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Investigating the infectious process in chickens infected with Newcastle disease virus genotype VII via different routes

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ABSTRACT

Introduction. Newcastle disease is a notifiable disease and is a major threat for commercial poultry. There are many known genotypes of the Newcastle disease virus (NDV), which differ in virulence. In recent years, there is an increasing interest in NDV genotype VII that stems from its prevalence and high pathogenicity in chickens and other species of commercial poultry, causing severe disease with up to 100% mortality.

Objective. Investigation of the infectious process and other clinical and post-mortem signs in chickens infected with Newcastle disease virus via different routes.

Materials and methods. Thirty-day-old chicks were experimentally infected with NDV genotype VII via three different routes: intranasal, oral and intramuscular. Forty eight hours post infection, six intact chickens were introduced in each group. Over the next 10 days, the clinical condition of the infected and contact poultry was assessed. Oropharyngeal and cloacal swabs were collected and tested by polymerase chain reaction. Dead chicks were subjected to post-mortem examination.

Results. The experiment demonstrated that NDV/chicken/rus/Saratov/2403-3/22 isolate causes poultry mortality within 5–7 days. Intramuscular infection led to faster disease progression and death in poultry compared to oral or intranasal routes. The NDV genome was identified in samples of oropharyngeal and cloacal swabs tested by polymerase chain reaction. While nonspecific signs of the disease were recorded in all individuals, the predominant clinical presentation varied with the infection route. Pronounced neurological symptoms were observed in birds infected via the intramuscular and oral routes. In contrast, respiratory signs were characteristic of infections via the oral and intranasal routes. The autopsy results indicate that specific pathological signs characteristic of Newcastle disease developed within 24 hours of the disease onset. A number of post-mortem lesions were found in the internal organs of individuals that died early. However, these lesions were not informative for a diagnosis of Newcastle disease.

Conclusion. The Newcastle disease virus NDV/chicken/rus/Saratov/2403-3/22 strain (genotype VII) was pathogenic to chickens during experimental infection. The disease was easily reproduced by intramuscular, intranasal, and oral routes of infection and was characterized by a peracute course with respiratory and neurological symptoms.

Keywords: Newcastle disease, infectious process, *Orthoavulavirus javaense, Paramyxoviridae,* genotype VII

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Изучение инфекционного процесса у кур при различных способах заражения вирусом ньюкаслской болезни генотипа VII

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РЕЗЮМЕ

Введение. Ньюкаслская болезнь птиц входит в перечень нотифицируемых болезней и является актуальной проблемой современного птицеводства. К настоящему времени известно о существовании различных генотипов возбудителя, отличающихся друг от друга по вирулентности. Все больший интерес в последние годы вызывает вирус ньюкаслской болезни генотипа VII, который инициирует тяжелую форму болезни среди кур и других видов коммерческой птицы вплоть до 100%-й летальности поголовья.

Цель исследования. Изучение инфекционного процесса, а также клинических и патолого-анатомических особенностей ньюкаслской болезни птиц при экспериментальном заражении кур разными способами.

Материалы и методы. Провели экспериментальное заражение вирусом ньюкаслской болезни генотипа VII 30-суточных цыплят тремя разными способами: интраназально, перорально и внутримышечно. Через 48 ч после инфицирования в каждую группу поместили по 6 интактных цыплят. В течение последующих 10 сут оценивали клиническое состояние зараженной и контактной птицы, собирали и исследовали методом полимеразной цепной реакции ротоглоточные и клоакальные смывы и проводили патолого-анатомическое вскрытие павшей птицы.

Результаты. В ходе поставленного эксперимента было установлено, что изолят NDV/chicken/rus/Saratov/2403-3/22 вызывает гибель птицы в течение 5–7 сут. При внутримышечном заражении болезнь и гибель птицы наступали быстрее, чем при пероральном и интраназальном инфицировании. В исследованных методом полимеразной цепной реакции образцах ротоглоточных и клоакальных мазков был выявлен геном вируса ньюкаслской болезни. Неспецифические признаки болезни были зафиксированы у всех особей, однако преобладание определенного симптомокомплекса зависело от способа заражения: у птиц, инфицированных внутримышечно и перорально, отмечались ярко выраженные неврологические симптомы; респираторные признаки были характерны при пероральном и интраназальном заражениях. Результаты вскрытия свидетельствуют о том, что специфические патолого-анатомические признаки, характерные для ньюкаслской болезни, развивались после 24 ч с момента начала болезни. У особей, павших ранее, был обнаружен ряд патологических изменений внутренних органов, которые тем не менее не являлись информативными для диагностики ньюкаслской болезни при вскрытии.

Заключение. Штамм вируса ньюкаслской болезни генотипа VII NDV/chicken/rus/Saratov/2403-3/22 является патогенным для кур при экспериментальном инфицировании. Болезнь легко воспроизводится при внутримышечном, интраназальном и пероральном способах заражения и характеризуется молниеносным течением с развитием респираторных и неврологических симптомов.

Ключевые слова: ньюкаслская болезнь птиц, инфекционный процесс, *Orthoavulavirus javaense, Paramyxoviridae,* генотип VII

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INTRODUCTION

Newcastle disease has threatened global poultry farming for over a century, continuing to cause significant economic damage worldwide. Despite ongoing vaccination efforts, recurrent Newcastle disease outbreaks in recent decades have underscored the virus's continued economic impact on the poultry industry [1]. Newcastle disease is caused by the virulent avian orthoavulavirus (*Orthoavulavirus javaense*, OAVJ). This virus is a member of the *Paramyxoviridae* family [2] and was previously referred to as avian paramyxovirus serotype 1 (APMV-1) [3]. Newcastle disease virus (NDV) genome is a negative-sense, single-stranded RNA virus in the order *Mononegavirales*, with a spiral (helical) capsid symmetry, and its replication occurs in the host cell's cytoplasm [4, 5]. Despite all NDV strains belong to OAVJ species, there is significant genetic and antigenic diversity between different genotypes [6]. NDV isolates are categorized into two classes, I and II, based on the genome length and unique genomic features [7, 8]. Class I strains are genotypically uniform, whereas the more diverse class II strains are currently divided into 21 genotypes based on the phylogenetic analysis of the F gene coding sequence [7, 9, 10, 11].

Many bird species are susceptible to NDV, but the disease's severity and outcome vary significantly by species [12]. Numerous bird species are susceptible to NDV, including domestic poultry like broilers, laying hens, ducks and turkeys, as well as game birds such as pigeons, peacocks, and pheasants, and even some non-poultry birds like ostriches and parrots (*Psittacidae*). First emerging in the 1990s, genotype VII has since become prevalent on multiple continents, including Asia, the Middle East, Europe, and parts of Africa and South America, garnering significant scientific attention in recent years [13]. Due to its high virulence, it causes a severe form of the disease in chickens and other commercial poultry, with mortality rates that can reach 100%. Initial classification divided genotype VII into two subgenotypes: VIIa, which emerged in the Far East in the 1990s and spread to Europe and other parts of Asia, and VIIb, which also originated in the Far East before spreading to South Africa [14]. The classification has since evolved, dividing genotype VII into eight subgenotypes. This includes the recently identified VII-L, which has been associated with Newcastle disease outbreaks in Iran [15] and other countries. Currently, based on new nomenclature criteria for NDV, genotype VII is now subdivided into three subgenotypes: VII.1.1, VII.1.2, and VII.2 [16]. It is known, that subgenotype VII.1.1 caused the third panzootic in pigeons in 1980s, and caused the fourth (starting in 1985) and the last, fifth panzootic [17].

Newcastle disease virus is traditionally divided into four pathotypes based on virulence: velogenic, mesogenic, lentogenic, and asymptomatic intestinal. However, the clinical signs associated with these pathotypes may not always be distinct [18]. Velogenic strains are further divided into two main categories: viscerotropic, which cause widespread hemorrhaging in the internal organs, and neurotropic, which are characterized by neurological and respiratory signs [19]. The disease is primarily transmitted via inhalation or ingestion of the virus shed in feces and respiratory secretions by infected birds for variable lengths of time [20, 21]. The virus can also be transmitted through the conjunctiva. Efficient bird-to-bird virus transmission requires the presence of infectious virus [22]. The infection may occur through inhalation of fine aerosols or large droplets containing the virus; however, the alimentary route of infection

is probably the main one [23]. The disease develops rapidly, with symptoms appearing in a flock within two days of aerosol transmission, but the incubation period can be longer, up to 15 days, with fecal-oral infection, especially in caged birds [24, 25]. Some sources state that the incubation period for Newcastle disease in experimental infection ranges from 2 to 6 days [23]. According to the World Organization for Animal Health (WOAH), the maximum incubation period for Newcastle disease is 21 days.

The disease manifests with signs affecting the respiratory, digestive, and nervous systems, with symptoms varying based on viral virulence, bird age, and immune status [26]. Classic symptoms of Newcastle disease in birds include depression, decreased appetite, ruffled feathers, conjunctivitis, and green or white diarrhea [23]. The green color is a result of the disease affecting the digestive system, likely due to factors like a viral-induced disruption of digestive enzymes and bile production [27]. Respiratory symptoms include coughing and wheezing, while neurological classic symptoms are tremors, wing and leg paralysis, and torticollis (twisted neck). The neurological signs typically appear later in the disease, often after respiratory and digestive signs have started or have become severe [23].

Newcastle disease causes a range of pathological changes in birds, with their severity dependent on the virus's virulence and the host's susceptibility. Significant pathological lesions are typically induced exclusively by velogenic strains of the NDV. Gross post-mortem lesions consist of petechiae on serous membranes and hemorrhages affecting the mucosal surface of the pancreas and the intestinal serosa. These are accompanied by multifocal, necrotic-hemorrhagic lesions, particularly within lymphoid tissues such as the intestinal (caecal) tonsils [25]. The spleen may be enlarged, blotchy, and necrotic [28]. The lung tissue may exhibit hyperemia, along with multifocal hemorrhages and necrotic areas ranging from punctate to ecchymotic. Clinical findings can include cyanosis and petechiation of the comb [29]. Congestion and hemorrhages in the trachea are common signs of velogenic viscerotropic Newcastle disease in chickens. It is also reported that unlike geese the virus replicates in the brain of chickens, causing neurological signs and lesions [30]. Other sources confirm that hyperemia and multi-focal point hemorrhages can occur in the brain's membranes of chickens infected with certain strains of the NDV [25]. Infection with lentogenic NDV strains typically causes airsacculitis, characterized by thickening of the air sac membranes, and can lead to pneumonia from the virus or secondary bacteria [29].

The Newcastle disease situation deteriorated sharply across the Russian Federation in 2019, following the rapid nationwide spread of the subgenotype VII-L (VII 1.1) virus, which spread from Primorsky Krai in the east to the Kursk Oblast in the west. As a result, 17 outbreaks were reported, all of them in backyards, where non-vaccinated poultry were kept [31]. According to the WOAH, a total of 289 outbreaks of Newcastle disease were reported globally in 2023, with thirteen of these occurring in the Russian Federation[1]. In 2024, Newcastle disease was reported in 15 countries across four continents, with a total of 518 recorded outbreaks, 399 of which were concentrated in Nigeria (212) and Iraq (187)[2]. Despite vaccination, sporadic outbreaks of Newcastle disease caused by genotype VII virus occur even in vaccinated poultry in South America [32] and Asia [33]. Moreover, genotype VII is expanding its distribution, leading to diseases in waterfowl [34]. Repeated outbreaks of Newcastle disease in vaccinated poultry flocks can be due to a mismatch between vaccine strains and circulating field strains, which can have different antigens or incorrect vaccination protocols used to control the disease [13].

Currently, the most widely used Newcastle disease vaccines rely on early genotypes (I and II) isolated about 70 years ago. However, the predominant field strains now belong to later genotypes – such as V in the Americas, VII in Asia and Africa, and the globally prevalent genotype VI in pigeons – which are genetically and antigenically distinct from the vaccine strains [35].

Analysis of the global situation indicates a high risk of the virus being introduced into the Russian Federation, underscoring the critical importance of robust preventive measures. Numerous scientific studies have been conducted worldwide by various authors on the infectious process of velogenic NDV in chickens. However, there are only few Russian publications addressing this problem. Therefore, a comparative assessment of the infectious process in chickens, using different routes of infection with a virulent NDV strain, is critically needed.

To advance specific prevention strategies against Newcastle disease, it is crucial to reproduce the genotype VII infectious process experimentally. This allows for a detailed study of viral properties and establishes standardized disease signs in susceptible animals, providing a foundation for vaccine and therapy development.

MATERIALS AND METHODS

Virus. For infection, a virulent NDV/chicken/rus/Saratov/2403-3/22 strain (subgenotype VII.1.1 (VII-L), genotype VII) was used. The infectious dose was 6.0 $lgEID_{50}$ according to the WOAH recommendations[3]. The NDV isolate is classified as velogenic, based on the presence of a polybasic cleavage site in the F protein sequence and an intracerebral pathogenicity index of 1.62 [36].

Poultry. The experiment was conducted in chicks hatched from SPF eggs (VALO BioMedia GmbH, Germany). At the time of inoculation of the virus-containing material, the chicks were 30 days old. The hemagglutination inhibition assay confirmed the absence of NDV-specific antibodies in serum samples collected from birds prior to infection.

Infection routes. To simulate the infectious process, three different routes of infection were applied. The virus was administered in a suspension at different infecting doses according to the route of inoculation: intranasal (0.1 mL instilled into the nose), oral (1.0 mL provided in drinking water), and intramuscular (0.5 mL injected into the thigh muscle).

The control birds were not infected.

Study design. The experimental birds were divided into three equal groups, 8 birds per group. The groups were inoculated applying the above routes. Forty eight hours post infection, 6 intact chickens were introduced in each group. The experimental groups were formed in accordance with

[1] Global Newcastle disease situation (WOAH, 2023). https://fsvps.gov.ru/wp-content/uploads/2023/10/БН-мир-2023.pdf (in Russ.)

[2] Global Newcastle disease situation (WOAH, 2024). https://fsvps.gov.ru/wp-content/uploads/2024/06/БН-мир-2024-2.pdf (in Russ.)

[3] 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

Table 1
Infectious process in Newcastle disease following different routes of exposure

Parameters	Experimentally infected birds			Contact birds	Control
	Inoculation route				
	Intramuscular	Oral	Intranasal		
Incubation period, days	2	2	3	3	–
Death following inoculation, days	5	6	7	6	–
Lethality, %	100	100	100	100	–
Virus genome in cloacal swabs	+	+	+	+	–
Virus genome in oropharyngeal swabs	+	+	+	+	–

the "Rules for Regulating the Veterinary Medicinal Products Circulation in the Customs Territory of the Eurasian Economic Union" (Council Decision No. 1 of the Eurasian Economic Commission dated January 21, 2022)[4]. Over the next 10 days, the clinical condition of infected and contact birds was evaluated in accordance with GOST R 58090-2018 "Clinical examination of unproductive animals. General requirements"[5]. Oropharyngeal and cloacal swabs were collected at the peak of clinical signs in accordance with the "Recommended practice of biological sample collection, storage and transportation for AIV and NDV diagnostic tests"[6]. Dead birds were necropsied in accordance with GOST R 57547-2017 "Services for non-productive animals. Pathological-anatomical study of corpses of non-productive animals. General requirements"[7]. The necropsy procedure consisted of an external examination of the carcass and an internal inspection of the viscera for pathological lesions. The reproductive organs of the experimental birds could not be adequately assessed due to age-related underdevelopment.

Birds were monitored daily for clinical signs. The progression of the disease, including the duration of each phase, the spectrum of clinical manifestations, and the incidence of mortality, was recorded. The dead birds were necropsied and all pathological lesions were recorded. The death specificity was confirmed by polymerase chain reaction (PCR).

All animal experiments were conducted in strict accordance with the interstate standard for laboratory animal keeping and handling GOST 33215-2014, adopted by the Interstate Council for Standardization, Metrology and Certification, and in accordance with 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 studies were approved by the Federal Centre for Animal Health Bioethics Commission (report of 25.07.2025).

Virus identification. The NDV genome in oropharyngeal and cloacal swabs was detected by real-time PCR in accordance with the "Recommended practice of RNA identification and differentiation of NDV virulent isolates by qRT-PCR"[8].

RESULTS AND DISCUSSION

Infectious process. The NDV used in the study was identified as genotype VII, subgenotype VII.1.1, and proved to be contagious for chickens across all experimental groups. The virus demonstrated efficient transmission from infected to contact birds, resulting in 100% lethality in both directly inoculated and contact-exposed poultry. Table 1 summarizes the key parameters of the infectious process for each experimental group.

The shortest incubation period (2 days) was observed with intramuscular and oral inoculation, while the longest (3 days) occurred with the intranasal route. This finding aligns with data from the WOAH and other sources [23, 25].

The time to death was shortest with intramuscular infection, intermediate with oral infection, and longest with intranasal infection (5, 6, and 7 days, respectively). Birds in all experimental groups, both infected and contact, shed the virus in their droppings and oropharyngeal secretions. The course of infection in contact chickens generally mirrored that of the experimentally infected birds.

Experimental inoculation routes that mimic natural infection – with the exception of the parenteral (intramuscular) route – resulted in an acute infectious process characteristic of velogenic NDV strains. Clinically, disease manifestation in contact chickens occurred almost simultaneously with that in directly inoculated birds, indicating high virus transmissibility and pathogenicity.

Clinical signs. The analysis included data from the infectious process as well as the clinical signs observed in both experimentally infected and contact birds. Table 2 demonstrates the variability of Newcastle disease clinical signs in chickens from different experimental groups.

Nonspecific signs, including hyperthermia (Fig. 1), diarrhea, inappetence, and depression (Fig. 2) were observed in all experimental groups. During episodes of diarrhea, the feces were watery and green in color.

Following the incubation period, intramuscularly infected birds developed a more severe and rapid disease course, leading to early mortality. However, the clinical presentation was often ambiguous and limited to non-specific signs. In addition, birds infected via the intramuscular and oral routes exhibited pronounced neurological signs. Respiratory signs – including coughing, sneezing, and

[4] https://www.alta.ru/tamdoc/22sr0001/?ysclid=mghl9evtve540241408 (in Russ.)
[5] https://files.stroyinf.ru/Data2/1/4293738/4293738274.pdf?ysclid=mghlIls82495439970 (in Russ.)
[6] Andreychuk D. B., Andriyasov A. V., Volkova M. A., Chvala Ir. A., Volkov M. S., Chvala Il. A. Recommended practice of biological sample collection, storage and transportation for AIV and NDV diagnostic tests: approved by the Federal Centre for Animal Health on 24.06.2019. Vladimir; 2019. 17 p. (in Russ.)
[7] https://files.stroyinf.ru/Data2/1/4293744/4293744536.pdf?ysclid=mghmowxuy341824070 (in Russ.)
[8] MU 47-16. Recommended practice of RNA identification and differentiation of NDV virulent isolates by qRT-PCR: approved by Rosselkhoznadzor on 06.06.2016. Vladimir; 2016. 11 p. (in Russ.)

wheezing – were observed in the orally and intranasally infected groups. Notably, nasal discharge was specific only to the intranasal infection route. Neither the directly inoculated chickens nor the contact-exposed birds exhibited ocular discharge or conjunctivitis. This finding contrasts with reports from numerous authors, who identify these clinical signs as characteristic of NDV infection [25, 37]. The clinical presentation of Newcastle disease in contact-exposed birds across all three groups mirrored that of the intramuscularly inoculated birds.

Post-mortem examination. Post-mortem examination revealed no gross pathological lesions characteristic of Newcastle disease in the viscera of birds that died within 24 hours of clinical onset. The birds exhibited below-average body condition and ruffled plumage. The beak and eyes were closed, no discharge observed. Minor petechiae were observed on the serosal surface of the sternum. Visceral changes included mild mucosal hyperemia of the intestine, without hemorrhages or necrotic foci, and slight splenomegaly. Cerebral edema with marked vascular hyperemia and scattered petechial hemorrhages were observed.

Birds that succumbed > 24 hours post-onset exhibited distinct pathological changes that varied with the route of exposure and clinical presentation. The birds exhibited below-average body condition and ruffled plumage. The cloaca was occluded, with the surrounding feathers soiled by greenish fecal material. Serous edema was present within the subcutaneous and interstitial tissues of the head, neck, and particularly the thoracic region. Pallor, sometimes progressing to cyanosis, was noted in the comb and wattles. Birds that succumbed following intranasal infection had exudate accumulated on the beak surface and around the nares. The oral mucosa in all experimental groups was cyanotic and exhibited catarrhal inflammation. The mucosa of the pharynx and esophagus was erythematous with multifocal hemorrhages. The oral cavity and pharynx contained abundant mucous exudate (Fig. 3). Hemorrhages were noted on the posterior pharyngeal wall and trachea (Fig. 4) in orally and intranasally infected birds. The blood within the cardiac chambers and major vessels was clotted. In some cases, the myocardium was flaccid. The lungs were hyperemic and edematous. The spleen was cyanotic and the capsule was tense. Diffuse hyperemia was present throughout the intestinal mucosa, accompanied by multifocal hemorrhages and areas of necrosis. The lymph nodes were enlarged. In some cases, the liver was flaccid and mottled, while the kidneys were enlarged, extending beyond the renal fossae. Cerebral edema with marked vascular hyperemia and scattered petechial hemorrhages were observed.

Birds that succumbed 72–96 hours post-onset exhibited distinct gross pathological lesions upon both external and internal examination. The birds were emaciated, with ruffled plumage soiled by liquid, greenish fecal material. Scalp, comb and wattles were cyanotic, without hemorrhage. Petechiae and small ecchymoses were present on the pancreatic serosal surface. The mucosal lining of the gizzard was loose and easily detached. In some birds, the mucosa at the junction of the pancreas and the proventriculus was hyperemic and exhibited band-like hemorrhages (Fig. 5). The spleen was dark in color, with nectrotic foci. The intestine exhibited catarrhal inflammation, with hemorrhages and necrotic foci involving the intestinal tonsils and associated lymphoid tissue. Cerebral edema

Table 2
Influence of exposure route on the clinical manifestation of Newcastle disease in chickens

Clinical signs	Experimentally infected birds			Contact birds
	Inoculation route			
	Intramuscular	Oral	Intranasal	
Depression	+	+	+	+
Loss of appetite	+	+	+	+
Diarrhea	+	+	+	+
Hyperthermia	+	+	+	+
Coughing, sneezing	–	+	+	–
Wheezing	–	+	+	–
Nasal discharge	–	–	+	–
Ocular discharge, conjunctivitis	–	–	–	–
Neurological symptoms (unsteady gait, torticollis, tremors of the head and limbs)	+	+	–	+

Fig. 1. Hyperthermia in infected chicks

Fig. 2. Ruffled feathers and general depression (right chick) and a clinically healthy contact chick (left)

Fig. 3. Accumulation of mucosal exudate in the mouth and pharynx

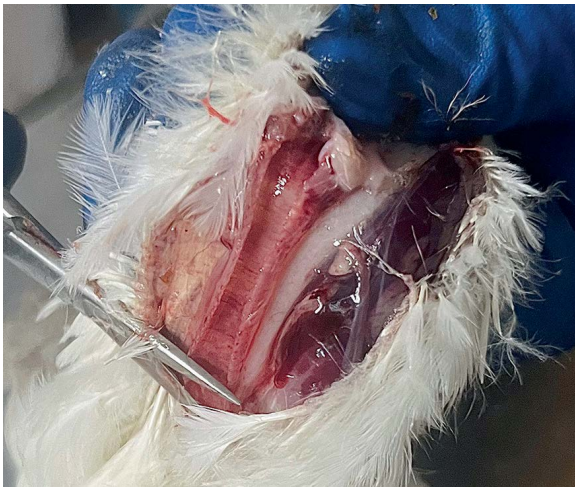

Fig. 4. Submucosal hemorrhages in the posterior oropharynx and trachea

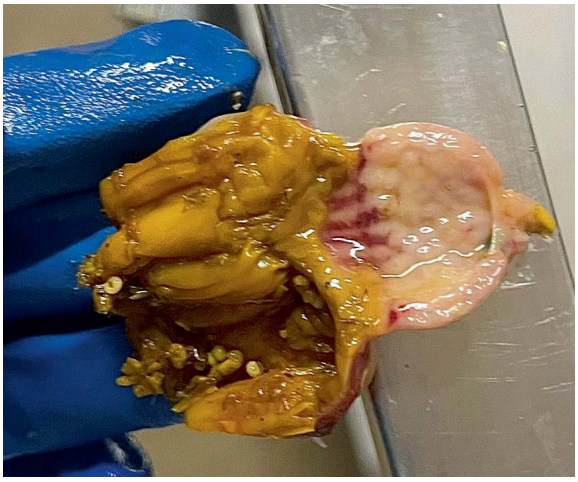

Fig. 5. Hemorrhagic banding at the proventricular-ventricular junction

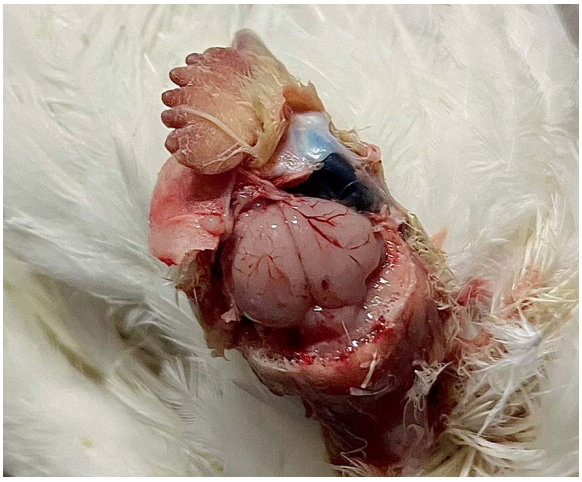

Fig. 6. Cerebral edema with associated hemorrhages

with marked vascular hyperemia and scattered petechial hemorrhages were observed (Fig. 6).

The overall pathological presentation observed in this study aligns with the characteristic lesions of Newcastle disease documented in prior literature [23, 37]. Neuropathological changes were present in all experimental birds, irrespective of clinical presentation, infection route, or time of death. Although some sources report no significant splenic changes in Newcastle disease, splenomegaly and discoloration were frequently observed pathological signs in the present study [38]. The development of specific lesions was contingent on survival beyond the incubation period; in birds surviving more than 24 hours post-incubation, pathological changes appeared and intensified as the clinical disease advanced. In cases of acute disease resulting in early mortality, pathological changes were mild and nonspecific; consequently, gross necropsy was not a reliable method for postmortem diagnosis of Newcastle disease.

CONCLUSION

Investigating the biological properties of NDV genotype VII strains and the resulting infectious process is essential for refining prevention strategies and preventing new outbreaks.

This study experimentally confirmed the contagiousness of the NDV isolate NDV/chicken/rus/Saratov/2403-3/22 (genotype VII, subgenotype VII-L) in chickens via intramuscular, oral, and intranasal infection routes. It also demonstrated the virus's ability to be shed into the environment through the respiratory and digestive tracts. An infecting dose of 6.0 lg EID_{50} was uniformly lethal for 30-day-old SPF chicks, resulting in 100% mortality under the experimental conditions.

The specific clinical presentation of Newcastle disease varied with the infection route. Neurological signs predominated in intramuscularly infected and contact-exposed birds, while respiratory signs were primary following intranasal inoculation. Orally infected birds exhibited a mixed clinical presentation, featuring both neurological and respiratory symptoms. Necropsy revealed that the pathological presentation of Newcastle disease is highly variable. The spectrum of lesions was influenced more by the time from disease onset to death than by the route of infection.

The velogenic isolate NDV/chicken/rus/Saratov/2403-3/22 (genotype VII-L) induced the most severe disease and highest mortality in birds following intramuscular administration. Although intramuscular infection is a non-natural route, it is a standard method for challenge tests and is recommended by the WOAH for evaluating

Newcastle disease vaccine efficacy. Therefore, intramuscular route for inoculation of a virus-containing suspension of NDV/chicken/rus/Saratov/2403-3/22 isolate is optimal for the experimental modeling of Newcastle disease

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