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Isolation and characteristics of influenza viruses circulating among swine populations in Kazakhstan during 2018–2019

Swine influenza is regarded as a highly contagious and acute infection. Swine can serve as a tool for the emergence of new influenza viruses that are potentially dangerous to humans. This paper shows the results of the isolation of influenza virus strains circulating among swine in Kazakhstan during 2018–2019 and the characterization of their biological properties. Biological samples were collected from pigs of 2 to 5 months of age in peasant livestock farms in East Kazakhstan, North Kazakhstan, Almaty, and Pavlodar regions. During the execution of the polymerase chain reaction for 662 collected samples, the genetic material of the influenza virus was found in 3.17 % of cases, of which the influenza A/H1N1 virus RNA was detected in 1.08 % and that of the A/H3N2 virus in 0.30 %. With performing virological studies of the obtained samples, three hemagglutinating agents were retrieved and indicated as influenza A viruses of the H1N1 and H3N2 subtypes. The isolated strains of swine influenza A virus revealed similarities with each other in a number of characteristics (temperature sensitivity of hemagglutinin, rates of adsorption and elution, sensitivity to nonspecific serum inhibitors), but differed in infectious activity. The results achieved in the detection of virus genetic material in the polymerase chain reaction and the data of virological studies confirmed the circulation of influenza A/H1N1 and A/H3N2 viruses among the swine population in different regions of Kazakhstan during 2018–2019. These findings highlight the importance of continuous monitoring of swine influenza viruses for detection of possibility of interspecies transmission of this infectious agent.

Keywords: virus, influenza, strain, isolate, antigen, hemagglutinin, swine, Kazakhstan.

Introduction

The influenza virus (IV) belongs to the *Orthomyxoviridae* family and is the major causative agent of infectious diseases of respiratory tract of humans and animals. Today, it is common to classify influenza viruses into four types: A, B, C, D.

Influenza A virus (IAV) can infect epithelial cells of almost all species of birds and mammals including humans [1]. Swine IV mainly affects the epithelium of the respiratory tract, causing cough, fever, somnolence, and anorexia. IAV is one of the most widespread exciters of respiratory tract diseases in pigs throughout the world [2]. The rate of IAVs infection in swine is high (up to 100 %); the disease is accompanied by low lethality: only 1 % of infected animals die. However, reproductive disorders along with weight loss and exacerbation of secondary infections characteristic of swine influenza result in some serious problems in animals and huge economic losses for the swine industry [3].

Currently, three influenza A subtypes are the most commonly circulating in swine: H1N1, H3N2, and H1N2 [4]. The swine population plays a significant role in the IAVs' evolution since they are a unique reservoir for the re-assortment of multi-host infectious agents. The presence of cellular receptors for human, mammalian, and avian IAVs in swine explains the possibility of their transmission from humans and birds to swine and vice versa [5]. There is abundant evidence that all IV subtypes can persist in swine. The simultaneous infection of an animal with viruses of various origins can lead to their re-assortment. The resulting IV re-assorting strains may give rise to a new influenza pandemic. Such an example is presented by two influenza pandemics of the XX century (IV A/H2N2 and A/H3N2), as well as the triple re-assorting H1N1pdm09, which has overcome the interspecies barrier, rapidly spread among the population of all countries of the world [6].

In recent years, A/H1N1pdm09 virus which caused the 2009–2010 influenza pandemic and subsequently almost displaced the previously circulating seasonal A/H1N1 strains continues to circulate in Kazakhstan. At the same time, the circulation of viruses with the antigenic formula A/H3N2 and two type B lines is going on in parallel with IV A/H1N1pdm09 [7].

Human and swine IVs exhibit a number of common features which suggests their relationship and common origin [8]. The issue of interspecies transmission of human and swine IAV (H1N1) is an important factor in the studies of the evolution, ecology, and epidemiology of the pathogen.

In the Republic of Kazakhstan, influenza epizootics among swine are a matter of great concern. In 1984, as a result of virological examination of piglets with clinical signs of respiratory diseases in swine farms located in East Kazakhstan, three IV A/H1N1 strains were isolated. The circulation of influenza A/H1N1 and A/H3N2 viruses was revealed in 2014–2018 among pigs of different ages in peasant farms located in different regions of the country [9, 10].

Determination of the biological and antigenic characteristics of circulating viruses makes it possible to identify the key directions in variation, phylogenetic relationships of the strains that were observed earlier and constantly appear in different countries and regions. The presence of mechanisms for antigenic variation in the influenza virus can lead to a radical change in their biological properties [11–15].

To understand the origin and structure of pandemic IVs, predict and prevent epidemics and pandemics, the surveillance of influenza infection among swine and the identification of new reassortant variants will therefore never lose their relevance.

In this regard, the goal of this investigation is to obtain and describe swine IV among animal populations in Kazakhstan during 2018–2019.

Experimental

Nasopharyngeal swabs from pigs were gathered in flasks with 2 mL of medium 199; 0.5 % bovine serum albumin and a complex of antibiotic drugs (50,000 units/mL of penicillin; 50 μg/mL of streptomycin; 3,000 μg/mL of gentamicin; 5,000 units/mL of nystatin). The specimens were retained for a day at 4 °C and preserved in liquid nitrogen (–196 °C).

RT-PCR was implemented on a Rotor-Gene Q 6Plex amplifier (QIAGEN, Germany) using RIBO-prep, AmpliSens® Influenza virus A/B-FL, AmpliSens® Influenza virus A-type FL, and AmpliSens® Influenza virus A/H1-swine-FL kits (Moscow).

9–11-day-old developing chicken embryos (CE) were used as a model for the isolation of hemagglutinating agents (HAA). To indicate the virus in the hemagglutination assay, a 0.75 % suspension of chicken and human 0 (1) blood group erythrocytes was used.

New viruses were typed in the hemagglutination inhibition (HAI) assay and the neuraminidase inhibition (NAI) assay according to the WHO recommendations with the panels of polyclonal serums [16].

The infectious activity of the strains was established in accordance with the generally accepted method [17], their titer was manifested in lgEID50/0.2 mL.

Antigenic surface glycoproteins were examined in the HAI assay according to the WHO recommendations [18] with rabbit immune serums.

The adsorbing properties of viruses were studied for 18 hours on formalized chicken erythrocytes with constant stirring at 4 °C. The rate of elution from erythrocytes of the Kazakhstan swine IV strains was determined in buffered saline at 37 °C with a time interval of 30–240 min.

The sensitivity of hemagglutinin (HA) to the temperature factor was determined by the ability of viruses to agglutinate erythrocytes after heating at 56 °C for 5, 10, 15, 30, and 60 min [19].

The spectrum of hemagglutinating activity of the strains was established in the hemagglutination assay with 0.75 % suspensions of guinea pig, hen, ram, and human blood group 0 (1) red blood cells [20].

The responsiveness of strains to nonspecific inhibitors was recognized in the HI assay with guinea pig, hen, and rabbit blood serums with native (without heating) and after heating (at 62 °C for 30 min and 100 °C for 10 min).

Statistical processing of the results was implemented by obtaining the geometric mean of the data collected in the thrice-repeated experiments and determining their standard deviations.

Results and Discussion

To carry out virological studies, in 2018–2019 biological samples were collected from 2–5-month-old pigs in peasant livestock farms located in the Almaty, North Kazakhstan, East Kazakhstan, and Pavlodar regions. A total of 662 nasopharyngeal swabs were obtained. The results of the primary investigation of nasopharyngeal samples for present of genetic material of IVs in RT-PCR assays are presented in Table 1.

Table 1

Characterization and RT-PCR screening of biological samples collected from swine in 2018–2019

Place of collecting	Number of collected samples	The number of PCR-positive samples to			
		Influenza virus of type A	Influenza virus subtypes		Influenza type A virus with an unspecified subtype
			A/Hsw1N1	A/H3N2	
Almaty region	97	7	3	2	2
Karaganda region	110	2	0	0	2
East Kazakhstan region	103	1	1	0	0
Pavlodar region	172	7	2	0	2
North-Kazakhstan region	180	4	1	0	3
Total	662	21	7	2	9

As shown in Table 1, while carrying out studies of samples in RT-PCR, the IV genetic material was revealed in 21 one (3.17 % of the total amount of investigated samples). When subtyping IV-positive samples, influenza A/Hsw1N1 virus RNA was detected in seven samples (1.08 %), while influenza A/H3N2 virus RNA in two samples (0.30 %). The subtype could not be identified in nine biological samples (1.36 %).

Thus, the primary screening of nasopharyngeal swabs showed the circulation of influenza A/Hsw1N1 and A/H3N2 viruses among the swine population in Kazakhstan.

In the case of primary infection of CEs with samples collected from different regions of Kazakhstan in 2018–2019, three HAAs were identified (A/swine/Pavlodar/43/19, A/swine/Pavlodar/44/19, and A/swine/Almaty/45/19) with hemagglutination titers which varied in the range of 1:32–1:128.

The antigenic formula of the isolates was determined in the HAI and NAI assays and RT-PCR. Table 2 is presented the results of HA subtype identification in the HAI assay.

Table 2

Identification of hemagglutinin subtypes of swine influenza viruses in HAI assay in Kazakhstan 2019

Diagnostic sera to reference strains	Homologous titers	Isolat		
		swine/Pavlodar/ 43/19	swine/Pavlodar/ 44/19	swine/Almaty/ 45/19
A/Michigan/45/2015 (H1N1) pdm	160	80	40	<20
A/USA/1976/31 (H1N1)	1280	160	80	<20
A/California/04/09 (H1N1) pdm	640	40	40	<20
A/New Jersey/8/76 (H1N1)	640	80	80	<20
A/Singapore/INFIMH-16–0019/2016 (H3N2)	160	<20	<20	40
A/Perth/16/09 (H3N2)	640	<20	<20	80
A/Panama/2007/99 (H3N2)	640	<20	<20	160
B/Colorado/06/2017	160	<20	<20	<20

Table 2 shows that the hemagglutinating activity of the A/swine/Pavlodar/43/19 and A/swine/Pavlodar/44/19 isolates from 1/16 to 1/4 of the homologous titer (1:40–1:160) was inhibited by diagnostic serums against reference viruses with antigenic formula A/H1N1. This made it possible to assignHAA from the Pavlodar region (43/19 and 44/19) to IAV with the H1 HAsubtype. Isolate A/swine/Almaty/45/19 reacted with immune serums against reference variants with antigenic formula A/H3N2 in 1/8–1/4 of the homologous titer (1:40–1:160), which identified it as IV with the H3HA subtype.

Identification of the neuraminidase subtype, carried out in the NAI assay, showed that the enzymatic activity of isolates with the A/H1 HA subtype (A/swine/Pavlodar/43/19 and A/swine/Pavlodar/44/19) was inhibited by polyclonal diagnostic serum against A/H1N1 virus, while that with the A/H3 HA subtype (A/swine/Almaty/45/19) by the serum against A/H3N2 virus in the titers of 1:100.

RT-PCR studies confirmed the belonging of two strains isolated from biological samples collected in Pavlodar (43/19 and 44/19) to IV A/H1N1 and that of the 45/19 strain isolated from samples obtained in Almaty to influenza A/H3N2 virus.

According to the results of identification in the HAI and NAI assays and RT-PCR, the Pavlodar isolates: 43/19 and 44/19 were authenticated as influenza A/H1N1 viruses, the Almaty isolate 45/19 was allocated to influenza A virus with antigenic formula H3N2.

Isolates were cloned by the limiting dilution method on developing CE. As the results showed, the hemagglutinating activity of strains (A/swine/Pavlodar/43/19, A/swine/Pavlodar/44/19, and A/swine/Almaty/45/19) was in the range of 1:512–1:1024.

In Table 3 the results of studies of the major biological properties (infectious activity, HA thermal sensitivity, adsorption-elution) of the regional and reference IV strains are shown.

Table 3

Biological properties of swine influenza virus strain isolated in 2019 in comparison with reference viruses

Isolat	Infectious activity (lg $EID_{50/0.2}$)	Thermostability of hemagglutinin		Adsorption of influenza viruses on CE, %	Elution speed at 37 °C in h.
		Initial	60 min		
A/swine/Pavlodar/43/19 (H1N1)	5.4	8.9±0.2*	9.8±0.4	90	1.0
A/swine/Pavlodar/44/19 (H1N1)	4.8	9.8±0.4	9.6±0.4	100	1.0
A/swine/Almaty/45/19 (H3N2)	3.8	9.7±0.2	9.6±0.2	90	0.5
A/swine/Iowa/15/30 (H1N1)	8.7	9.7±0.6	6.3±0.6	100	1.0
A/swine/USA/1976/31 (H1N1)	8.0	8.7±0.6	6.6±0.6	90	0.5
A/California/04/09 (H1N1) pdm	6.0	8.7±0.6	6.6±0.6	100	1.0
A/Wisconsin/67/05 (H3N2)	6.8	7.7±0.4	6.3±0.4	90	1.0
Note — * the geometric mean logarithms to the base 2 for hemagglutinin inverse titers after heating at 56 °C are presented					

Table 3 shows that the infectious activity of swine IV strains on EC ranged from 3.8 to 5.4 lg $EID_{50/0.2\ mL}$. On this basis, the Kazakhstan isolates were slightly inferior to the reference strains, the infectivity of which was 6.0–8.7 lg $EID_{50/0.2\ mL}$.

While studying the HA sensitivity of the Kazakhstan 2019 swine influenza viruses to heating at 56 °C, it was found that, as the reference strains, they were classified as thermostable variants, since after heating for 60 min they retained the ability to agglutinate chicken erythrocytes in high titers.

All examined viruses had good adsorption and elution capacity in relation to chicken erythrocytes, since the percentage of their adsorption on erythrocytes was 90–100 %, the elution process was completed after 30–60 min of incubation at 37 °C.

While examining the spectrum of hemagglutinating activity with erythrocytes from humans with 0 (1) blood group and various animal species, it was found that the Kazakhstan strains, as the reference variants, actively agglutinated in high titers all types of erythrocytes taken in the experiment (Tab. 4).

Table 4

Hemagglutinating activity of isolates and reference strains in relation to erythrocytes from humans and various animal species

Isolat	Red blood cells			
	hen	guinea pig	ram	Human of group “0”
A/swine/Pavlodar/43/19 (H1N1)	8.2±0.6	10.1±0.0	9.8±0.0	10.8±0.6
A/swine/Pavlodar /44/19(H1N1)	8.6±0.6	9.8±0.6	10.0±0.0	10.2±0.0
A/swine/Almaty/45/19 (H3N2)	8.8±0.6	11.0±0.6	10.2±1.0	10.1±0.0
A/swine/Iowa/15/30(H1N1)	9.7±0.6	12.0±0.0	9.5±1.0	9.6±1.5
A/swine/USA/1976/31(H1N1)	8.7±0.6	11.0±0.0	8.2±1.0	9.3±1.0
A/California/04/09 (H1N1) pdm	8.7±0.6	9.3±0.6	6.6±0.0	8.0±0.0
A/Wisconsin/67/05 (H3N2)	7.7±0.6	8.6±1.0	9.8±0.6	9.5±1.5
Note — the geometric mean logarithms to the base 2 for hemagglutinin inverse titers are presented.				

The sensitivity of isolated strains to serum inhibitors was determined in the HAI assay with the guinea pig, chicken, and rabbit blood serums. The data are illustrated in Table 5.

Table 5

Sensitivity of isolates and reference strains to nonspecific inhibitors of blood serums

Isolat	Animal blood serum								
	Guinea pig			hen			rabbit		
	native	warmed up (62°C)	warmed up (100°C)	native	warmed up (62°C)	warmed up (100°C)	native	warmed up (62°C)	warmed up (100°C)
A/swine/Pavlodar/43/19 (H1N1)	<20	40	80	<20	40	80	<20	20	40
A/swine/Pavlodar /44/19(H1N1)	<20	20	80	<20	40	40	<20	40	80
A/swine/Almaty/45/19 (H3N2)	<20	40	160	<20	80	160	<20	80	160
A/swine/Iowa/15/30	<20	40	80	<20	<20	40	<20	80	160
A/swine/USA/1976/31	<20	80	80	<20	<20	40	<20	40	160
A/California/04/09 (H1N1) pdm	<20	40	80	<20	<20	40	<20	80	80
A/Wisconsin/67/05 (H3N2)	<20	40	160	<20	80	160	<20	80	160
Note — the reciprocals of specific anti-hemagglutinin titers are presented/									

It is indicated in Table 5 that all strains were inhibitor-resistant. Their hemagglutinating activity was not inhibited by native chicken, guinea pig, and rabbit serums. At the same time, the heating of blood serums promoted an increase in their inhibitory activity. Titers increased in the HAI assay with serums heated at 62ºC and even more during boiling (1:20–1:160).

Thereby, influenza virus strains isolated from pigs nasal swabs of Pavlodar and Almaty regions in 2018–2019 showed similarities with each other in terms of HA thermal sensitivity, adsorption and elution rates, sensitivity to nonspecific serum inhibitors, but differenced in infectious activity.

Conclusions

Pigs as a mixing vessel play a major role in the interspecies adaptation and spread of IVs. In order to reduce the likelihood and minimize the role of these animals as a source of the next pandemic causative agent, a decisive role is therefore assigned to the continuous surveillance of influenza in the populations of these animals. Obtaining the comprehensive picture of the infectious process of the influenza infection process will make it possible to predict the epidemiological and epizootic situation; thus, to choose the correct strategy and tactics for conducting preventive measures, identifying the unique biological properties of swine IV is an important component.

References

1 Sandbulte M.R. Optimal use of vaccines for control of influenza A virus in swine / M.R. Sandbulte, A.R. Spickler, P.K. Zaabel, J.A. Roth // Vaccines. — 2015. — Vol. 3, No. 1. — P. 22–73. https://doi.org/10.3390/vaccines3010022

2 Brown I.H. The epidemiology and evolution of influenza viruses in pigs / I.H. Brown // Vet Microbiol. — 2000. — Vol. 74, No. 1–2. — P. 29–46. https://doi.org/10.1016/s0378–1135(00)00164-4

3 Olsen C.W. Swine influenza // In book: *Diseases of Swine,* 9th Edition / C.W. Olsen, I. Brown, B.C. Easterday, K. Van Reeth, K. — Iowa: Iowa State University Press, 2006. — P. 469–482.

4 Kyriakis C.S. Virogical Surveillance and Preliminary Antigenic Characterization of Influenza Viruses in Pigs in Five European Countries from 2006 to 2008 / C.S. Kyriakis, I.H. Brown, E. Foni, G. Kuntz-Simon, J. Maldonado, F. Madec, S.C. Essen, C. Chiapponi, K. Van Reerh // Zoonoses and Public Health. — 2011. — Vol. 58, No. 2. — P. 93–101. https://doi.org/10.1111/j.1863–2378.2009.01301.x

5 Ma W. Swine influenza virus: Current status and challenge / W. Ma // Virus Research. — 2020. — Vol. 288, Iss. 198118. https://doi.org/10.1016/j.virusres.2020.198118

6 Neumann G., Noda T., Kawaoka Y. Emergence and pandemic potential of swineorigin H1N1 influenza virus // Nature. — 2009. — Vol. 459, No. 7249. — P. 931–939. DOI: 10.1038/nature08157

7 Klivleyeva N.G. Detection of influenza virus and pathogens of acute respiratory viral infections in population of Kazakhstan during 2018–2019 epidemic season / N.G. Klivleyeva, N.S. Ongarbayeva, A.M. Baimukhametova, N.T. Saktaganov, G.V. Lukmanova, T.I. Glebova, M.K. Sayatov, V.E. Berezin, G.E. Nusupbaeva, A.M. Aikimbayev // Russian Journal of Infection and Immunity. — 2021. — Vol. 11, No. 1. — P. 137–147. https://doi.org/10.15789/2220-7619-DOI-1348

8 Покровский В.И. Грипп птиц: основы патогенности и вклад в эволюцию пандемических вирусов / В.И. Покровский, О.И. Киселев // Грипп птиц: происхождение инфекционных биокатастроф. — СПб.: Росток, 2005. — С. 15–60.

9 Кливлеева Н.Г. Обнаружение вирусов гриппа А(H1N1) у людей и свиней в регионе Северного Казахстана в 2014–2016 гг. / Н.Г. Кливлеева, Н.Т. Сактаганов, Т.И. Глебова, Г.В. Лукманова, М.Г. Шаменова, М.Х. Саятов, Н.С. Онгарбаева, М.Қ. Қалқожаева, А.М. Баймухаметова, Л.К. Амирашева, М.К. Мустафин, Б.М. Мустафин, Г.А. Баисеев // Изв. НАН РК. Серия биол. и мед. — 2017. — № 5 (323). — С. 106–114.

10 Klivleyeva N.G. Circulation of Influenza Viruses Among Humans and Swine in the Territory of Kazakhstan During 2017–2018 / N.G. Klivleyeva, N.S. Ongarbayeva, N.T. Saktaganov, T.I. Glebova, G.V. Lukmanova, M.G. Shamenova, M.Kh. Sayatov, V.E. Berezin, G.E. Nusupbaeva, A.M. Aikimbayev, R.J. Webby // Bulletin of National Academy of Sciences of the Republic of Kazakhstan. — 2019. — Vol. 2, No. 378. — P. 6–13. https://doi.org/10.32014/2019.2518–1467.35

11 Gregory V. Emergence of influenza A H1N2 reassortant viruses in the human population during 2001 / V. Gregory // Virology. — 2002. — Vol. 300, № 1. — P. 1–7.

12 Xu X. International circulation of human influenza A (H1N2) reassortant viruses during the 2001–2002 influenza season / X. Xu, C.B. Smith, B.A. Mungall, S.E. Lindstrom, H.E. Hall, K. Subbarao, N.J. Cox, A. Klimov // J. Infect. Dis. — 2002. — Vol. 186, No. 10. — P. 490–493.

13 Киселев О.И. Грипп A/H1N1 как типичная эмерджетная инфекция / О.И. Киселев, В.П. Малый, В.В. Зарубабаев, А.Л. Коваленко, Т.В. Сологуб, М.Г., Романцов, О.В. Волобуева, О.В. Голобова, Т.И. Лядова, М.А. Тымкович [Электронный ресурс]. — Режим доступа: https://medi.ru/info/426/

14 Сактаганов Н.Т. Характеристика биологических свойств изолятов вируса гриппа свиней A/H1N1, выделенных в Актюбинской области в 2014 г. / Н.Т. Сактаганов, Н.Г. Кливлеева, Т.И. Глебова, М.Қ. Қалқожаева, Г.В. Лукманова, Н.С. Онгарбаева, С.Б. Байсейіт, А.М. Баймухаметова, М.Г. Шаменова // Ветеринария. — 2019. — № 2. — С. 21–25. https://doi.org/10.30896/0042-4846.2019.22.2.21-25

15 Saktaganov N.T. Study on antigenic relationships and biological properties of swine influenza a/h1n1 virus strains isolated in Northern Kazakhstan in 2018 / N.T. Saktaganov, N.G. Klivleyeva, N.S. Ongarbayeva, T.I. Glebova, G.V. Lukmanova, A.M. Baimukhametova // Сельскохозяйственная биология. — 2020. — Vol. 55, No. 2. — P. 355–363. https://doi.org/10.15389/ agrobiology.2020.2.355eng

16 Douwdal W.A. Influenza virus / W.A. Douwdal, A. Kendal, G.R. Noble // Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infection. — Washington, 1979. — P. 585–609.

17 Ramakrishnan M.A. Determination of 50 % endpoint titer using a simple formula / M.A. Ramakrishnan // World J. Virol. — 2006. — No. 5. — P. 85–86.

18 WHO Global Influenza Surveillance Network. Manual for the laboratory diagnosis and virological surveillance of influenza. — Geneva: WHO, 2011. — 153 p.

19 Cloud S. Characterisation of nine avian paramyxoviruses / S. Cloud, J. Rosenberger // Avian Dis. — 1986. — Vol. 24. — P. 139–152.

20 Ito T. Receptor specificity of influenza A viruses correlates with the agglutination of erythrocytes from different animal species / T. Ito, Y. Suzuki, L. Mitnaul, A. Vines, H. Kida, Y. Kawaoka // J. Virol. — 1997. — Vol. 227. — P. 493–499. https://doi.org/10.1006/viro.1996.8323

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2018–2019 жылдары Қазақстанда шошқа популяциясында таралған тұмау вирусын оқшаулау және сипаттамасы

Шошқа тұмауы өте жұқпалы және өткір инфекция болып саналады. Сонымен қатар, шошқалар адамдар үшін ықтимал қауіпті тұмаудың жаңа вирустарының пайда болуының құралы бола алады. Мақалада 2018-2019 жылдары Қазақстанда шошқаларда таралған тұмау вирусының штамдарын оқшаулау нәтижелері және олардың биологиялық қасиеттерінің сипаттамасы келтірілген. Биологиялық сынамаларды жинау Шығыс Қазақстан, Солтүстік Қазақстан, Алматы және Павлодар облыстарының шошқа өсірумен айналысатын шаруа қожалықтарындағы 2-5 айлық шошқаларға жүргізілді. Жиналған 662 үлгінің полимеразды тізбекті реакциясы кезінде тұмау вирусының генетикалық материалы 3,17 % жағдайда анықталды, оның ішінде A/H1N1 тұмау вирусының РНҚ 1,08 %, ал A/H3N2 вирусының РНҚ 0,30 % анықталды. Биосынамаға вирусологиялық зерттеулер жүргізу кезінде A/H1N1 және A/H3N2 тұмауының вирустары ретінде анықталған үш гемагглютинациялаушы агент табылды. Шошқаның А тұмауы вирусының оқшауланған штамдары бірқатар белгілері бойынша (гемагглютининнің термосезімталдығы, адсорбция және элюция жылдамдығы, қан сарысуының сипатты емес тежегіштеріне сезімталдық) бір-біріне ұқсас болды, бірақ инфекциялық белсенділігімен ерекшеленді. Полимеразды тізбекті реакциядағы вирустық генетикалық материал детекциясының нәтижелері және вирусологиялық зерттеулердің деректері 2018–2019 жылдары Қазақстанның әртүрлі өңірлеріндегі шошқа популяциясында A/H1N1 және A/H3N2 тұмауы вирусының айналымын растайды. Алынған мәліметтер осы инфекциялық агенттің тұраралық берілу мүмкіндігін анықтау үшін шошқа тұмауы вирусын үнемі бақылаудың маңыздылығын көрсетеді.

Кілт сөздер: вирус, тұмау, штамм, изолят, антиген, гемагглютинин, шошқа, Қазақстан.

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Изоляция и характеристика вирусов гриппа, циркулировавших в популяциях свиней в Казахстане в 2018–2019 гг.

Грипп свиней считается высококонтагиозной и остропротекающей инфекцией. Свиньи могут служить инструментом возникновения новых потенциально опасных для человека вирусов гриппа. В статье приведены результаты изоляции штаммов вируса гриппа, циркулировавших у свиней в Казахстане в 2018–2019 гг., и характеристика их биологических свойств. Сбор биологических проб проводили от свиней 2–5-месячного возраста в крестьянских животноводческих хозяйствах Восточно-Казахстанской, Северо-Казахстанской, Алматинской и Павлодарской областей. В ходе полимеразной цепной реакции генетический материал вируса гриппа был обнаружен в 3,17 % случаев, из которых РНК вируса гриппа A/H1N1 была обнаружена в 1,08 %, а вирус A/H3N2 — в 0,30 %. При проведении вирусологических исследований биопроб выделены три гемагглютинирующих агента, идентифицированные как вирусы гриппа A/H1N1 и A/H3N2. Изолированные штаммы вируса гриппа А свиней по ряду признаков (термочувствительность гемагглютинина, скорость адсорбции и элюции, чувствительность к неспецифическим ингибиторам сывороток крови) проявляли сходство между собой, но различались по инфекционной активности. Результаты детекции вирусного генетического материала в полимеразной цепной реакции и данные вирусологических исследований подтверждают циркуляцию в 2018–2019 гг. в популяции свиней в различных регионах Казахстана вирусов гриппа A/H1N1 и A/H3N2. Полученные данные подчеркивают важность постоянного мониторинга вирусов гриппа свиней для выявления возможности межвидовой передачи этого инфекционного агента.

Ключевые слова: вирус, грипп, штамм, изолят, антиген, гемагглютинин, свинья, Казахстан.

References

1 Sandbulte, M.R., Spickler, A.R., Zaabel, P.K., & Roth, J.A. (2015). Optimal Use of Vaccines for Control of Influenza A Virus in Swine. *Vaccines*, *3(1)*; 22–73. https://doi.org/10.3390/vaccines3010022

2 Brown, I.H. (2000). The epidemiology and evolution of influenza viruses in pigs. *Veterinary microbiology*, *74* (*1–2)*; 29–46. https://doi.org/10.1016/s0378-1135(00)00164-4

3 Olsen, C.W., Brown, I., Easterday, B.C., & Van Reeth, K. (2006). Swine influenza. *Diseases of Swine* (9th Ed.), Iowa: Iowa State University Press, 469–482.

4 Kyriakis, C.S., Brown, I.H., Foni, E., Kuntz-Simon, G., Maldonado, J., & Madec, F., et al. (2011). Virological surveillance and preliminary antigenic characterization of influenza viruses in pigs in five European countries from 2006 to 2008. *Zoonoses and public health*, *58* (*2)*; 93–101. https://doi.org/10.1111/j.1863–2378.2009.01301.x

5 Ma, W. (2020). Swine influenza virus: Current status and challenge. *Virus research*, *288*; 198118. https://doi.org/10.1016/j.virusres.2020.198118

6 Neumann, G., Noda, T., & Kawaoka, Y. (2009). Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature, 459;* 931–939. https://doi.org/10.1038/nature08157

7 Klivleyeva, N.G., Ongarbayeva, N.S., Baimukhametova, A.M., Saktaganov, N.T., Lukmanova, G.V., & Glebova, T.I., et al. (2021). Detection of influenza virus and pathogens of acute respiratory viral infections in population of Kazakhstan during 2018–2019 epidemic season. *Russian Journal of Infection and Immunity*, *11(1)*; 137–147. https://doi.org/10.15789/2220-7619-DOI-1348

8 Pokrovskii, V.I., & Kiselev, O.I. (2005). Gripp ptits: osnovy patogennosti i vklad v evoliutsiiu pandemicheskikh virusov [Avian Influenza: Fundamentals of Pathogenicity and Contribution to the Evolution of Pandemic Viruses]. *Gripp ptits: proiskhozhdenie infektsionnykh biokatastrof — Avian influenza: the origin of infectious bio-disasters*. Saint Petersburg: Rostok [in Russian].

9 Klivleeva, N.G., Saktaganov, N.T., Glebova, T.I., Lukmanova, G.V., Shamenova, M.G., & Saiatov, M.Kh., et al. (2017). Obnaruzhenie virusov grippa A(H1N1) u liudei i svinei v regione Severnogo Kazakhstana v 2014–2016 gg. [Detection of influenza A (H1N1) viruses in humans and pigs in the region of northern Kazakhstan in 2014–2016]. *Izvestiia NAN RK. Seriia biologiia i meditsina — Bulletin of the National Academy of Sciences of the Republic of Kazakhstan. Series Biology and Medicine*, *5(323),* 106–114 [in Russian].

10 Klivleyeva, N.G., Ongarbayeva, N.S., Saktaganov, N.T., Glebova, T.I., Lukmanova, G.V., & Shamenova, M.G., et al. (2019). Circulation of influenza viruses among humans and swine in the territory of Kazakhstan during 2017–2018. *Bulletin of national academy of sciences of the Republic of Kazakhstan*, *2(378)*; 6–13. https://doi.org/10.32014/2019.2518–1467.35

11 Gregory, V., Bennett, M., Orkhan, M.H., Al Hajjar, S., Varsano, N., & Mendelson, E., et al. (2002). Emergence of influenza A H1N2 reassortant viruses in the human population during 2001. *Virology*, *300 (1)*; 1–7. https://doi.org/10.1006/viro.2002.1513

12 Xu, X., Smith, C.B., Mungall, B.A., Lindstrom, S. E., Hall, H.E., Subbarao, K., Cox, N.J., & Klimov, A. (2002). Intercontinental circulation of human influenza A (H1N2) reassortant viruses during the 2001–2002 influenza season. *The Journal of infectious diseases*, *186 (10)*; 1490–1493. https://doi.org/10.1086/344738

13 Kiselev, O.I., Malyi, V.P., Zarubabaev, V.V., Kovalenko, A.L., Sologub, T.V., & Romantsov, M.G., et al. Gripp A/H1N1 kak tipichnaia emerdzhetnaia infektsiia [Influenza A/H1N1 as a typical emerging infection]. *medi.ru* Retrieved from https://medi.ru/info/426/ [in Russian].

14 Saktaganov, N.T., Klivleeva, N.G., Glebova, T.I., Qalqozhaeva, M.Q., Lukmanova, G.V., & Ongarbaeva, N.S., et al. (2019). Kharakteristika biologicheskikh svoistv izoliatov virusa grippa svinei A/N1N1, vydelennykh v Aktiubinskoi oblasti v 2014 g. [Characteristics of the biological properties of the swine influenza virus A / H1N1 isolated in the Aktobe region in 2014]. *Veterinariia — Veterinary medicine*, *2,* 21–25. https://doi.org/10.30896/0042-4846.2019.22.2.21-25 [in Russian].

15 Saktaganov, N.T., Klivleyeva, N.G., Ongarbayeva, N.S., Glebova, T.I., Lukmanova, G.V., & Baimukhametova, A.M. (2020). Study on antigenic relationships and biological properties of swine influenza a/H1N1 virus strains isolated in Northern Kazakhstan in 2018. *Agricultural Biology*, *55 (2)*; 355–363. https://doi.org/10.15389/agrobiology.2020.2.355eng

16 Douwdal, W.A., Kendal, A., & Noble, G.R. (1979). *Influenza virus. Diagnostic Procedures for Viral, Rickettsialand Chlamydial Infection*. Washington.

17 Ramakrishnan, M.A. (2016). Determination of 50 % endpoint titer using a simple formula. *World journal of virology*, *5(2)*; 85–86. https://doi.org/10.5501/wjv.v5.i2.85

18 World Health Organization (2011). *WHO Global Influenza Surveillance Network. Manual for the laboratory diagnosis and virological surveillance of influenza*. Geneva: WHO.

19 Cloud, S., & Rosenberger, J. (1986). Characterisation of nine avian paramyxoviruses. *Avian Dis*., *24:* 139–152.

20 Ito, T., Suzuki, Y., Mitnaul, L., Vines, A., Kida, H., & Kawaoka, Y. (1997). Receptor specificity of influenza A viruses correlates with the agglutination of erythrocytes from different animal species. *Virology*, *227(2)*; 493–499. https://doi.org/10.1006/viro.1996.8323