappeared after hyaluronidase treatment. Using agglutination test it was

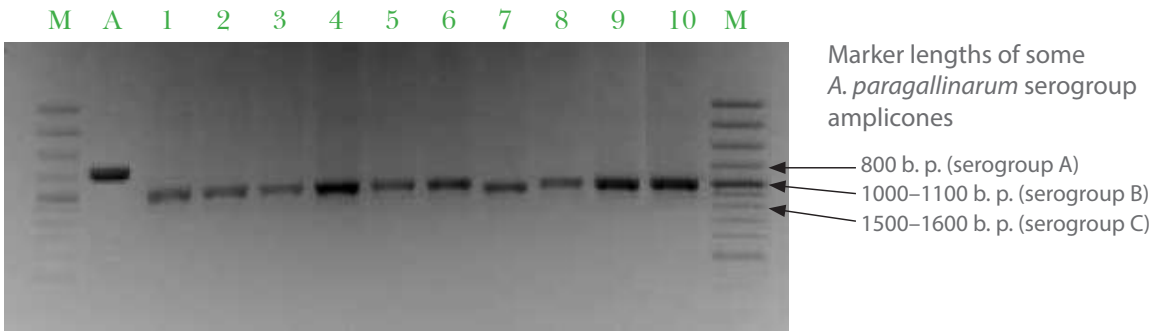

Table 4
Antigenic relationship between *A. paragallinarum* by hemagglutination inhibition test
n = 3

Antigen (isolate, strain No.)	Reciprocal relationship, R% (mean value)										
	Antigen specific serum										
	1	2	3	4	5	6	7	8	9	10	29545
1	**100**	100	96.8	86.8	92.4	86.4	88.8	92.4	96.7	96.2	6.4
2	92.6	**100**	94.6	92.6	98.2	94.6	86.6	100	90.2	82.4	7.2
3	98.6	96.4	**100**	100	84.2	94.8	90.4	98.4	86.2	84.8	10.4
4	100	94.8	95.2	**100**	94.2	88.4	96.8	100	94.6	98.8	3.6
5	87.6	93.2	87.4	97.8	**100**	98.2	84.6	90.2	97.2	100	4.8
6	90.2	100	79.6	96.2	86.2	**100**	88.2	92.6	100	96.4	8.2
7	94.6	96.6	91.3	100	96.8	100	**100**	98.2	99.3	86.8	2.2
8	88.2	100	96.4	96.3	98.4	92.8	96.8	**100**	100	88.2	4.8
9	86.6	90.8	87.8	78.4	92.6	94.8	94.2	96.2	**100**	100	6.2
10	96.6	92.4	92.8	86.4	88.4	86.2	98.0	100	98.4	**100**	4.2
29545	6.3	4.8	8.6	12.2	6.3	5.2	10.3	4.2	6.8	8.2	**100**

established that seven tested isolates out of ten belonged to one serological group; herewith reciprocal antigenic relationship value was ≥78.4%. Hemagglutination inhibition test demonstrated ≥92.6% antigenic relationship between tested isolates, which suggests that they belong not only to one serogroup, but also to one serovar. Agglutination test (≤12.2%) and hemagglutination inhibition test (≤23.6%) showed no serological relatedness between the tested isolates and serogroup A reference strain was detected. Using PCR it was established that all isolates, recovered in the Russian Federation and Republic of Belarus in 2014–2016 belonged to a genetically distinct serogroup B.

REFERENCES

1. Andreychuk D. B., Chvala Il. A., Potehin A. V. Methodical guidelines for *Avibacterium paragallinarum* genome detection and typing using multiplex-PCR [Metodicheskie rekomendacii po vyyavleniyu genoma i tipirovaniyu mikroorganizma *Avibacterium paragallinarum* s pomoshch'yu PCR v formate mul'tipleks].Vladimir: FGBI "ARRIAH", 2016 (in Russian).
2. Yevgrafova V. A., Potehin A. V. Virulence of chicken infectious coryza agent isolates [Virulentnost' izolyatov vozbuditelya infekcionnogo rinita kur]. *Veterinary Science Today*. 2017; 4 (23): 28–32 (in Russian).
3. Archetti I., Horsfall F. L. Persistent antigenic variation of influenza A virus after incomplete neutralization in ovo with heterologous immune serum. *J. Exp. Med*. 1950; 92 (5): 441–462; DOI: 10.1084/jem.92.5.441.
4. Blackall P. J. The avian haemophili. *Clin. Microbiol. Rev*. 1989; 2 (3): 270–277; DOI: 10.1128/CMR.2.3.270.
5. Blackall P. J. Infectious coryza: overview of the disease and new diagnostic options. *Clin. Microbiol. Rev.* 1999; 12 (4): 627–632; DOI: 10.1128/CMR.12.4.627.
6. Blackall P. J., Eaves L. E., Rogers D. G. Proposal of a new serovar and altered nomenclature for *Haemophilus paragallinarum* in the Kume hemagglutinin scheme. *J. Clin. Microbiol.* 1990; 28 (6): 1185–1187; PMCID: PMC267902.
7. Blackall P. J., Matsumoto M. Infectious coryza. *Diseases of Poultry*. ed. Y. M. Saif [et al.]. 11th ed. Ames, Iowa, 2003: 691–703.
8. Bragg R. R. Effects of differences in virulence of different serovars of *Haemophilus paragallinarum* on perceived vaccine efficacy. *Onderstepoort J. Vet. Res*. 2005; 72 (1): 1–6; PMID: 15991700.
9. Characterization of isolates of *Haemophilus paragallinarum* from Indonesia. S. Poernomo, Sutarma, M. Rafiee, P. J. Blackall. *Australian Vet. J.* 2000; 78 (11): 759–762; DOI: 10.1111/j.1751-0813.2000.tb10447.x.
10. Eaves L. E., Rogers D. G., Blackall P. J. Comparison of hemagglutinin and agglutinin schemes for the serological classification of *Haemophilus paragallinarum* and proposal of a new hemagglutinin serovar. *J. Clin. Microbiol*. 1989; 27 (7): 1510–1513; PMCID: PMC267605.
11. Evaluation of a panel of monoclonal antibodies in the subtyping of *Haemophilus paragallinarum*. P. J. Blackall, Y. Z. Zheng, T. Yamaguchi [et al.]. *Avian Dis.* 1991; 35 (4): 955–959; PMID: 1786026.
12. Further studies on the use of a polymerase chain reaction test for the diagnosis of infectious coryza. X. Chen, C. Song, Y. Gong, P. J. Blackall. *Avian Pathol.* 19984; 27 (6): 618-624; DOI: 10.1080/03079459808419393.
13. Pathogenicity and serovar-specific hemagglutinating antigens of *Haemophilus paragallinarum* serovar B strains. T. Yamaguchi, P. J. Blackall, S. Takigami [et al.]. *Avian Dis.* 1990; 34 (4): 964–968; PMID: 2149263.
14. Rapid and sensitive detection of *Avibacterium paragallinarum* in the presence of other bacteria using a 5' Taq nuclease assay: a new tool for diagnosing infections coryza. B. G. Corney, I. S. Diallo, L. Wright [et al.]. *Avian Pathol.* 2008; 37 (6): 599–604; DOI: 10.1080/03079450802449139.
15. Reclassification of *Pasteurella gallinarum*, [*Haemophilus*] *paragallinarum*, *Pasteurella avium* and *Pasteurella volantium* as *Avibacterium gallinarum* gen. nov., comb. nov., *Avibacterium paragallinarum* comb. nov., *Avibacterium avium* comb. nov. and *Avibacterium volantium* comb. Nov. P. J. Blackall, H. Christensen, T. Beckenham [et al.]. *Int. J. Syst. Evol. Microbiol*. 2005; 55 (Pt): 353–362; DOI: 10.1099/ijs.0.63357-0.
16. Sakamoto R., Kino Y., Sakaguchi M. Development of a multiplex PCR and PCR-RFLP method for serotyping of *Avibacterium paragallinarum*. *J. Vet. Med. Sci.* 2012; 74 (2): 271–273; DOI: 10.1292/jvms.11-0319.
17. Sawata A., Kume K. Relationships between virulence and morphological or serological properties of variants dissociated from serotype 1 *Haemophilus paragallinarum* strains. *J. Clin. Microbiol.* 1983; 18 (1): 49–55; PMCID: PMC270743.
18. Sawata A., Kume K., Nakai T. Serologic typing of *Haemophilus paragallinarum* based on serum bactericidal reactions. *Jpn. J. Vet. Sci.* 1984; 46 (6): 909–912; PMID: 6521153.
19. Sawata A., Kume K., Nakase Y. Biologic and serologic relationships between Page's and Sawata's serotypes of *Haemophilus paragallinarum*. *Am J. Vet. Res*. 1980; 41 (11): 1901-1904; PMID: 7212424.
20. Thornton A. M., Blackall P. J. Serological classification of Australian isolates of *Haemophilus paragallinaru. Aust. Vet J*. 1984; 61 (8): 251–253; DOI: 10.1111/j.1751-0813.1984.tb15533.x.
21. Virulence of the nine serovar reference strains of *Haemophilus paragallinarum*. V. E. Soriano, G. M. Longinos, R. P. Fernández [et al.]. *Avian Dis*. 2004; 48 (4): 886–889; DOI: 10.1637/7188-033104R1.

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