DOI: 10.29326/2304-196X-2021-3-38-224-229

Comparative testing of vaccines based on viruses of genetic lineages G1 and Y280 for their potency against low pathogenic avian influenza H9N2

S. V. Frolov[1], L. O. Scherbakova[2], N. V. Moroz[3], V. N. Irza[4], V. Yu. Kulakov[5]
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia
[1] *ORCID 0000-0001-6802-9940, e-mail: frolov@arriah.ru*
[2] *ORCID 0000-0001-5434-6179, e-mail scherbakova@arriah.ru*
[3] *ORCID 0000-0002-9672-8594, e-mail: moroz@arriah.ru*
[4] *ORCID 0000-0001-7489-1772, e-mail: irza@arriah.ru*
[5] *ORCID 0000-0002-1898-4576, e-mail: kulakov@arriah.ru*

SUMMARY
Due to the genetic diversity of low pathogenic avian influenza (LPAI) viruses of subtype H9N2, it deemed appropriate to study the potency of the vaccines based on the antigens of strains A/chicken/Amursky/03/12 and A/chicken/Chelyabinsk/314-1/20 that represent currently circulating in the Russian Federation genetic lineages Y280 and G1, respectively. While low pathogenicity of the agent does not allow demonstrating the vaccine protective properties by the direct methods generally used for potency assessment (e.g. morbidity and mortality), the indirect methods were used: determination of antigenic relatedness of the strains, level of the postvaccinal homologous and heterologous humoral immunity, analysis of the virus genome synthesis inhibition (reduction) in vaccinated birds following their challenge. The strains used in the vaccines were determined to have some antigenic differences, which were demonstrated in the hemagglutination inhibition (HI) assay during control of the postvaccinal immunity in birds. Both vaccines generally induced strong humoral immunity in vaccinated birds (9–10 $\log_2$ determined using HI assay) with some difference in the levels of the immune response following the use of homologous or heterologous antigens. It was also reliably determined that homologous immunity facilitated more expressed inhibition of the virus reproduction after the challenge. The level of inhibition (reduction) of the virulent LPAI virus genome synthesis in vaccinated birds following their challenge with H9N2 virus of genetic lineage G1 was higher in birds following homologous vaccination, while the time periods of the genome detection in the biomaterial samples were the same. It was demonstrated that due to antigenic and immunogenic differences between LPAI H9N2 strains, use of both antigenic components in the inactivated vaccines is appropriate.

Keywords: vaccines, low pathogenic avian influenza (LPAI), vaccine potency, humoral immunity

Acknowledgements: The study was funded by the FGBI "ARRIAH" within the framework of "Veterinary Welfare" research work.

For citation: Frolov S. V., Scherbakova L. O., Moroz N. V., Irza V. N., Kulakov V. Yu. Comparative testing of vaccines based on viruses of genetic lineages G1 and Y280 for their potency against low pathogenic avian influenza H9N2. *Veterinary Science Today*. 2021; 10 (3): 224–229. DOI: 10.29326/2304-196X-2021-3-38-224-229.

Conflict of interests: The authors declare no conflict of interests.

For correspondence: Sergey V. Frolov, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory for Avian Diseases Prevention, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: frolov@arriah.ru.*

УДК 619:616.98:578.832.1:636.52/.58:615.371:616-097.3

Сравнение иммуногенной активности вакцин против низкопатогенного гриппа птиц подтипа H9N2, изготовленных на основе вирусов генетических линий G1 и Y280

С. В. Фролов[1], Л. О. Щербакова[2], Н. В. Мороз[3], В. Н. Ирза[4], В. Ю. Кулаков[5]
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия
[1] *ORCID 0000-0001-6802-9940, e-mail: frolov@arriah.ru*
[2] *ORCID 0000-0001-5434-6179, e-mail scherbakova@arriah.ru*
[3] *ORCID 0000-0002-9672-8594, e-mail: moroz@arriah.ru*
[4] *ORCID 0000-0001-7489-1772, e-mail: irza@arriah.ru*
[5] *ORCID 0000-0002-1898-4576, e-mail: kulakov@arriah.ru*

РЕЗЮМЕ

В связи с генетическим разнообразием вирусов низкопатогенного гриппа птиц H9N2 представлялось целесообразным изучение иммуногенной активности вакцин, изготовленных на основе антигенов штаммов A/chicken/Amursky/03/12 и A/chicken/Chelyabinsk/314-1/20 – представителей генетических линий Y280 и G1 соответственно, циркулирующих на территории Российской Федерации. Поскольку низкая патогенность возбудителя не позволяет продемонстрировать протективные свойства вакцин прямыми методами оценки иммуногенности препаратов (например, заболеваемость и смертность), применяли косвенные методы: определение антигенной родственности штаммов, напряженности поствакцинального гуморального гомо- и гетерологичного иммунитета птиц, оценка подавления (редукции) синтеза генома вируса после контрольного заражения в организме вакцинированных птиц. Было установлено, что использованные в составе вакцин штаммы имели некоторые антигенные различия, которые были обнаружены в реакции торможения гемагглютинации при контроле поствакцинального иммунного ответа птиц. В целом обе вакцины индуцировали напряженный гуморальный иммунитет у привитых птиц (9–10 $\log_2$ в реакции торможения гемагглютинации) с некоторой разницей в величине иммунного ответа при использовании гомо- и гетерологичного антигенов. Также было достоверно установлено, что гомологичный иммунитет обеспечивал более выраженное подавление репродукции вируса при экспериментальном заражении. Степень подавления (редукции) синтеза генома вирулентного вируса низкопатогенного гриппа птиц в организме вакцинированных особей после их заражения вирусом H9N2 генетической линии G1 была выше у птиц, привитых гомологичной вакциной при одинаковых сроках детекции генома в пробах биоматериала. Показано, что с учетом антигенных и иммуногенных различий между штаммами вируса низкопатогенного гриппа птиц H9N2 целесообразно использование обоих антигенных компонентов в составе инактивированных вакцин.

Ключевые слова: вакцина, низкопатогенный вирус гриппа птиц подтипа H9N2, иммуногенность вакцины, гуморальный иммунитет

Благодарность: Работа выполнена за счет средств ФГБУ «ВНИИЗЖ» в рамках тематики научно-исследовательских работ «Ветеринарное благополучие».

Для цитирования: Фролов С. В., Щербакова Л. О., Мороз Н. В., Ирза В. Н., Кулаков В. Ю. Сравнение иммуногенной активности вакцин против низкопатогенного гриппа птиц подтипа H9N2, изготовленных на основе вирусов генетических линий G1 и Y280. *Ветеринария сегодня*. 2021; 10 (3): 224–229. DOI: 10.29326/2304-196X-2021-3-38-224-229.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Фролов Сергей Владимирович, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории профилактики болезней птиц ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: frolov@arriah.ru.*

INTRODUCTION

Low pathogenic avian influenza (LPAI) viruses H9N2 are widely spread in poultry in Africa, Asia and the Middle East. Based on the genetic features, there are two main genetic groups of H9N2 viruses – 'North American' and 'Eurasian', circulating in poultry and wild birds. The Eurasian group is divided into three main genotypes: G1, Y280 and Y439. Viruses of the G1 and Y280 genetic lineages demonstrate the widest geographical distribution, from East Asia to the Middle East [1–4].

In 2012, 2017 and 2018, LPAI cases in the poultry industry of the Russian Federation were caused by H9N2 virus of Y280 lineage [5–7]. In 2018, low pathogenic avian influenza virus H9N2 of G1 genetic lineage was isolated for the first time from wild birds in the Amur Oblast [8].

In 2019–2020, low pathogenic H9N2 influenza virus of G1 genetic lineage was detected in poultry raised on commercial poultry farms of the Ural region (the Chelyabinsk Oblast and the Perm Krai), as well as on farms of the European part of the country [5–7, 9].

Due to significant economic losses caused by this infection, some countries have been actively vaccinating against LPAI H9N2: Israel (since 2003), South Korea (since 2007). China has been a leader in prevention of LPAI H9N2 subtype since 1998 [1].

In 2012, the FGBI "ARRIAH" developed inactivated combined emulsion vaccine against LPAI H9N2 and Newcastle disease and launched its the serial production. H9N2 virus of Y280 lineage, isolated in 2012 from chickens on a poultry farm in the Amur region is used as a production virus strain. Currently, this vaccine is successfully used in commercial poultry farming of the Russian Federation.

Due to the genetic diversity of LPAI H9N2 viruses, it is appropriate to study the immunogenic activity of vaccines against H9N2 avian influenza, based on the antigens of different virus sublineages circulating in the Russian Federation. Low pathogenicity of the pathogen makes it impossible to demonstrate protective properties of the vaccines using direct methods of immunogenicity assessment (for example, morbidity and mortality). Therefore, indirect methods were used, i.e. determination of strain antigenic relatedness, assessment of humoral homo- and heterologous immunity levels in birds after vaccination, assessment of the virus genome synthesis inhibition in the vaccinated birds after challenge.

MATERIALS AND METHODS

Antigens. Antigens of the following strains of LPAI H9N2 subtype were used in the experiment:

– A/chicken/Chelyabinsk/314-1/20 (G1 genotype), infectivity before inactivation 8.75 lg EID_{50}/cm^3, hemagglutinating activity 9 $\log_2$ GAE, named as "Chelyabinsk-20";

– A/chicken/Amursky/03/12 (Y280 genotype), infectivity before inactivation 8.25 lg EID_{50}/cm^3, hemagglutinating activity 9 $\log_2$ GAE, named as "Amur-12".

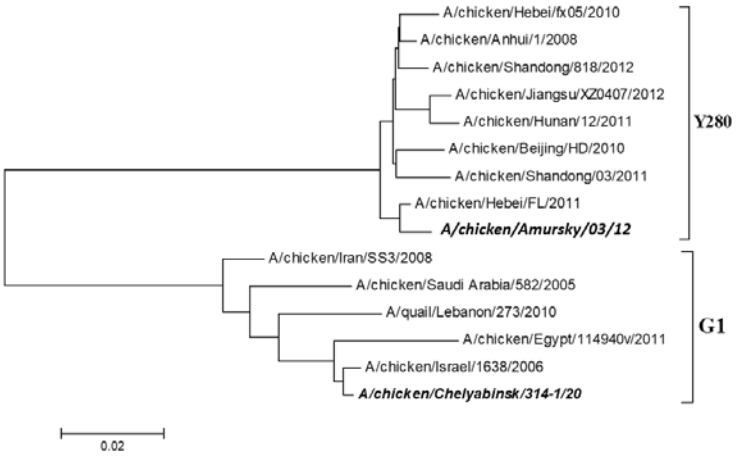

Fig. Phylogenetic tree constructed on the basis of LPAI H9 HA gene alignment using NJ method and MEGA 6.0 software

In order to standardize the antigen content in the inoculation dose (determined before inactivation on the basis of the infectious titer), the inactivated suspension of "Chelyabinsk-20" virus strain was diluted (by 3 times) before emulsification, so that the vaccine inoculation dose contained equal number of LPAI virus antigens.

Different genetic lineages of the viruses are shown in the figure.

Vaccines. The two vaccines used in the experiment contained a mixture of LPAI and Newcastle disease virus antigens (taken at equal volumes), which is the active component of the vaccine:

– an experimental vaccine with an antigen of "Chelyabinsk-20" strain, hereinafter referred to as "Chelyabinsk-20 (G1) Vaccine";

– an experimental vaccine with an antigen of "Amursky-12" strain, hereinafter referred to as "Amursky-12 (Y280) Vaccine".

During vaccine production, the active component (antigen) was combined with an oil adjuvant Montanide ISA 70 (Seppic, France) in the proportion 30:70 (% by weight) and emulsified in a high-speed laboratory mixer Silverson (England) at a speed of 6,000 rpm for 5 minutes.

LPAI virus for challenge. A virus of G1 genetic lineage, i.e. A/chicken/Chelyabinsk/314-1/20 H9N2 ("Chelyabinsk-20"), was used in the experiment in the form of a suspension prepared from a freeze-dried product with an original infectivity of 8.95 lg EID_{50}/cm^3.

Poultry. The experiment was carried out in egg laying chickens at the age of 80 days, taken from a farm free from acute forms of avian infectious diseases.

All animal experiments were performed in strict compliance with interstate standard on laboratory animal keeping and handling GOST 33215-2014, adopted by Interstate Council on Standardization, Metrology and Certification and pursuant to the requirements of Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

The experiment scheme. According to the tested vaccines, the poultry were divided into two groups (20 chickens in each). The vaccines were administered intramuscularly into the chest area in a volume of 0.5 cm^3.

On Day 28 post vaccination, the chickens of both groups were challenged with "Chelyabinsk-20" virus. The virus-containing suspension was administered orally in a volume of 1 cm^3, the infectious dose was 7.3 lg EID_{50}.

During the experiment, oropharyngeal and cloacal swabs were taken in both groups after the challenge, either daily or sometimes with a one day interval. The samples were tested in real-time reverse transcription polymerase chain reaction (real-time RT-PCR) to detect the challenge virus genome. Totally, 12 samples of each type of material were taken.

Before the challenge and on Day 15 after it, blood samples were taken from all the chickens to compare antibody titers to the LPAI virus in the hemagglutination inhibition test (HI test) with virus antigens of two different genetic lineages.

Test methods. The virus genome in the samples was detected and the amplification cycle threshold values were estimated in accordance with Methodical Instructions 45-16 "Instructions for RNA detection of avian influenza virus type A using real time RT-PCR" [10].

Serological tests. The hemagglutination inhibition test (HI) was performed according to a generally accepted procedure using a diagnostic kit manufactured by the FGBI "ARRIAH" to detect antibodies to avian influenza virus subtype H9 (H9N2 virus antigen of Y280 genetic lineage) and H9N2 virus antigen of G1 genetic lineage obtained during the vaccine production. Antibody titers of ≥ 4 $\log_2$ (≥ 1:16) were considered positive.

Statistical analysis. The significance of differences between quantitative indicators was analyzed. We used Miller's nonparametric multiple-comparison procedure [11] for the *k*-number of samples (groups), based on checking feasibility of the following inequality $|H_1 - H_2| / (\sqrt{(k\,(kn + 1) / 12)}) \geq q$, where H_1 and H_2 stand for the mean ranks of the compared samplings 1 and 2 in the general ordered series; *n* stands for samplings volumes ($n_1 = n_2 = n$); *q* is the table coefficient for *k* number and the given probability of forecast error (*p*) [11]. The differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Studying antigenic relationship between LPAI H9N2 strains and assessing strength of the post-vaccination immunity in poultry. On Day 28 post vaccination blood sera from poultry were tested with homologous and heterologous antigens, sera after challenge were tested with "Chelyabinsk-20" strain antigen. The results obtained are shown in Table 1. To facilitate processing of the data given in the table, vaccines and antigens are indicated by their clusters in the genetic lineages of H9N2 virus.

Based on the data in Table 1, it can be concluded that:

a) the antibody titers determined in homologous systems when testing blood sera taken on Day 28 after vaccination of poultry with both vaccines were almost the same, as demonstrated by the equality of logarithmic medians. The mean titer (*T*) was 9 $\log_2$ (1:512). It means that the poultry have demonstrated quite strong and roughly equal humoral immune response to each of the tested antigens;

b) when testing sera specified in point "*a*", the mean rank equivalents of (*H*) titers established in homologous systems for both antigens were reliably ($p < 0.05$) different

from the corresponding values observed when using heterologous antigens. For the "Chelyabinsk-20 (G1) Vaccine", *H* value was: $H_{homol.} = 24.2 > H_{heterol.} = 16.8$ and for the "Amursky-12 (Y280) Vaccine": $H_{homol.} = 24.4 > H_{heterol.} = 16.6$. It meant that, on average, heterologous reactions demonstrated significantly less activity, which, in turn, indicated certain antigenic differences between the studied strains.

For both antigens, the difference between medians of log titers in hetero- and homologous reactions was negative ($8 \log_{2(heterol.)} - 9 \log_{2(homol.)} = -1 \log_2$), i.e. the heterologous reaction in both cases was twice less active (anti-$\log_2(-1) = 1/2$). This value can be interpreted by Archetti and Horsfall as an assessment of antigenic relationship (*r*), which for each strain was $r = 1/2 \times 100 = 50\%$. The obtained value suggests a slight antigenic difference between the studied viruses;

c) a test with "Chelyabinsk-20" strain antigen and sera from the poultry challenged with this LPAI strain, demonstrated that the mean rank equivalents of titers determined for the sera from poultry vaccinated with experimental and mass produced vaccines differed significantly ($p < 0.01$): $H_{homol.} = 15.4 < H_{heterol.} = 25.7$. This effect can be explained by the fact that after administration of the vaccine based on "Chelyabinsk-20" strain antigen, the poultry immunity was quite strong in relation to the homologous virus, and the pathogen penetration into the body did not cause any impact on the poultry immune system. The poultry that were administered mass-produced vaccine

Table 1
HI titres of LPAI virus antibodies in sera of vaccinated birds

Estimate of antibody titers (*T*, $\log_2$) on Day 28 post vaccination and on Day 15 after challenge, established in HI tests using homologous and heterologous antigens											
Day 28 post vaccination								Day 15 after challenge			
"Chelyabinsk-20" strain (G1) Vaccine				"Amursky-12" strain (Y280) Vaccine				"Chelyabinsk-20" strain (G1) Vaccine		"Amursky-12" strain (Y280) Vaccine	
G1 antigen		Y280 antigen		Y280 antigen		G1 antigen					
9	*25*[*]	9	*25*	8	*15.5*	7	*6*	9	*11.5*	10	*24*
10	*34*	7	*4.5*	11	*39.5*	8	*15.5*	10	*24*	9	*11.5*
8	*14*	8	*14*	9	*25.5*	7	*6*	8	*3*	12	*38*
8	*14*	9	*25*	9	*25.5*	7	*6*	9	*11.5*	11	*32.5*
7	*4.5*	8	*14*	8	*15.5*	7	*6*	9	*11.5*	8	*3*
9	*25*	7	*4.5*	8	*15.5*	10	*35*	9	*11.5*	10	*24*
9	*25*	8	*14*	9	*25.5*	9	*25.5*	10	*24*	12	*38*
11	*39*	10	*34*	10	*35*	9	*25.5*	9	*11.5*	9	*11.5*
11	*39*	9	*25*	8	*15.5*	8	*15.5*	11	*32.5*	9	*11.5*
9	*25*	7	*4.5*	9	*25.5*	7	*6*	9	*11.5*	12	*38*
9	*25*	7	*4.5*	7	*6*	7	*6*	10	*24*	10	*24*
9	*25*	10	*34*	9	*25.5*	10	*35*	9	*11.5*	10	*24*
10	*34*	8	*14*	9	*25.5*	9	*25.5*	10	*24*	10	*24*
11	*39*	8	*14*	10	*35*	7	*6*	8	*4*	11	*32.5*
8	*14*	10	*34*	9	*25.5*	10	*35*	9	*11.5*	12	*38*
8	*14*	7	*4.5*	11	*39.5*	8	*15.5*	11	*32.5*	10	*24*
10	*34*	8	*14*	10	*35*	7	*6*	10	*24*	11	*32.5*
7	*4.5*	8	*14*	9	*25.5*	7	*6*	7	*1*	11	*32.5*
9	*25*	7	*4.5*	7	*6*	8	*15.5*	9	*11.5*	9	*11.5*
9	*25*	10	*34*	9	*25.5*	10	*35*	9	*11.5*	12	*38*
9[**]	***(24.2)***	**8**	***(16.8)***	**9**	***(24.4)***	**8**	***(16.6)***	**9**	***(15.4)***	**10**	***(25.7)***
$p < 0.05$[***]				$p < 0.05$				$p < 0.01$			

* in italic is the rank (serial number) of the value in the combined and ordered sample of titers determined using homologous and heterologous systems;

** in bold is median sample ("*T*), in brackets is mean rank of random values (H);

*** statement of significance of the difference between average trends of titres in the relative systems (prediction error).

based on "Amursky-12" strain antigen demonstrated a slight increase in antibody titers, i.e. when infected, the heterologous virus had an additional impact on the poultry immune system. The corresponding medians of log titers ($\log_2$) were "$T_{homol.} = 9$ and "$T_{heterol.} = 10$.

Studying replication of LPAI virus in the vaccinated poultry. Within 14 days after challenge, 12 oropharyngeal and cloacal swabs were randomly taken to be tested in real time RT-PCR in order to detect influenza virus genome and assess its concentration. Amplification cycles threshold values (Ct) were estimated. A lower Ct value corresponded to a higher original concentration of the virus genetic material in the sample. The value of Ct ≥ 37 was considered negative, i.e. the virus genome was missing. To facilitate the analysis, the test results obtained within the time interval (j) were expressed as deviations from the negative assessment in the form of values $d_j = 37 - Ct_j$. Thus, the deviation values could range between $0 \le d \le 36$.

Table 2
Real-time RT-PCR-detection of avian influenza virus genome in the samples collected from vaccinated birds after challenge

Estimate of amplification cycles threshold values (Ct_j) in the form of deviations from the negative reaction ($d_j = 37 - Ct_j$), corresponding to the day post challenge (j, day), the type of swabs (oropharyngeal and cloacal) and the type of vaccines used for poultry vaccination. According to the types of samples, the ranks of d values and the results of corresponding comparisons are given								
j	Oropharyngeal swabs				Cloacal sawbs			
	"Chelyabinsk-20" strain (G1) Vaccine		"Amursky-12" strain (Y280) Vaccine		"Chelyabinsk-20" strain (G1) Vaccine		"Amursky-12" strain (Y280) Vaccine	
1	0	*4.5**	0	*4.5*	0	*4.5*	0	*4.5*
2	0.91	*14*	11.97	*24*	0	*4.5*	0	*4.5*
3	0.94	*15*	10.17	*22*	1.01	*14*	4.54	*23*
4	4.26	*21*	10.25	*23*	3.43	*21*	4.65	*24*
5	1.62	*19*	1.23	*18*	1.54	*18*	3.49	*22*
6	0	*4.5*	0.73	*12*	0	*4.5*	0.68	*13*
7	0.43	*11*	1.17	*17*	0.31	*11*	1.42	*16*
8	0	*4.5*	0	*4.5*	0	*4.5*	0.39	*12*
9	0	*4.5*	0.83	*13*	0	*4.5*	1.18	*15*
10	0.16	*9*	3.83	*20*	0.06	*9*	1.53	*17*
11	0	*4.5*	0.95	*16*	0	*4.5*	1.55	*19*
12	0	*4.5*	0.39	*10*	0.15	*10*	2.58	20
		(9.67)*		***(15.33)***		***(9.17)***		***(15.83)***
	*$p < 0.05$***				*$p < 0.025$*			

* in italic is the rank (serial number) of the value in the combined and ordered sample of d values determined for specific type of samples collected from birds immunized with G1 and Y280 vaccines;

** in brackets is mean rank of random values (H);

*** statement of significance of the difference (prediction error) between average trends of sample values.

Based on the data in Table 2, it can be concluded that:

a) after the challenge of vaccinated birds, the viral genome concentration in all types of the studied samples increased, reached a peak and decreased. The presence of the pathogen genome in both oropharyngeal and cloacal swabs suggested the virus presence in the body;

b) the oropharyngeal d-indicators determined during the first 4 days after the challenge formally exceeded similar values in cloacal swabs. This could be evidence of a slightly more active virus replication in the larynx, pharynx or upper respiratory tract of the bird. However, the statistical reliability of such an assumption could not be determined, due to a high variability of the primary values and the small sampling size;

c) effectiveness of the challenge virus reproduction depended on the homology with the vaccine antigen. Homologous immune background significantly reduced the pathogen development. Mean rank equivalents of d values established in oropharyngeal swabs obtained from the birds vaccinated with different vaccines significantly ($p < 0.05$) differed: $H_{homol.} = 9.67 < H_{heterol.} = 15.33$. A similar result ($p < 0.025$) was obtained when comparing the corresponding equivalents of d-values established during the analysis of cloacal swabs: $H_{homol.} = 9.17 < H_{heterol.} = 15.83$. Within 12 days of observation, the cumulative indicators of the challenge virus replication expressed as sums of d values at the homologous immunity in oropharyngeal and cloacal swabs were 8.32 and 6.5, respectively, similar values at heterologous immunity were 41.52 and 22.01.

These data are consistent with the results earlier obtained by the FGBI "ARRIAH" in experiments with vaccines against highly pathogenic avian influenza based on H5N1 virus (clades 2.2 and 2.3.2), when their protective properties against H5N8 virus were studied (clade 2.3.4.4b). The results of serological tests of post-vaccination immunity in HI test using different diagnostic antigens also confirmed antigenic variability of the avian influenza virus subtype H5. The results of acute experiments have shown that the match between the hemagglutinin of the vaccine antigen and the hemagglutinin of the field virus and its concentration in the vaccine is a decisive factor in ensuring protective properties of vaccines [12].

In our experiments, both vaccines significantly exceeded the minimum value of 5 $\log_2$ recommended by the OIE Guidelines for the immunity strength [13].

CONCLUSION

Antigens of low pathogenic avian influenza virus of H9N2 subtype (strains A/chicken/Chelyabinsk/314-1/20 G1 genotype and A/chicken/Amursky/03/12 Y280 genotype), that are used as active components in the inactivated combined emulsion vaccine against avian influenza H9N2 and Newcastle disease, induce strong humoral immunity.

These strains have some antigenic differences that can be detected by hemagglutination inhibition test when monitoring the post-vaccination immune response in birds. Homologous immunity more evidently suppresses virus reproduction during experimental infection.

Taking into account the identified antigenic differences of the studied strains, as well as further evolution of the pathogen, it is advisable to include both antigens in the active component of the inactivated avian influenza (H9) vaccines.

REFERENCES

1. Peacock T. H. P., James J., Sealy J. E., Iqbal M. A. A global perspective on H9N2 avian influenza virus. *Viruses*. 2019; 11 (7):620. DOI: 10.3390/v11070620.

2. Guo Y. J., Krauss S., Senne D. A., Mo I. P., Lo K. S., Xiong X. P. Characterization of the pathogenicity of members of the newly established H9N2 influenza virus lineages in Asia. *Virology*. 2000; 267 (2): 279–288. DOI: 10.1006/viro.1999.0115.

3. Nagy A., Mettenleiter T. C., Abdelwhab E. M. A brief summary of the epidemiology and genetic relatedness of avian influenza H9N2 virus in birds and mammals in the Middle East and North Africa. *Epidemiol. Infect*. 2017; 145 (16): 3320–3333. DOI: 10.1017/S0950268817002576.

4. Dong G., Luo J., Zhang H., Wang C., Duan M., Deliberto T. J. Phylogenetic diversity and genotypical complexity of H9N2 influenza A viruses revealed by genomic sequence analysis. *PLoS One*. 2011; 6 (2):17212. DOI: 10.1371/journal.pone.0017212.

5. Varkentin A. V., Irza V. N., Volkov M. S., Demchenko L. A. Case study of low pathogenic avian influenza H9N2 on commercial egg layers farm. *Poultry and Poultry Products*. 2020; 3: 10–13. DOI: 10.30975/2073-4999-2020-22-3-10-13. (in Russian)

6. Volkov M. S., Varkentin A. V., Irza V. N. Spread of low pathogenic avian influenza A/H9N2 in the world and Russian Federation. Challenges of disease eradication. *Veterinary Science Today*. 2019; 3 (30): 51–56. DOI: 10.29326/2304-196X-2019-3-30-51-56.

7. Zinyakov N. G., Osipova O. S., Akshalova P. B., Sosipatorova V. Yu., Andriyasov A. V., Andreychuk D. B., Chvala I. A. Analysis of genetic characteristics of influenza virus a/chicken/Chelyabinsk/30/2019 H9N2 isolated in Chelyabinsk Oblast. *Veterinary Science Today*. 2019; 4 (31): 49–53. DOI: 10.29326/2304-196X-2019-4-31-49-53.

8. Sharshov K., Kurskaya O., Sobolev I., Leonov S., Kabilov M., Alikina T., et al. First detection of a G1-like H9N2 virus in Russia, 2018. *Korean J. Vet. Res*. 2019; 59 (1): 37–42. DOI: 10.14405/kjvr.2019.59.1.37.

9. Marchenko V. Yu., Goncharova N. I., Evseenko V. A., Susloparov I. M., Gavrilova E. V., Maksyutov R. A., Ryzhikov A. B. Overview of the epidemiological situation on highly pathogenic avian influenza virus in Russia in 2018. *Problems of Particularly Dangerous Infections*. 2019; 1: 42–49. DOI: 10.21055/0370-1069-2019-1-42-49. (in Russian)

10. MI 45-16 Methodical instructions for the detection of avian influenza A virus RNA using real-time RT-PCR: approved by the Rosselkhoznadzor on 06.06.2016. Vladimir: FGBI "ARRIAH"; 2016. 13 p. (in Russian)

11. Hollander M., Wolfe D. A. Nonparametric Statistical Methods. 1st ed. New York: Wiley Sons Limited, Inc.; 1973. 503 p.

12. Irza V. N., Volkov M. S., Varkentin A. V., Frolov S. V., Altunin D. A. Protective properties of the domestic commercial and experimental vaccines against highly pathogenic subtype H5N1 and H5N8 avian influenza viruses isolated in 2015–2016 [Protektivnye svojstva otechestvennyh serijnyh i eksperimental'nyh vakcin v otnoshenii virusov vysokopatogennogo grippa ptic podtipov H5N1 i H5N8, izolirovannyh v 2015–2016 gg.]. *Poultry and Poultry Products*. 2017; 6: 12–15. Available at: http://vniipp.ru/images/statya/20176/st4.pdf. (in Russian)

13. Avian influenza (including infection with high pathogenicity avian influenza viruses). Chapter 3.3.4. *In: OIE. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.* Available at: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.03.04_AI.pdf.

Received on 17.05.2021
Approved for publication on 28.07.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Sergey V. Frolov, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory for Avian Diseases Prevention, FGBI "ARRIAH", Vladimir, Russia.

Lidia O. Scherbakova, Candidate of Science (Biology), Leading Researcher, Reference Laboratory for Avian Viral Diseases, FGBI "ARRIAH", Vladimir, Russia.

Natalya V. Moroz, Candidate of Science (Veterinary Medicine), Head of Laboratory for Avian Diseases Prevention, FGBI "ARRIAH", Vladimir, Russia.

Viktor N. Irza, Doctor of Sciences (Veterinary Medicine), Chief Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Vladimir Yu. Kulakov, Candidate of Science (Veterinary Medicine), Leading Researcher, Laboratory for Avian Diseases Prevention, FGBI "ARRIAH", Vladimir, Russia.

Фролов Сергей Владимирович, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории профилактики болезней птиц ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Щербакова Лидия Олеговна, кандидат биологических наук, ведущий научный сотрудник референтной лаборатории вирусных болезней птиц ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Мороз Наталья Владимировна, кандидат ветеринарных наук, заведующий лабораторией профилактики болезней птиц ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Ирза Виктор Николаевич, доктор ветеринарных наук, главный научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Кулаков Владимир Юрьевич, кандидат ветеринарных наук, ведущий научный сотрудник лаборатории профилактики болезней птиц ФГБУ «ВНИИЗЖ», г. Владимир, Россия.