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Assessment of immunogenic activity of Newcastle disease vaccines

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ABSTRACT

Introduction. Newcastle disease (ND) is reported in many countries worldwide, where it sometimes assumes an epizootic nature. Newcastle disease virus (NDV) genotype VII, which has been actively circulating in recent years, is highly virulent and raises concern due to its ability to undergo rapid mutation. In the context of intensive poultry farming, special attention must be paid to the specific prevention of this disease, including the use of effective vaccines to protect poultry flocks.

Objective. To determine the immunogenic activity of three inactivated Newcastle disease vaccines in chickens following challenge with NDV genotype VII.

Materials and methods. Three vaccines against Newcastle disease were tested: a monovalent vaccine (La Sota antigen), ARRIAH-AviNew Multi, and ARRIAH-AviNewFlu Multi (containing ARRIAH G7 and La Sota antigens). The antigens were diluted with saline solution at ratios of 1:25, 1:50, and 1:100 or used undiluted, and then emulsified with Coralvac RZ 528 adjuvant. Each vaccine sample was administered at a volume of 0.5 cm³ intramuscularly into the pectoral muscle to 10 four-week-old egg-type chickens per group. The control group remained unvaccinated. The antibody titers were determined using the hemagglutination inhibition test at day 28 post-vaccination, and the chickens were challenged with a virulent strain of NDV genotype VII.

Results. All tested vaccines induced strong post-vaccination immunity against NDV by day 28 post inoculation to birds and met the requirements of the World Organisation for Animal Health. However, the ARRIAH-AviNew Multi and ARRIAH-AviNewFlu Multi vaccines demonstrated a higher level of protective efficacy following challenge with an NDV genotype VII isolate compared to the monovalent vaccine based on La Sota antigen.

Conclusion. The polyvalent vaccines ARRIAH-AviNew Multi and ARRIAH-AviNewFlu Multi, prepared using a mixture of NDV antigens of genotypes II and VII, provide a high degree of protection in chickens and can be used for prevention of Newcastle disease caused by genotype VII viruses.

Keywords: Newcastle disease, Newcastle disease virus genotype VII, inactivated vaccines, immunogenic activity, PD₅₀, humoral immunity

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Оценка иммуногенного действия вакцин против ньюкаслской болезни

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РЕЗЮМЕ

Введение. Ньюкаслская болезнь регистрируется во многих странах мира, иногда принимая характер эпизоотии. Активно циркулирующий в последние годы вирус ньюкаслской болезни генотипа VII отличается высокой вирулентностью и вызывает беспокойство в силу способности активно мутировать. В условиях интенсивного птицеводства важно уделять особое внимание вопросам специфической профилактики данной болезни и применять эффективные вакцины для защиты птицепоголовья.

Цель исследования. Определить иммуногенную активность трех инактивированных вакцин против ньюкаслской болезни птиц при контрольном заражении цыплят вирусом ньюкаслской болезни генотипа VII.

Материалы и методы. Исследованы три вакцины против ньюкаслской болезни: моновалентная (антиген «Ла-Сота»), «ВНИИЗЖ-АвиНью Мульти» и «ВНИИЗЖ-АвиНьюФлу Мульти» (антигены «ВНИИЗЖ G7» и «Ла-Сота»). Антигены разбавляли физиологическим раствором в соотношениях 1:25, 1:50, 1:100 или использовали неразведенными, затем эмульгировали с адъювантом Coralvac RZ 528. Каждый образец соответствующей вакцины вводили 10 цыплятам яичного кросса 4-недельного возраста внутримышечно в область груди в объеме 0,5 см³. Контрольную группу не вакцинировали. Через 28 сут определяли титр поствакцинальных антител в реакции торможения гемагглютинации и проводили контрольное заражение вирулентным штаммом вируса ньюкаслской болезни генотипа VII.

Результаты. Все исследуемые препараты вызывали формирование напряженного поствакцинального иммунитета к вирусу ньюкаслской болезни спустя 28 сут после введения птицам, а также соответствовали требованиям Всемирной организации здравоохранения животных. Тем не менее вакцины «ВНИИЗЖ-АвиНью Мульти» и «ВНИИЗЖ-АвиНьюФлу Мульти» показали более высокий уровень протективного действия при контрольном заражении изолятом вируса ньюкаслской болезни генотипа VII в сравнении с моновалентной вакциной из антигена «Ла-Сота».

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Заключение. Поливалентные вакцины «ВНИИЗЖ-АвиНью Мульти» и «ВНИИЗЖ-АвиНьюФлу Мульти», изготовленные на основе смеси антигенов вируса ньюкаслской болезни генотипов II и VII, обеспечивают высокую степень защиты цыплят и могут использоваться для профилактики ньюкаслской болезни, вызванной вирусами генотипа VII.

Ключевые слова: ньюкаслская болезнь, вирус ньюкаслской болезни генотипа VII, инактивированные вакцины, иммуногенная активность, PD₅₀, гуморальный иммунитет

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INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease that affects various bird species depending on their degree of susceptibility [1] and causes enormous economic damage to the poultry industry [2].

The ND cases were first recorded in 1926 in Indonesia and in 1927 in Newcastle-upon-Tyne (England, UK) [3]. Since then, major ND outbreaks have been reported in many countries, including Korea, the Philippines, India, Sri Lanka, and Japan [4].

The clinical signs of the disease often differ depending on the bird species and the specific pathogen strain. Chickens are known to be highly susceptible, whereas ducks and geese are less susceptible to the virus [5]. The pathogen can infect more than 236 bird species belonging to at least 20 orders, and both domestic and wild birds may be infected [6, 7]. In poultry, the characteristic clinical signs of ND include anorexia, depression, polydipsia, weakness, edema and reduced egg production. Respiratory signs may comprise sneezing, dyspnea, nasal discharge and coughing, whereas greenish diarrhea is a prominent intestinal manifestation [8]. Neurological signs include torticollis, paresis, paralysis, ataxia and tremors of the head and limbs. In acute ND, death is sudden, and may occur without premonitory clinical signs [8].

The causative agent of the disease is a member of the genus *Orthoavulavirus*, subfamily *Avulavirinae*, family *Paramyxoviridae* [2]. It is an enveloped virus possessing a non-segmented, negative-sense, single-stranded RNA genome of approximately 15,000 nucleotides [9, 10]. The key factor in the pathogenicity of the Newcastle disease virus (NDV) is the formation of active fusion proteins F1 and F2 upon cleavage of the F0 precursor protein, as well as the presence of certain basic residues at the fusion protein cleavage site (FPCS) [11].

Since its emergence, the NDV has undergone significant evolution, manifested in substantial genetic, virulence, and antigenic diversity, as well as in the expansion of its geographic range [12]. Phylogenetic analysis of the F protein gene sequence allows classification of NDV strains into two classes [13]. Class I comprises predominantly avirulent virus strains whose natural reservoir is wild waterfowl, whereas class II includes virus variants with higher genetic variability and virulence, comprising at least 20 genotypes that infect various species of domestic and wild birds [14]. Furthermore,

class II virus strains are considered responsible for most ND outbreaks known to date [15]. Genotypes II, III, and IV were responsible for the first ND epizootics that occurred from 1920 to 1960, while subgenotype VIb (subgenotype VI.1.1 according to modern classification) led to the third panzootic among pigeons in the 1980s [16]. Genotype VII, in turn, caused the fourth ND panzootic, affecting Asia, Africa, Europe and South America [2], as well as the fifth and most recent panzootic [16]. NDV genotype VII variants are considered the most rapidly evolving within their class [17], and their high virulence and ability to cause disease even in waterfowl justifiably raise concern in the global community [18].

Since the efficacy of any therapeutic agents for the treatment of ND in birds has not been proven, the only way to protect flocks is prevention achieved through biosecurity measures and vaccination [19].

On commercial poultry farms, the main biological risks are improper disposal of dead birds and incomplete disinfection of litter, mixed housing of birds of different ages and multiple species, as well as inadequate control of movements and sanitation on the farm [20]. In this regard, the main focus of biosecurity is to minimize contact of domestic poultry, feed, water and working equipment with any hazards located outside the farm, particularly with wild birds.

Vaccination is fundamental to the strategy for controlling ND and protecting domestic poultry populations in endemic regions [21]. The most commonly used ND vaccines are based on live or inactivated virus strains isolated in the last century, including strains La Sota, B1, Ulster/67, Mukteswar and VG/GA [22]. Although current vaccines, when properly applied, can prevent disease outbreaks and mortality, they do not always prevent virus spread [23]. A potential antigenic mismatch between vaccine and field strains may affect the vaccination efficacy. Under these conditions, field virus isolates may still spread among birds and cause atypical disease [24]. Available evidence suggests that vaccines based on circulating strains demonstrate a higher level of protection [25] and contribute to reduced virus shedding compared to vaccines prepared from strains that differ significantly from field isolates [24]. It has also been reported that the significant genetic difference between currently circulating field isolates of genotype VII and vaccine strains of genotype I or II plays

a critical role in the frequent occurrence of outbreaks of virulent NDV on poultry farms despite implemented vaccination strategies [26].

These findings highlight the necessity to improve the specific prevention strategies for ND, particularly through the updating of vaccine production strains. Accordingly, this study aims to investigate the immunogenic activity of a monovalent vaccine based on the conventionally used NDV genotype II antigen, in comparison with the ARRIAH-AviNew Multi and ARRIAH-AviNewFlu Multi vaccines, which are produced using NDV antigens of genotypes II and VII.

MATERIALS AND METHODS

Objects of study:

1) ND-La Sota inactivated emulsion vaccine against ND; active component is NDV antigen of the La Sota strain contained in extraembryonic fluid (EEF) of infected embryonated chicken eggs;

2) ARRIAH-AviNew Multi inactivated emulsion vaccine against ND; active component is NDV antigen consisting of a mixture of the La Sota and ARRIAH G7 strains in EEF of infected embryonated chicken eggs;

3) ARRIAH-AviNewFlu Multi combined inactivated emulsion vaccine against ND (G7 and La Sota) and avian influenza H9N2 (Y280 and G1); active component is the NDV antigen consisting of a mixture of the La Sota and ARRIAH G7 strains in EEF of infected embryonated chicken eggs.

Production virus strains. The following strains were used in the experiment:

- ARRIAH G7 strain of NDV genotype VII, subgenotype VII.1.1 (hereinafter referred to as ARRIAH G7);
- La Sota strain of NDV genotype II (hereinafter referred to as La Sota);
- A/chicken/Amursky/03/12/H9N2 strain of low pathogenic avian influenza virus subtype H9N2;
- Chelyabinsk-20 strain of low pathogenic avian influenza virus subtype H9N2.

The viruses were inactivated with aminoethylethylene imine (0.25%). The specific activity of the obtained NDV antigens was $7 \log_2$ (1:128) HAU for ARRIAH G7 and $10 \log_2$ (1:1024) HAU for La Sota. The specific infectious activity of the viruses before inactivation was $9.0 \text{ EID}_{50}/\text{cm}^3$ for ARRIAH G7; $10.5 \text{ EID}_{50}/\text{cm}^3$ for La Sota.

Preparation of vaccine dilutions with specified antigen concentrations. Whole (undiluted) La Sota strain antigen was used as the initial NDV antigen for preparation of ND-La Sota vaccine, while a 1:1 mixture of La Sota and ARRIAH G7 strain antigens was used for preparation of the ARRIAH-AviNew Multi and ARRIAH-AviNewFlu Multi vaccines. The antigen concentrations (D) in the vaccine inoculation volume were adjusted by diluting the initial antigen preparations with saline solution at 1:25, 1:50 and 1:100, and undiluted antigens were also used.

After that, the resulting antigen preparations were emulsified at 30:70 in Coralvac RZ 528 oil adjuvant (Turkey).

To prepare ARRIAH-AviNewFlu Multi vaccine dilutions, low pathogenic avian influenza virus subtype H9N2 antigens (A/chicken/Amursky/03/12/H9N2 and Chelyabinsk-20 strains) were also used.

The aforementioned NDV and avian influenza virus antigens were used at 1:1, while the quantity of antigens of different strains was taken in equal proportions.

Mixing with the diluent was performed using a laboratory tissue homogenizer at 6,000 rpm for 10 minutes. The stability of the resulting emulsion was assessed by centrifugation at 1,000 g for 10 minutes. The emulsion was considered stable if the separation of the light (oil) fraction did not exceed 5% by volume and no separation of the heavy (aqueous) fraction occurred.

Birds. The study was conducted using 4-week-old Lohmann Brown hybrid layer chickens obtained from a farm free from acute infectious diseases and seronegative for NDV. The absence of NDV-specific antibodies was confirmed by testing serum samples obtained prior to bird challenge using the hemagglutination inhibition (HI) test.

Immunization of birds. Each vaccine sample with a specified antigen concentration was tested using a separate group of 10 birds. The vaccine (0.5 cm^3) was administered intramuscularly into the pectoral area. Additionally, a virus control group of 10 non-vaccinated (intact) birds was included. Experimental groups were formed in accordance with the "Rules for regulating the circulation of veterinary medicinal products in the customs territory of the Eurasian Economic Union" (approved by Decision No. 1 of the Council of the Eurasian Economic Commission of 21 January 2022)¹. The birds were kept in groups in isolated tabletop boxes with independent ventilation, water and feed supply.

Hemagglutination assay (HA). Samples of antigen-containing materials were tested using HA test according to the procedure described in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Organization for Animal Health (WOAH)². The titer was expressed in hemagglutinating units (HAU).

Hemagglutination inhibition test. The HI test was used to determine anti-NDV antibody titers in sera obtained from birds in 28 days post-vaccination, using diagnostic kits manufactured by the Federal Centre for Animal Health, in accordance with the Methodological guidelines for the identification of avian influenza and Newcastle disease viruses using hemagglutination inhibition test³.

Challenge of birds. Immunized and intact birds were challenged at day 28 post-vaccination using the virulent NDV isolate NDV/chicken/rus/Saratov/2403-3/22. The viral material ($6.0 \text{ lg EID}_{50}/0.5 \text{ cm}^3$) was administered intramuscularly into the thigh.

Over the subsequent 10 days, the condition of challenged and contact birds was assessed according to GOST R 58090-2018 Clinical examination of unproductive animals. General requirements⁴. Daily observations were conducted to monitor the clinical condition of the experimental birds, recording the phases of disease progression, various manifestations and signs of illness, as well as mortality.

At the end of the observation period, the total number of clinically diseased and dead birds recorded during

¹ <https://docs.cntd.ru/document/728138234> (in Russ.)

² Newcastle disease (infection with Newcastle disease virus). In: WOAH. *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 3.3.10.* https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.03.10_NEWCASTLE_DIS.pdf

³ Sosipatorova V. Yu., Chvala Ir. A., Tsivanyuk M. A., Altunin D. A., Chvala Il. A. Methodological guidelines for the identification of avian influenza and Newcastle disease viruses using haemagglutination inhibition test. No. 27-16. Vladimir: ARRIAH; 2016. 15 p. (in Russ.)

⁴ <https://docs.cntd.ru/document/1200158776> (in Russ.)

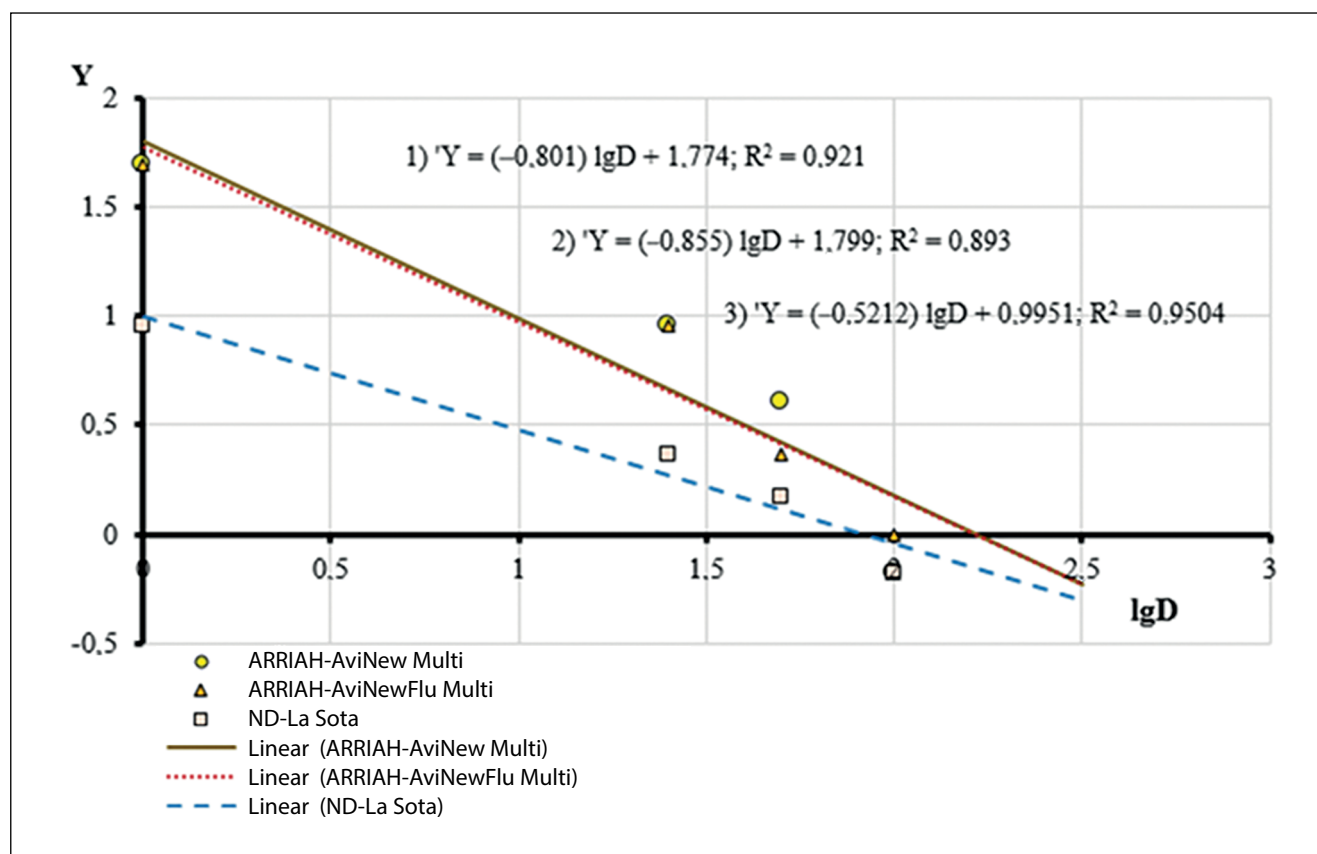


Fig. Relationship between the tested antigen dilutions ($\lg D$) included in the vaccines and the protection index equivalents (Y) of immunized birds after challenge with the NDV/chicken/rus/Saratov/2403-3/22 isolate

Note: The position of experimental Y estimates along the $\lg D$ axis is indicated. The Y -axis is intersected at the point $Y = 0$, which corresponds to the 50% protective effect. Regression equations (models) are provided, where Y is the expected protection index equivalent for a given $\lg D$ value: 1) ARRIAH-AviNew Multi; 2) ARRIAH-AviNewFlu Multi; 3) ND-La Sota. The model adequacy coefficients (R^2) characterizing the degree of correspondence between the models and the experimental data points are provided.

the experimental period (Σc) was determined for each group. Based on these data, protective indices (Pr) were calculated using the following formula:

$$Pr = 1 - (\Sigma c) / n,$$

where n is the number of birds in the group prior to challenge.

All experiments were carried out in strict accordance with the Interstate Standard for accommodation and care of animals. Environment, housing and management (GOST 33215-2014), adopted by the Interstate Council for Standardization, Metrology and Certification, as well as in compliance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22 September 2010 on protecting animals used for scientific purposes. The study was approved by the Bioethics Committee of the Federal Centre for Animal Health (Conclusion of September 26, 2025).

Indicators of post-vaccination immunity strength. The mean logarithmic antibody titers (T) against NDV determined by the HI test served to assess post-vaccination humoral immunity against NDV. At the same time, ARRIAH G7 and La Sota antigens were used for testing. The results of clinical observations in the groups following challenge were expressed as clinical indices and protection indices.

Interpretation of experimental data. Conventional methods for processing samples of variable elements were employed. The mean values, standard deviations

and standard errors of the means were determined. Regression analysis was employed [27]. To approximate a linear relationship between variables, D values (antigen dilution factor) were log-transformed ($\lg D$), while Pr estimates were converted into linear equivalents. Linear equivalents were calculated using the Berkson logit transformation [28]. For empirical values of $P = 1$, conditional estimates were used:

$$P_1 = 1 - (1 / 5n) [29].$$

The 50% protective dose (PD_{50}) was calculated using the formula:

$$\lg PD_{50} = b / (-k),$$

where b is the intercept term of the regression equation (regression line ordinate);

k is the regression coefficient.

Statistical uncertainty of $\lg PD_{50}$ [30] was expressed as the standard error of measurement ($\pm S$).

According to the WOAHP Manual, PD_{50} was taken as the vaccine dilution at which 50% protection of animals is observed. A vaccine was considered to meet the standard if PD_{50} was at least 50 per dose and if the lower confidence limit was at least 35 PD_{50} per dose⁵.

Computational operations and graphical constructions were performed using Microsoft Excel.

⁵ Newcastle disease (infection with Newcastle disease virus). In: WOAHP. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 3.3.10. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.03.10_NEWCASTLE_DIS.pdf

Table 1

Protective efficacy estimates for three inactivated vaccines containing specified doses of NDV ARRIAH G7 and LaSota antigens after challenge with the NDV/chicken/rus/Saratov/2403-3/22 isolate

Vaccine	D* (lgD)	n	Σc^{**}	$Pr = 1 - (\Sigma c) / n$	$Y = \log (Pr / (1 - Pr))$
ARRIAH-AviNew Multi	1 (0)	10	0	0.98 [#]	1.690
	25 (1.4)	10	1	0.90	0.954
	50 (1.7)	10	3	0.70	0.368
	100 (2.0)	10	5	0.50	0.000
ARRIAH-AviNewFlu Multi	1 (0)	10	0	0.98	1.690
	25 (1.4)	10	1	0.90	0.954
	50 (1.7)	10	3	0.70	0.368
	100 (2.0)	10	6	0.40	-0.176
ND-La Sota	1 (0)	10	1	0.90	0.954
	25 (1.4)	10	3	0.70	0.368
	50 (1.7)	10	4	0.60	0.176
	100 (2.0)	10	6	0.40	-0.176
Control	–	10	10	–	–

* the antigen dilution value in the vaccine inoculation volume is indicated (D = 1 corresponds to undiluted antigen);

** the number of clinically diseased and dead birds recorded in the group during the experimental period;

[#] for empirical estimates of $Pr = 1 - (\Sigma c) / n = 1$, a conditional value of $Pr_1 = 1 - (1 / 5n)$ was taken.

RESULTS AND DISCUSSION

The experiment demonstrated that the tested ND vaccines exhibited different immunogenic properties against the virulent NDV genotype VII isolate. Table 1 shows the primary data on the protective activity of the ND vaccines. The antigen dilutions tested on groups of n birds, the protective indices (Pr) and their corresponding linear equivalents (Y) have been presented.

Accordingly, the relationship between the equivalent values (Y) and the tested inoculation dose levels of antigens (D) was examined for each tested product. Regression models were constructed to objectively reflect the relationship between the studied parameters considering the overall statistics of all primary data, and PD₅₀ values characterizing the protective potentials of the tested vaccines were calculated.

The results of the regression analysis are presented graphically in the figure.

As shown, the regression equations demonstrated a high level of adequacy ($R^2 > 0.8$), enabling their use for subsequent calculation of concentrations of the 50% protective doses (PD₅₀), contained in the 0.5 cm³ inoculation volume of the tested preparations under the specified experimental conditions, that are presented in Table 2.

In an experiment to evaluate the protective effect of vaccines against challenge with the highly virulent

NDV isolate NDV/chicken/rus/Saratov/2403-3/22, it was established that the ARRIAH-AviNew Multi preparation possessed the greatest protective potential, containing 164 PD₅₀/0.5 cm³ in the inoculation volume. The next preparation by this parameter was ARRIAH-AviNewFlu Multi, containing 127 PD₅₀/0.5 cm³. The vaccine ND-La Sota had the lowest estimate (81 PD₅₀/0.5 cm³).

Further serological tests were performed. The HI test was used to determine NDV antibody titers (T) in chicken serum samples collected at day 28 post-immunization. The relationship between antibody titers and the specified inoculation doses of NDV antigens was analyzed, and the titers obtained in homologous and heterologous tests were compared. The relevant results are presented in Table 3.

Based on the obtained data, it can be concluded that the intensity of post-vaccination humoral immunity depended on the antigen concentration in the vaccine inoculation volume. Antibody titers in groups of chickens vaccinated with the undiluted antigen contained in the vaccine ranged from 6.4 to 10.0 log₂. It was also noted that antibody titers in the HI test slightly differed depending on the antigens used in the test. The highest antibody titers against the La Sota antigen were detected in groups of chickens vaccinated with ARRIAH-AviNew Multi and ND-La Sota: (9.30 ± 0.11) and (9.30 ± 0.10) log₂, respectively. The highest antibody titers

Table 2
The $\lg PD_{50} \pm S$ values determined following challenge of birds with the NDV/chicken/rus/Saratov/2403-3/22 isolate for each of the tested vaccines

Vaccine	50% protective dose values ($\lg PD_{50} \pm S$) for the tested vaccines	PD_{50} per inoculation dose ($PD_{50}/0.5 \text{ cm}^3$)
ARRIAH-AviNew Multi	$1.7738/0.8006 = 2.215 \pm 0.25$	164 (92)*
ARRIAH-AviNewFlu Multi	$1.7991/0.8550 = 2.104 \pm 0.32$	127 (61)
ND-La Sota	$0.9951/0.5212 = 1.909 \pm 0.13$	81 (60)

* the lower interval of $PD_{50}/0.5 \text{ cm}^3$ for each of the tested vaccines is shown in parentheses.

Table 3
Hemagglutination inhibition test results of chicken sera in 28 days post-immunization with three inactivated vaccines containing specified doses of NDV ARRIAH G7 and La Sota antigens

Vaccine	Vaccine antigen dilution	$\log_2 T$ by antigen used	
	D ($\lg D$)	ARRIAH G7 (homologous)	La Sota (heterologous)
ARRIAH-AviNew Multi	1 (0)	10.0 ± 0.12	9.30 ± 0.11
	25 (1.4)	9.20 ± 0.12	6.80 ± 0.12
	50 (1.7)	7.90 ± 0.12	2.80 ± 0.14
	100 (2.0)	5.30 ± 0.12	2.50 ± 0.10
ARRIAH-AviNewFlu Multi	1 (0)	8.10 ± 0.10	6.40 ± 0.11
	25 (1.4)	5.40 ± 0.10	3.90 ± 0.10
	50 (1.7)	5.40 ± 0.11	3.70 ± 0.12
	100 (2.0)	4.40 ± 0.10	2.60 ± 0.10
ND-La Sota	D ($\lg D$)	ARRIAH G7 (heterologous)	La Sota (homologous)
	1 (0)	7.20 ± 0.10	9.30 ± 0.10
	25 (1.4)	6.20 ± 0.12	8.10 ± 0.09
	50 (1.7)	5.50 ± 0.08	7.00 ± 0.12
	100 (2.0)	5.20 ± 0.09	6.80 ± 0.14

against the ARRIAH G7 antigen were detected following vaccination with ARRIAH-AviNew Multi.

Furthermore, it was established that post-vaccination antibody titers in homologous tests ($\log_2 T_{\text{hom}}$) exceeded those in heterologous tests ($\log_2 T_{\text{het}}$), indicating lower efficiency of immune complex formation in the heterologous system.

Thus, based on the results of challenge and the assessment of humoral immunity, it was determined that all vaccines complied with the WOA requirements, as the obtained 50% protective dose values exceeded $50 PD_{50}/0.5 \text{ cm}^3$, and the lower interval was above $35 PD_{50}/0.5 \text{ cm}^3$. Nevertheless, among the tested preparations, ARRIAH-AviNew Multi proved to be the more immunogenic product for the control of ND in chickens caused by genotype VII viruses. The data obtained in this study confirm results previously reported by other authors [31, 32], indicating the potential ineffectiveness

of vaccination when there is insufficient genetic match between NDV vaccine and field strains. This identified problem, in turn, makes the task of improving ND-specific prevention measures and updating vaccine strains in accordance with the epizootic situation, – both globally and in the specific region of application, – a relevant priority.

CONCLUSION

As a result of the experiment, it was found that all tested vaccines containing undiluted (whole) antigen induced the formation of strong post-vaccination immunity by day 28 after administration to chickens and complied with the WOA requirements for ND products. Nevertheless, the vaccines based on NDV genotypes II and VII, namely ARRIAH-AviNew Multi and ARRIAH-AviNewFlu Multi, demonstrated a higher level of protective activity when using diluted antigen following challenge with the highly virulent NDV genotype VII isolate.

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