технологиялық схеманың үлгісіне сәйкес, мүйіздер мен тұяқтарды гидролиздеу әдісімен ақуыз өнімін алу үшін ғылыми және тәжірибелік жұмыстар жүргізілді. Нәтижесінде ақуыз өнімінің және жоғары эмульсиялық қабілеті бар сұйық ақуыз концентратының тәжірибелік үлгілері алынды. Алынған жем қоспасы құрамында кемінде 70% ақуыз және 9% ылғалдылықпен 6% май болады. Жаңа мүйізді тұяқты шикізаттың өнімі 65-70% құрады.

### **РЕЗЮМЕ**

В статье представлены результаты исследования по анализу рациональных технологии переработки кератин и коллагенсодаржащих отходов мясопереработки касательно получения востребованных продуктов. Установлено, что одним из эффективных путей переработки кератин и коллагенсодержащих отходов является получение белковых кормовых добавок на основе различных технологических процессов. Выявлены проблемы утилизации и переработки кератин и коллагенсодержащих отходов в существующих убойных цехах расположенных в сельских населенных пунктах, заключающиеся в отсутствии специальных помещений для хранения и переработки указанных отходов. В результате эти отходы вывозится на свалки без соблюдения ветеринарно-санитарных требований и рекомендации, что могут привести размножению вредных вирусов для здоровья людей и животных. Для решения данной проблемы разработана технологическая схема переработки коллаген и кератинсодержащего сырья, образующихся в подобных убойных пунктах с разработкой конкретных ветеринарносанитарных требований и рекомендаций на примере рогов и копыт. В лабораторных условиях по модели разработанной технологической схемы проведены научно-экспериментальные работы по получению белкового продукта по методу гидролиза рогов и копыт. В результате получены опытные образцы белкового продукта и жидкого белкового концентрата обладающего высокой эмульгирующей способностью. Полученная кормовая добавка содержит не менее 70% протеина и 6% жира при 9% влаги. Выход продукта составляло 65-70% от массы свежего рогокопытного сырья.

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# STUDY OF THE BIOLOGICAL PROPERTIES OF THE NEWCASTLE DISEASE VIRUS STRAINS

## Abstract

Newcastle disease is the most contagious and dangerous viral infection of domestic and wild birds.

Kazakhstan has a huge territory crossed by large overpasses, and hundreds of bird species are concentrated in natural landscapes during periods of migration and reproduction

The data obtained in many years of research indicate a poor situation in Kazakhstan due to Newcastle disease among domestic birds of both industrial and household content.

Circulation in populations of synanthropic birds epizotically topical, mesogenic strains the virus of Newcastle disease, which antigenetically differs from previously circulating variants, determines the need for regular monitoring of this pathogen to Kazakhstan

From the scientific data it should be emphasized that the most significant viral infection for the poultry industry of the Republic of Kazakhstan, Newcastle disease continues to be in sight, leading positions in terms of the degree of danger and economic losses. The analysis of the spread of these diseases in the Republic of Kazakhstan is characterized by the annual registration of new foci of diseases.

This article presents the results of a study of the biological properties of two strains of the Newcastle disease virus, isolated from chickens contained in a private compound and one virus isolated from a fallen pigeon in the territory of Almaty.

**Keywords**: Newcastle's disease, type 1 paramyxovirus of birds, genotype.

**Introduction.** Newcastle's disease is the most contagious and dangerous viral infection of domestic and wild birds. The causative agent causes devastating outbreaks in all regions of the world and causes significant damage to the poultry industry [1, 2].

The Newcastle disease virus (NDV) belongs to the Avulavirus genus of the Paramyxoviridae family and is characterized by a minus-strand RNA genome. Its virion RNA is represented by six genes encoding hemagglutinin - neuraminidase (HN), nucleoprotein (NP), phosphoprotein (P), matrix protein (M), RNA-dependent RNA polymerase (L) and fusion protein (F), also there are two non-structural proteins V and W [3].

The disease was first described by F. Kranveld in 1926 on island of Java, and the virus itself was isolated by T. Doyle in 1927 [4, 5].

According to D.J. Alexander, NDV is able to infect birds belonging to 241 species, which creates a significant ecological niche for its preservation in nature, another distinctive feature of PMV-1 is a wide range of virulence, including asymptomatic variants, as well as low pathogenic (lentogenic), moderate pathogenic (mesogenic) and highly pathogenic (velogenic) strains, the infection of which occurs via alimentary and inhalation routes [6]. It has been established that the decisive role in the formation of pathogenic properties is played by the amino acid sequence of the F protein cleavage site, which directly affects the efficiency of virus penetration into target cells [7].

Molecular genetic studies conducted in many countries show heterogeneity of populations of NDV circulating in the world. There are several phylogenetic classifications of PMV-1 with division into lines or genotypes. Studies have shown that viruses of various phylogenetic lines, representing different geographical regions of the world, simultaneously undergo evolutionary changes, which makes it difficult to control and diagnose the disease [8, 9].

**Materials and Methods.** Virus isolation from field material was carried out according to a standard procedure by inoculation of tissue homogenates into the allontoic cavity of embryonated hen's eggs (EHE) (Miller, 2010).

Virus isolation. Isolation of virus was performed in 10-day-old embryos of SPF-chickens (CE). A 10% suspension on a phosphate buffer solution was prepared from a biological material (pH 7.2) and was introduced into the allantoic cavity of CE in a volume of 0.2 ml (Cattoli et al., 2010). Embryos that died after 24 hours of incubation and more were used to collect extra-embryonic fluid (EEG) and conduct further research. HAI test. To identify the NDV isolate, the HAI test was performed using antigens and hyperimmune sera against influenza viruses and Newcastle disease of birds produced by the Federal Center for Animal Health (FGBI ARRIAH, Vladimir). Determination of infectivity titer of Newcastle disease virus. A tenfold serial dilution method was used (from 10<sup>-1</sup> to 10<sup>-1</sup> 9). Each virus dilution was inoculated into the allantoic cavity of four CEs. The titer of the virus in the source material was determined by the method of Kerber and expressed in units of AID<sub>50</sub>/cm<sup>3</sup>. Determination of the pathogenicity index of ND virus during intracerebral infection. Each of the tenday SPF - chickens was injected intracerebrally with 0.05 cm<sup>3</sup> of the virus-containing extraembryonic fluid under study at a dilution of 1:10 on sterile PBS. Within 8 days of the experiment, the clinical condition of each bird was assessed daily, and the coefficient was assigned: 0 - the bird is clinically healthy; 1 - the bird is sick, signs of disease are noted (depression, refusal of food and water, disruption of the activity of the respiratory or digestive tracts, the nervous system); 2 - the bird is dead. Dead birds were assigned a coefficient of 2 daily for 8 days of the experiment [10, 11].

The pathogenicity index (1CP1) was calculated by the formula:

$$ICPI = \frac{\sum_{i=1}^{n} (DA_i * 1 + D_i * 2)}{8*N}, \quad (1)$$

where  $DA_i$ , - number of diseased animal per day i;

 $D_I$  – number of died per day i;

n - total number of birds in the experiment

As a negative control, 5 daily SPF-chickens were used, which were intracerebrally injected with 0.05 cm<sup>3</sup> of sterile PBS.

**Results and Discussion.** Results of clinical observation are presented in table 1.

Table 1 - Identification of hemagglutinating agents isolated from poultry and pigeon in HAI test

	The titer of anti-hemagglutinins to isolates					
Immune serum	from «c	from «chickens»				
	05/17	11/17	19/17			
PMV-1/Lasota/46	640	640	320			
PMV-1/chicken/Almaty/47/98	1280	640	640			
PMV-2/Ukeypa/California/56	<20	<20	<20			
PMV-3/Turkey/Wisconsin/68	<20	<20	<20			
PMV-4/duck/Hong Kong/DZ/75	<20	<20	<20			
PMV-6/duck/Hong Kong/199/77	<20	<20	<20			
PMV-7/Dove/Tennessee/4/75	<20	<20	<20			
PMV-8/Delaware/1053/76	<20	<20	<20			
PMV-9/duck/New York/22/78	<20	<20	<20			

Thus, the identification carried out in the HAI test with a set of diagnostic sera to nine serotypes of the PMV of birds allowed us to classify three isolates from domestic and synatropic birds to NDV. Identification in PCR with primers to the conserved region of the F-gene of PMV-1 will be published in subsequent studies. As a result of cloning on embryonated hen's eggs by the method of limiting dilutions of NDV isolates isolated from chickens (05/17,11/17) and pigeon (19/17), viruses with a hemagglutinating activity of 1: 64-1: 1024 and infectivity of 6.24 lg EID50/<sub>0,2</sub> ere obtained. The following biological properties were studied: thermosensitivity, spectrum of hemagglutinating activity, infectivity. The data on the thermosensitivity of HA and infectivity of PMV-1 isolates isolated in 2017 are presented in table 2.

Table 2 - Thermosensitivity of HA of PMV-1 isolates isolated in 2017

Strain under study Infectivity in lg EID50/ <sub>0,2</sub>	Virus titre							
	Before	efore After heating at 56°C in (in minutes)						
	heating	30	60	90	120			
PMV-1/chicken/Almaty/05/17 6,		6,120	$6,120\pm0,00$	6,120±0,00	6,120±0,20	6,120±0,20		
PMV-1/chicken/Almaty/11/17		6,240	6,240±0,30	6,120±0,00	6,10±0,40	$6,10\pm0,00$		
PMV-1/Dove/Almaty/19/17			3,20±0,80	0	0	0		

In relation to the temperature factor of the three studied isolates of PMV-1, viruses isolated from chickens did not differ significantly among themselves and were characterized as thermostable HA (05/17, 11/17), since they did not lose the ability to cause agglutination of chicken red blood cells after heating at 56°C for 120 min. Isolate 19/17 from pigeon after 30 min. of heating in the HAR did not interact with 0.75% fresh chicken red blood cells and, therefore, was attributed to a strain with thermolabile HN proteins.

The results of determining the spectrum of the hemagglutinating activity of the studied NDV isolates with red blood cells of various animal species are shown in table 3.

Table 3 - The spectrum of hemagglutinating activity of PMV-1 isolates, 2017

Isolate	The titer of the hemagglutination activity of viruses with red blood cells of							
Isolate	chicken	guinea pig	sheep	bovine	mice	horse		
PMV-1/chicken/Almaty/05/17	$6,00\pm0,80$	6,30±0,40	$4,70\pm0,00$	5,80±0,20	3,00±0,10	0		
PMV-1/chicken/Almaty/11/17	5,80±0,40	5,00±0,30	4,60±0,40	5,10±0,30	4,40±0,60	0		
PMV-1/Dove/Almaty/19/17	4,60±0,60	4,20±0,40	4,50±0,20	4,0±0,40	2,20±0,20	0		
Note – shows the geometric mean virus titers in log <sub>2</sub>								

Table 4 shows that the NDV strains, as expected, did not interact with horse red blood cells and significantly varied in the degree of avidity to the red blood cells of the other five species of animals.

	Clinical	Observation period, days					ICPI			
Isolate under study	condition of the bird	1	2	3	4	5	6	7	8	
PMV 1/chicken/Almaty/05/17	healthy	10	10	6	0	0	0	0	0	
	sick	0	0	4	8	0	0	0	0	1,20
	fallen	0	0	0	2	10	10	10	10	
PMV 1/chicken/Almaty/11/17	healthy	10	10	5	0	0	0	0	0	1,21
	sick	0	0	5	6	2	0	0	0	
	fallen	0	0	0	4	8	10	10	10	
PMV-1/Dove/Almaty/19/17	healthy	10	10	7	6	3	2	2	1	
	sick	Λ	Λ	3	1	7	1	2	1	0.91

Table 4 - The results of the observation of chickens after intracerebral infection

fallen

**Conclusion**. An external examination of infected chickens and pigeons showed signs of disease such as depression, refusal of food and water, paresis of limbs and paralysis; at necropsy, hyperimea of the tissues and hemorrhages in the brain, intestines, and swelling of the lungs were observed. The incubation period lasted at least 2 days, and all chickens and pigeons died within 4-5 days. an experiment. As can be seen from the presented data, the pathogenicity index had a value of 1.20 for PMV-1 isolate/chicken/Almaty/05/17; 1.21 for PMV-1 isolate/chicken/Almaty/11/17 and 0.91 for PMV-1 isolate/Dove/Almaty/19/17, which made it possible to identify the isolates as a virulent Newcastle disease virus.

## REFERENCES

- 1. Alexander D.J. Newcastle disease and other avian paramyxoviruses // Rev. Sci. Tech. 2000. № 19(2). P. 443-462.
- 2. Webster R.G., Bean, W.J., Gorman, O.T., Chambers, T.M., Kawaoka, Y. Evolution and ecology of influenza A viruses // Microbiol Rev. -1992. № 56. P. 152-179.
- 3. Kaverin N.V., Lvov, D.K. Paramiksovirusy (*Paramyxoviridae*). M., 2000. P. 183-189. (in Russian)
- 4. Kranveld F.E. About a poultry disease in the Netherlands Indies // Ned. Indies. -1926.  $N_{\odot}$  38. P. 448-450.
- 5. Doyle T.M. A hitherto unrecorded disease of fowls due to a filter passing virus // Journal of Comparative Pathology. 1927.- № 40. P. 162-171.
- 6. King D.J., Seal B.S. Biological and molecular characterization of Newcastle disease virus isolates from surveillance of live bird markets in the United states // Avian Dis. Vol. − 1997. № 43. − P. 683-689.
- 7. Bogoyavlenskiy A.P., Beresin V.E., Prilipov A.G. Newcastle disease outbreaks in Kazakhstan and Kyrgyzstan during 1998, 2000, 2001, 2003, 2004 and 2005 were caused by viruses of the genotypes VII и and Vlld // Virus Genes. 2009. Vol.39. № 1. P. 94-101.
- 8. Miller P.J., Decanini E.L., Afonso C.L. Newcastle disease: Evolution of genotypes and the related diagnostic challenges Infect // Gen. Evol. -2010. N 0 10(1). P. 26-35. DOI: 10.1016/j.meegid.2009.09.012.
- 9. Cattoli G., Fusaro A., Monne I. Emergens of a new genetic kineage of Newcastle disease virus in West and Central Africa implications for diagnosis and control // Vet. Microbiol. 2010. Vol. 142 (3-4). P. 168-176.
- 10.Hu S., Ma H., Wu Y. et al. A vaccine candidate of attenuated genotype VII Newcastle disease virus generated by reverse genetics // Vaccine. 2009. № 27 (6). P. 904-910.
- 11.Newcastle disease // O.I.E. Manual of standarts for diagnostic tests and vaccines. [Electronic resource]. access mode: https://www.oie.int/doc/ged/D7722.PDF.

# ТҮЙІН

Ньюкасл ауруы – жабайы құстар мен үй құстарының контагиозды, қауіпті, жұқпалы вирусты індет болып табылады.

Қазақстанның үлкен территориялық аумағы ұшу жолдарымен қиылысады. Құстардың жүздеген көп түрлері көбею мен көшіп қону кезеңдері аясында табиғи ландшафтарда шоғырланады.

Көп жылдық зерттеулер барысында алынған мәліметтерде Қазақстан аумағы Ньюкасл ауруы бойынша үй құстары мен өнеркәсіптік, аула маңайы құстарына да қатысты қолайсыз жағдай туралы ақпарат береді.

Эпизоотиялық өзекті, мезогенді штаммдардың синантропты құстар популяциясындағы циркуляциясы антигендік айналымдағы нұсқалардан ерекшеленетін Ньюкасл ауруының вирусы Қазақстанда осы қоздырғышқа тұрақты мониторинг жүргізу қажеттілігін айқындап беріп отыр.

Ғылыми мәліметтерге назар аудара отырып, Ньюкасл ауруының вирустық инфекциясы Қазақстан Республикасының өндірістік құс шаруашылығында өзекті мәселе қатарында қалып, зардаптылығы мен өзектілігі жағынан экономикалық шығындарға әкеп соқтыруда. Бұл аурулардың таралуына талдау жасай келе, әр жыл сайын Қазақстан Республикасының аумағында жаңа індет ошағының пайда болуымен және тіркелуімен сипатталады.

Бұл мақалада Алматы қаласының жеке меншік ауласында орналасқан тауықтан және көгершіннен оқшауланған Ньюкасл ауруы вирусының екі штаммының биологиялық қасиеттерінің нәтижелері келтірілген.

### **РЕЗЮМЕ**

Болезнь Ньюкасла является наиболее контагиозным и опасным вирусным инфекцием домашних и диких птиц.

Казахстан имеет огромную территорию, пересекаемую большими пролетными путями, и сотни видов птиц концентрируются в природных ландшафтах в периоды миграции и размножения.

Полученные в ходе многолетних исследований данные свидетельствуют о неблагополучной обстановке в Казахстане по болезни Ньюкасла среди домашних птиц как промышленного, так и приусадебного содержания.

Циркуляция в популяциях синантропных птиц эпизоотически актуальных, мезогенных штаммов вирус болезни ньюкасла антигенно отличающихся от ранее циркулировавших вариантов, определяет необходимость проведения регулярного мониторинга этого возбудителя в Казахстане.

Из научных данных следует подчеркнуть, что наиболее значимые для птицеводства Республики Казахстан вирусная инфекция болезнь Ньюкасла продолжает оставаться на виду, занимая лидирующие позиции по степени опасности и экономических потерь. Анализ распространения этих болезней в Республике Казахстан характеризуется ежегодной регистрацией новых очагов болезней.

В данной статье приведены результаты исследовании биологических свойств двух штаммов вируса болезни Ньюкасла, изолированных от кур содержащегося в частном подворье и одного вируса изолированных от павшего голубя на террритории города Алматы.