AGRICULTURAL BIOLOGY,
2020, V. 55, № 2, pp. 355-363
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.4:619:578(574.2)

doi: 10.15389/agrobiology.2020.2.355eng
doi: 10.15389/agrobiology.2020.2.355rus

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

Research and Production Center for Microbiology and Virology, A26T6C0 Republic of Kazakhstan, Almaty, st. Bogenbai Batyr, 105, e-mail biochem_vir@mail.ru, nsaktaganov1984@mail.ru (✉ corresponding author), i_nailya@list.ru, nuray.syrlybay@gmail.com, taty1962@mail.ru, gal_l@bk.ru, a_baimukhametova@mail.ru
ORCID:
Saktaganov N.T. orcid.org/0000-0001-6526-956X
Klivleyeva N.G. orcid.org/0000-0002-4094-137X
Ongarbayeva N.S. orcid.org/0000-0001-9022-331X
Glebova T.I. orcid.org/0000-0002-1139-9868
Lukmanova G.V. orcid.org/0000-0002-9809-6674
Baimukhametova A.M. orcid.org/0000-0002-8959-126X
The authors declare no conflict of interests
Received October 21, 2019

Abstract

Swine influenza is a highly contagious acute disease characterized by pronounced fever, general weakness, and disorders of the respiratory system. Swine influenza virus can cause disease in humans and, on the contrary, swine may be infected by human influenza virus. In the pig's organism, simultaneously infected with viruses of different origin, genetic reassortment takes place with the risk of occurrence of new dangerous highly pathogenic strains. The study of influenza viruses circulating in the pig population therefore plays an important role in preventing the development of dangerous outbreaks of the disease and planning preventive measures. In this work we studied the characteristics of the newly isolated strains of swine influenza virus which are epizootically relevant in the specified region at the present time. Our purpose was to identify the biological and antigenic characteristics of strains of swine influenza A/H1N1virus, circulating in the North Kazakhstan oblast of the Republic of Kazakhstan in 2018. Influenza A/H1N1 virus strains were studied including those isolated from pigs in pig farms of the North Kazakhstan oblast, the A/swine/Petropavlovsk/01/18, A/swine/Petropavlovsk/02/18, and A/swine/Petropavlovsk/03/18, and also the reference strains A/swine/Iowa/15/30, A/swine/USA/1976/31, and A/California/04/09 pdm. The strains were cloned in 10-day-old developing chicken embryo systems. The antigenic properties of surface glycoproteins of the strains were examined by cross-reactivity hemagglutination inhibition assay with rabbit immune sera. Infectivity was determined in chicken embryos (CE) and MDCK cell culture. The adsorption properties were studied on formalinized chicken red blood cells under constant stirring at 4°C for 18 hours. Elution from red blood cells was determined after 30, 60, 120, 180, and 240 min in buffered saline at 37°C. The heat sensitivity of hemagglutinin was assessed by the ability to agglutinate red blood cells after heating at 56 °C for 5, 10, 15, 30, and 60 minutes. The hemagglutinating activity of strains was assayed using 0.75% suspensions of chicken, guinea pig, ram, horse erythrocytes, and blood group I(0) erythrocytes of human. The susceptibility of isolates to nonspecific inhibitors was determined in the hemagglutination inhibition assay with native and heated (30 min at 62 °C and 10 min at 100 °C) blood sera of guinea pig, chicken, and rabbit. The susceptibility of virus strains to different concentrations of antiviral drugs was evaluated by the level of reproductive suppression of lg 100 EID_{50}/0.2 ml of virus in CE. The antigenic relationship of the examined variations of influenza A/H1N1 virus between each other and with the reference strains A/swine/USA/1976/31 and A/swine/Iowa/15/30 was revealed as well as their difference from the strain A/California/04/09 pdm. The studied strains in high titers agglutinated all types of red blood cells taken in the experiment. The infectious activity of swine influenza virus strains ranged within 6.5-7.9 lg EID_{50}/0.2 ml in chicken embryos, and 3.5-4.3 lg TCD_{50}/0.2 ml in MDCK cell culture. After heating at 56 °C, all strains agglutinated chicken erythrocytes in high titers ($\log_2 = 6.3\pm0.6$-9.6 ± 0.8) and were characterized as thermostable. The isolated strains possessed good adsorption ability against chicken erythrocytes (90-100 %) and eluted from them after 30-60 min incubation at 37 °C. The strains revealed inhibitory resistance with native nonspecific sera and were suppressed by inhibitors of the heated sers only. The studied strains proved to be susceptible to Tamiflu and Remantadine (the inhibitory concentrations were 5.6-6.6 and 3.7-12.7 μg/ml, respectively). Viruses exhibited resistance to the drugs Arbidol and Ingavirin. Thus, the study revealed similarity of isolated and reference A/H1N1 strains in ther-

mostability of the hemagglutinin, adsorption rate and susceptibility to antiviral drugs, as well as differences in infectious activity and the rate of elution from chicken red blood cells.

Keywords: swine influenza virus, A/H1N1, strain, isolate, antigen, hemagglutinin, infectivity, thermostability, resistance, drug susceptibility

Influenza A viruses are unique infectious agents in humans, as well as in other mammals and birds [1, 2]. Interspecific transmission of influenza A/H1N1 viruses in humans and animals is important for studying the evolution, ecology and epidemiology of the pathogen. Theoretically, influenza A virus transmission is possible between birds of the near-water complex and other marine inhabitants, birds and pigs, seals and humans, pigs and humans [3, 4].

In pigs infected by influenza virus clinical signs often do not appear, and the mortality rate does not exceed 1-4%. In temperate climates, animals can be infected by influenza virus year-round, but in cold weather the probability of infection increases [5)].

Swine flu virus has been found in many countries with developed livestock farming, including Kazakhstan [6-9]. Currently, three subtypes of influenza A virus have been identified in pig populations: H1N1, H3N2, H1N2. Antigenically different bird H1N1 variation has been isolated in pigs since 1979. The most common is A/H1N1, antibodies to which were detected in pigs in all countries of the world [10]. Strains of the influenza virus with the antigenic formula A/H3N2, which became the result of interspecific transmission of the virus from humans to pigs, were first discovered in 1970. The A/H1N2 strains, resulting from reassortment of swine, human, and bird flu viruses, were isolated from pigs in 1994 and now continue to circulate [11].

Pigs can be infected with human and bird flu viruses. Under simultaneous infection with these two viruses, genetic material is exchanged between strains of various origins; as a result, newly emerged virions acquire the ability to be transmitted from person to person with the likelihood of pandemic [12].

Monitoring of the virus spread, understanding etiology of the disease, and a comprehensive description of the infectious agent are important to prevent possible epizootics. Determination of biological and antigenic features of circulating viruses allows us to identify the main parameters of variability, phylogenetic relationships of strains that were previously discovered and constantly appearing in different countries and regions. Information on epidemiologically relevant strains makes it possible to understand the origins of current and future epidemics and to find the most accurate means and methods for preventing influenza, as well as a treatment strategy

In this work, giving characterization of biological properties of influenza A/H1N1 strains isolated in a pig population in Northern Kazakhstan, we first established a higher degree of similarity of these strains with the classic A/H1N1 swine influenza virus (A/Swine/USA/1976/31 and A/Swine/Iowa/15/30) than with related to A/California/4/09 pdm strains which are currently circulating in the human population. In addition, the sensitivity of isolated strains to anti-influenza drugs of the adamantane series (Rimantadine) and neuraminidase inhibitors (Tamiflu®), as well as resistance to Arbidol® and Ingavirin®, were found.

Our goal was to identify the biological and antigenic features of strains of swine influenza virus A/H1N1, circulated in the North Kazakhstan region of the Republic of Kazakhstan in 2018.

Materials and methods. Influenza A/H1N1 virus isolates from samples collected in 2018 in pig farms in the North Kazakhstan region (A/pig/Petropavlovsk/01/18, A/pig/Petropavlovsk/02/18 and A/pig/Petropavlovsk/03/18), as well as reference

strains A/Swine/Iowa/15/30, A/Swine/USA/1976/31 and A/California/04/09 pdm (collection of the Laboratory of Virus Biochemistry, Research and Production Center for Microbiology and Virology) were investigated. Viruses were cultivated in 10-day-old developing chicken embryos (CE) with inoculation of viral material into the chorioallantoic cavity. The collected allantoic fluid was centrifuged at 24,000 rpm for 180 min at + 4 °C. Further purification and concentration of viruses was carried out in a sucrose density gradient (a Beckman centrifuge, Beckman Coulter, USA; Ti 45 rotor, 37,000 rpm, 90 min, + 4 °C) [13].

Antigenic properties of viral glycoproteins were studied by a cross-reactivity in hemagglutination inhibition assay (HIA) as per the recommendations of the World Health Organization [14] with rabbit immune sera [15]. Specific hyperimmune rabbit sera were obtained by 3-fold immunization of chinchilla rabbits weighing 2.5-3 kg. The concentrated virus was injected subcutaneously, 150 μg per animal with a 21-day interval. The specific activity of the obtained hyperimmune rabbit serum was determined in HIA with a set of antigens for the diagnosis of influenza viruses (LLC PDPP, St. Petersburg, Russia) with antigenic formulas A/H1N1, A/H3N2 and type B.

Virus infectivity of the isolated strains was determined on 10-day CE and in an MDCK cell culture (continuous line of Madin-Darby canine kidney epithelial cells, ATCC Catalog of cell lines & hybridomas, 7th edn. Rockville, MD, 1992) according to L. Reed and H. Muench [16] by the assessment of embryonic infectious dose and tissue cytopathogenic dose and expressed in lg EID_{50}/0.2 ml and lg TCD_{50}/0.2 ml, respectively.

The adsorption properties were investigated on 50% formalized chicken erythrocytes for 18 h at 4 °C with constant stirring. Formalized chicken erythrocytes were obtained by treating pre-washed chicken erythrocytes with a 40% formaldehyde solution (1:1). After erythrocyte contact with formalin for 6 days with periodic resuspension, formalin was removed by repeated washing with sterile saline followed by centrifugation at 3000 rpm (Rotanta 460, Hettich, Germany). Elution from red blood cells at 37 °C was evaluated in buffered saline after 30, 60, 120, 180, and 240 min. The heat sensitivity of hemagglutinin (HA) was assessed by the ability of viruses to agglutinate red blood cells after heating at 56 °C for 5, 10, 15, 30, and 60 min [17]. Viral hemagglutinating activity was assayed with 0.75% suspensions of erythrocytes of chicken, guinea pig, ram, horse and human with I(0) blood group in hemagglutination tests (HT) [18]. Red blood cells were washed thrice in a sterile buffered physiological solution by sedimentation for 10 min at 1500 rpm (CM-6M, Elmi, Latvia). A 0.75% suspension was prepared from the precipitate of washed red blood cells.

Sensitivity of the isolates to nonspecific inhibitors was assayed in HIA with guinea pig, chicken and rabbit sera (native and warmed 30 min at 62 °C and 10 min at 100 °C).

The sensitivity of the isolates to different concentrations of antiviral drugs was evaluated by suppression of viral reproduction in CE (100 EID_{50}/0.2 ml). Remantadine (100 mg/capsule, JSC Olainfarm, Latvia), Tamiflu® (75 mg/capsule, Cenexi SAS, France, packed by F. Hoffmann-La Roche AG, Switzerland), Arbidol® (100 mg/capsule, umifenovir hydrochloride monohydrate expressed as umifenovir hydrochloride, Pharmstandard-Leksredstva OJSC, Russia); Ingavirin® (90 mg/capsule, imidazolyl ethanamide pentandioic acid — vitaglutam, Valenta Pharmaceuticals OJSC, Russia). Drugs were dissolved in a phosphate-buffered solution (50 mg/ml), the resultant solutions were used as initial ones. The dose that suppresses 2 times the titer of the virus in HT as compared

to control was deemed an inhibitory concentration (IC_{50}) [19].

The results were statistically processed using Microsoft Office Excel 2010 software. For all series of results, the geometric mean inverse binary logarithms of hemagglutination titers (geometric mean titer, GMT) were found and their standard deviations (±SD) were calculated.

Results. To study the antigenic relationships of the isolated strains, viral preparations obtained in CE after purification were concentrated in a 2.0-2.7 ml phosphate-buffer solution. The protein content in the samples was 0.336-0.683 mg/ml, the hemagglutinating activity was 256000-512000 HAU/ml. When rabbits were immunized with purified virus-containing suspensions (150 μg protein per animal), hyperimmune polyclonal sera were obtained. For further work, the serum was diluted (1:10) and warmed up according to the standard procedure at 56 °C for 30 min to inactivate the heat-labile proteins of the complement system. The titers of specific antibodies of the obtained sera in HIA with homologous strains were 1:320 and 1:5120

Immune rabbit sera to Kazakhstan strains of swine influenza virus in titers 1:160 inhibited the hemagglutinating activity of influenza virus A/H1N1. Serum did not interact with heterologous A/H3N2 and type B viruses.

1. Cross-reaction of inhibition of hemagglutination (HIA**) of the reference strains of swine influenza virus A/H1N1 and isolates form pig farms of Northern Kazakhstan** (2018)

Isolate, strain	Antiserum to viruses					
	1	2	3	4	5	6
A/swine/Petropavlovsk/01/18	320	160	2560	1280	320	40
A/swine/Petropavlovsk/02/18	160	320	2560	1280	160	40
A/swine/Petropavlovsk/03/18	160	160	2560	1280	160	40
A/Swine/USA/1976/31	80	160	1280	640	640	20
A/Swine/Iowa/15/30	320	80	2560	640	640	40
A/California/04/09 pdm	20	40	20	20	40	160

N o t e. Inverse titers of anti-hemagglutinins are presented. 1 — A/pig/Petropavlovsk/01/18, 2 — A/pig/Petropavlovsk/02/18, 3 — A/pig/Petropavlovsk/03/18, 4 — A/pig/Petropavlovsk/, 5 — A/Swine/Iowa/15/30, 6 — A/California/04/09 pdm.

The isolated strains A/swine/Petropavlovsk/01/18, A/swine/Petropavlovsk/02/18, and A/swine/Petropavlovsk/03/18 interacted with immune sera to the reference viruses A/Swine/USA/1976/31 (H1N1) and A/Swine/Iowa/15/30 (H1N1) in high titers (Table 1), while with the antiserum to the drift variation A/California/04/09 (H1N1) pdm they reacted in lower titers. According to the antigenic structure of HA, the studied strains did not differ significantly from each other. We revealed the antigenic relationship of influenza A(H1N1) virus isolates from pigs among the isolates themselves and with reference strains A/H1N1 (A/Swine/USA/1976/31 and A/Swine/Iowa /15/30), and also their difference from strain A/California/04/09 pdm.

The infectious activity of the isolates varied within 6.5-7.9 lg EID_{50}/0.2 ml on CE, and within 3.5-4.3 lg TCD_{50}/0.2 ml on MDCK cell culture. Thence, the isolates were slightly inferior to the reference strains A/Swine/Iowa/15/30 and A/Swine/USA/1976/31 with 8.7 and 8.0 lg EID_{50}/0.2 ml and 5.2 and 5.1 lg TCD_{50}/0.2 ml, respectively (Table 2). Strain A/swine/Petropavlovsk/01/18 in its infectious activity was close to the reference strain A/California/04/09 pdm.

As to heat sensitivity of HA, the tested isolates of the swine influenza virus, similar to reference strains, were assigned to thermostable, since they retained the ability to agglutinate chicken erythrocytes in high titers (log2 from 6.3±0.6 to 9.6±0.8) after heating at 56 °C for 60 min (see Table 2). It was established that all the studied strains can well adsorb chicken erythrocytes (90-

100%); their elution occurred within 30-60 min of incubation at 37 °C.

2. Biological properties of swine influenza virus A/H1N1 isolates from pig farms of Northern Kazakhstan (2018)

Isolate, strain	IA		HT		Ad	El
	1	2	3	4		
A/swine/Petropavlovsk/01/18	6.5	3.5	9.7±0.2	9.6±0.8	90	1.0
A/swine/Petropavlovsk/02/18	7.8	4.3	9.6±0.4	9.6±0.8	100	1.0
A/swine/Petropavlovsk/03/18	7.9	4.3	9.7±0.6	9.3±0.9	100	0.5
A/Swine/USA/1976/31	8.8	5.2	9.7±0.6	6.3±0.6	100	1.0
A/Swine/Iowa/15/30	8.0	5.2	8.7±0.6	6.6±0.6	90	0.5
A/California/04/09 pdm	6.0	3.8	8.7±0.6	6.6±0.6	100	1.0

N o t e. IA — infectious activity: 1 — for chicken embryo, lg EID_{50}/0.2 ml, 2 — for MDCK cells, lg TCD_{50}/0.2 ml; HT — hemagglutinin thermal stability (GMT±SD): 3 — intact viral preparation, 4 — viral preparation heated at 56 °C for 60 min; Ad — adsorption on chicken erythrocytes, %; El — time of elution from chicken erythrocytes at 37 °C, hours.

The isolates, as well as the reference strains A/Swine/Iowa/15/30 and A/Swine/USA/1976/31, activated all types of red blood cells in high titers (Table 3).

3. Hemagglutinating activity of swine influenza virus A/H1N1 reference strains and isolates from pig farms of Northern Kazakhstan (GMT±SD, 2018)

Isolate, strain	Chicken	Guinea pig	Sheep	Horse	Human I(0)
A/swine/Petropavlovsk/01/18	9.5±0.4	10.1±0.2	10.0±0.0	9.6±0.4	10.4±0.2
A/swine/Petropavlovsk/02/18	9.6±0.3	10.5±0.4	10.0±0.0	9.7±0.2	10.4±0.2
A/swine/Petropavlovsk/03/18	9.5±0.4	10.5±0.4	10.0±0.0	9.5±0.4	10.3±0.0
A/Swine/USA/1976/31	9.7±0.6	12.0±0.0	9.5±1.4	8.7±0.3	9.6±1.5
A/Swine/Iowa/15/30	8.7±0.6	11.0±0.0	8.2±1.6	9.7±0.4	9.3±1.2
A/California/04/09 pdm	8.7±0.6	9.3±0.6	6.6±0.6	1.0±0.0	8.0±0.0

N o t e. Geometrical mean binary logarithm for reverse hemagglutinin titers (GMT) are presented.

In all tested isolates, hemagglutinating activity was not suppressed by nonspecific tested native sera (Table 4). However, heating of the sera contributed to an increase in their inhibitory activity. Inhibitor titers increased in sera heated for 30 min at 62 °C, and even more when boiling for 10 min at 100 °C.

4. Sensitivity of swine influenza virus A/H1N1 isolates from pig farms of Northern Kazakhstan (2018) **to different nonspecific inhibitors**

Isolate, strain	Serum								
	guinea pig			chicken			rabbit		
	1	2	3	1	2	3	1	2	3
A/swine/Petropavlovsk/01/18	< 20	20	160	< 20	40	80	< 20	20	20
A/swine/Petropavlovsk/02/18	< 20	40	80	< 20	80	80	< 20	80	160
A/swine/Petropavlovsk/03/18	< 20	40	80	< 20	160	160	< 20	80	160
A/Swine/USA/1976/31	< 20	40	80	< 20	< 20	40	< 20	80	160
A/Swine/Iowa/15/30	< 20	80	80	< 20	< 20	40	< 20	40	160
A/California/04/09 pdm	< 20	40	80	< 20	< 20	40	< 20	80	80

N o t e. 1 — intact serum, 2 — serum heated for 30 min at 62 °C, 3 — serum boiled for 10 min at 100 °C. Inverse titers of nonspecific inhibitors are presented.

A preliminary study on CE did not reveal the embryotoxic effect of the used antiviral drugs in all the doses studied (20, 21).

5. Sensitivity of swine influenza virus A/H1N1 isolates from pig farms of Northern Kazakhstan (2018) **to different to antiviral drugs** (GMT±SD)

Isolate, strain	Inhibiting concentration, μg/ml			
	Tamiflu®	Remantadine	Arbidol®	Ingavirin®
A/swine/Petropavlovsk/01/18	6.6±0.1	6.9±0.0	No inhibition	No inhibition
A/swine/Petropavlovsk/02/18	5.6±2.0	12.7±0.1	No inhibition	No inhibition
A/swine/Petropavlovsk/03/18	5.7±1.7	3.7±0.2	No inhibition	No inhibition
A/Swine/USA/1976/31	6.5±0.1	6.7±0.2	No inhibition	No inhibition
A/Swine/Iowa/15/30	6.6±0.6	7.0±0.1	No inhibition	No inhibition
A/California/04/09 pdm	3.5±0.0	No inhibition	No inhibition	No inhibition

N o t e. The concentration causing a 2-fold decrease in virus reproduction in chicken embryos is indicated.

The studied isolates of A/H1N1 virus and the reference strains were sensitive to Tamiflu® and Remantadine (Table 5). The inhibitory concentration was 5.6-6.6 and 3.7-12.7 μg/ml, respectively. Also, the isolates, as well as the reference strains, turned out to be resistant to Arbidol® and Ingavirin® which did not inhibit viral reproduction even in high concentrations (50 μg/ml).

To summarize, it should be noted that a comparison of the swine influenza viruses isolated in the Republic of Kazakhstan in 2018 and in 2010-2016 [9, 22] revealed similarities in the main biological characteristics that allows us to assign these isolates into a single rather homogeneous group. However, the isolates show slight antigenic heterogeneity that indicates a prolonged circulation of antigenically homogeneous swine flu virus strains among pigs. There is also a greater similarity of the isolates with the reference strains of swine influenza virus (A/Swine/USA/1976/31 and A/Swine/Iowa/15/30) than with A/H1N1 strains which have been circulating in the human population since 2009 and are genetically related to A/California/04/09 pdm. The susceptibility to avian and human influenza A viruses, detected in pigs, allows us to refer them as intermediate hosts which provide a reassortment between the genes of influenza viruses of various origins. This can lead to the emergence of new antigenic variations of the influenza virus with epidemic potential [23]. The degree of pathogenicity and epidemic activity is not the same for different influenza viruses and depends both on their molecular biology and ecological features. Comparison of cross-reactivity of influenza viruses by HIA discloses the nature of their serological relations which may reflect small antigenic differences in hemagglutinin between similar strains and/or indicate differences between groups of strains [24, 25].

Thus, the isolates of swine influenza virus of 2018 from pigs in Northern Kazakhstan are antigenically similar to each other, to the strains that circulated earlier in the pig population in the Republic of Kazakhstan, and to classical standards of swine flu virus, but different from strains circulating in the human population. The isolates of 2018 are similar to each other and to reference strains in thermostability of hemagglutinin and the rate of adsorption on chicken erythrocytes, but they differ in the rate of elution from red blood cells, sensitivity to antiviral drugs, and also in infectious activity. Two strains, the A/swine/Petropavlovsk/02/18 and A/swine/Petropavlovsk/03/18, are similar to the reference strains A/Swine/Iowa/15/30 and A/Swine/USA/1976/31 in infectivity, while strain A/swine/Petropavlovsk/01/18 shows similarities with the reference strain A/California 04/09 pdm. The tested isolates are sensitive to antiviral drugs Tamiflu® and Remantadine and resistant to Arbidol® and Ingavirin®. Data on the antigenic and biological properties of animal influenza viruses reveal the patterns of their circulation and the development of infection, which is necessary to predict an epidemic situation and an appropriate strategy and tactics for preventive and anti-epidemic measures. Identification of emerging variations of the influenza viruses in populations of susceptible animal species, especially pigs, is extremely important, therefore, we plan to continue monitoring studies.

REFERENCES

1. Lvov D.K. Influenza A viruses — a sum of populations with a common protected gene pool. In: *Soviet medical reviews. Section E. Virology reviews.* V.M. Zhdanov (ed.). Bell and Bain Ltd., Glasgow, 1987, V. 2: 15-37.
2. Webster R.G., Bean W.J., Gorman O.T., Chambers T.M., Kawaoka Y.C. Evolution and ecology of influenza A viruses. *Microbiological Reviews*, 1992, 56(1): 152-179 (doi: 10.1128/MMBR.56.1.152-179.1992).
3. Hinshaw V.S., Webster R.G., Bean W.J., Downie J., Senne D.A. Swine influenza-like viruses in turkeys: potentional source of virus for humans? *Science*, 1983, 220(4593): 206-208 (doi:

10.1126/science.6298942).
4. *Swine Influenza (Influenza in Swine).* Available: https://www.cdc.gov/flu/swineflu/influenza-in-swine.htm. Accessed: 20.08.2019.
5. VOZ. *Vspyshka grippa sredi lyudei, vyzvannaya grippom A/H1N1, — soobrazheniya po povodu perekhoda virusa ot zhivotnogo k cheloveku. 30 aprelya 2009 goda* [WHO. A/H1N1 influenza outbreak in humans — Considerations for the transition of the virus from animal to human. April 30, 2009]. Available: https://www.google.ru/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=2ahUKEwi6sdz9s6niAhUNuIsKHep6AngQFjAAegQIARAC&url=http%3A%2F%2Forigin.who.int%2Fentity%2Ffoodsafety%2Ffs_management%2FNo_02_influenza_Apr09_ru_rev1.pdf&usg=AOvVaw1l43P4XoJ-JgAeEUsOOJDI. Accessed: 20.05.2019 (in Russ.).
6. Simon G., Larsen L.E., Dürrwald R., Foni E., Harder T., Van Reeth K., Markowska-Daniel I., Reid S.M., Dan A., Maldonado J., Huovilainen A., Billinis C., Davidson I., Agьero M., Vila T., Hervé S., Breum S.Ø., Chiapponi C., Urbaniak K., Kyriakis C.S., ESNIP3 consortium, Brown I.H., Loeffen W. European surveillance network for influenza in pigs: surveillance programs, diagnostic tools and Swine influenza virus subtypes identified in 14 European countries from 2010 to 2013. *PloS ONE*, 2014, 9(12): e115815 (doi: 10.1371/journal.pone.0115815).
7. Lukmanova G.V., Klivleeva N.G., Shamenova M.G., Saktaganov N.T., Glebova T.I., Mustafin M.K., Baiseev G.A., Mustafin B.M. *Veterinariya*, 2016, 1(45): 61-63 (in Russ.).
8. Klivleyeva N.G., Saktaganov N.T., Glebova T.I., Lukmanova M.G., Shamenova M.G., Sayatov M.H. Detection of influenza A(H1N1) viruses in humans and pigs in the Northern Kazakhstan during 2014-2016. *Int. Conf. Symp. «Influenza 2018: Centenary of the 1918 Pandemic»*. London, 2018: 38.
9. Saktaganov N.T., Klivleeva N.G., Glebova T.I., Kalkozhaeva M.K., Lukmanova G.V., Ongarbaeva N.S., Baiseiit S.B., Baimukhametova A.M., Shamenova M.G. *Veterinariya*, 2019, 2: 21-25 (doi: 10.30896/0042-4846.2019.22.2.21-25) (in Russ.).
10. Brown I.N., Done S.N., Spencer Y.I., Cooley W.A., Harris P.A., Alexander D.J. Pathogenicity of a swine influenza H1N1 virus antigenically distinguishable from classical and European strains. *Veterinary Record*, 1993, 132(24): 598-602 (doi: 10.1136/vr.132.24.598).
11. *Factsheet on swine influenza in pigs.* Available: https://www.ecdc.europa.eu/en/swine-influenza/facts/swine-influenza-pigs. Accessed: 20.05.2019.
12. Ma J., Shen H., McDowell C., Liu Q., Duff M., Lee J., Lang Y., Hesse D., Richt J.A., Ma W. Virus survival and fitness when multiple genotypes and subtypes of influenza A viruses exist and circulate in swine. *Virology*, 2019, 532: 30-38 (doi: 10.1016/j.virol.2019.03.016).
13. Sominina A.A., Burtseva E.I., Lobova T.G., Konovalova N.I., Gudkova T.M., Litvinova O.M., Slepushkin A.N., Ivanova V.T. *Vydelenie virusov grippa v kletochnykh kul'turakh i kurinykh embrionakh i ikh identifikatsii. Metodicheskie rekomendatsii* (utverzhdeny FS po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiya cheloveka). 18 aprelya 2006 g. № 0100/4430-06-34 [Isolation of influenza viruses in cell cultures and chicken embryos and their identification. Methodical recommendations (approved by the Federal Assembly for Supervision of Consumer Rights Protection and Human Well-being). April 18, 2006 No. 0100/4430-06-34]. Available: http://docs.cntd.ru/document/420214503. Accessed: 20.05.2019 (in Russ.).
14. WHO Global Influenza Surveillance Network. *Manual for the laboratory diagnosis and virological surveillance of influenza.* WHO Press, Geneva, 2011. Available: http://apps.who.int/bookorders/MDIbookPDF/Book/11500806.pdf?ua=1. Accessed: 28.04.2018.
15. Archetti I., Horsfall F.L. Jr. Persistent antigenic variation of influenza A viruses after incomplete neutralization in ovo with heterologous immune serum. *Journal of Experimental Medicine*, 1950, 92(5): 441-462 (doi: 10.1084/jem.92.5.441).
16. Reed L., Muench H. A simple method of estimation fifty percent and pints. *American Journal of Tropical Medicine and Hygiene*, 1938, 27(20): 493-497.
17. Cloud S.S., Rosenberger J.K. Characterization of nine avian paramyxoviruses. *Avian Diseases*, 1986, 24(1): 139-152 (doi: 10.2307/1589773).
18. Ito T., Suzuki Y., Mitnaul L., Vines A., Kida H., Kawaoka Y. Receptor specificity of influenza A viruses correlates with the agglutination of erythrocytes from different animal species. *Virology*, 1997, 227(2): 493-499 (doi: 10.1006/viro.1996.8323).
19. *Rukovodstvo po eksperimental'nomu (doklinicheskomu) izucheniyu novykh farmakologicheskikh veshchestv* /Pod redaktsiei R.U. Khabrieva [Guidelines for the experimental (preclinical) study of new pharmacological substances. R.U. Khabriev (ed.)]. Moscow, 2005 (in Russ.).
20. Tare D.S., Pawar S.D. Use of embryonated chicken egg as a model to study the susceptibility of avian influenza H9N2 viruses to oseltamivir carboxylate. *Journal of Virological Methods*, 2015, 224: 67-72 (doi: 10.1016/j.jviromet.2015.08.009).
21. Loginova S.Ya., Borisevich S.V., Maksimov V.A., Bondarev V.P. *Antibiotiki i khimioterapiya*, 2009, 54(3-4): 11-14 (in Russ.).

22. Ongarbaeva N.S., Saktaganov N.T., Kalkozhaeva M.K., Lukmanova G.V., Baimukhametova A.M., Glebova T.I., Klivleeva N.G. *Materialy IV Mezhdunarodnoi nauchnoi konferentsii «Dostizheniya molodykh uchenykh v veterinarnuyu praktiku», posvyashchennoi 55-letiyu aspirantury FGBU VNIIZZH* [Proc. IV Int. Conf. «Achievements of young scientists in veterinary practice», dedicated to the 55th anniversary of postgraduate studies at FGBU VNIIZZh]. Vladimir, 2016: 99-103 (in Russ.).
23. Kiselev O.I. *Genom pandemicheskogo virusa grippa A/N1N1v-2009* [The genome of the pandemic influenza virus A/H1N1v-2009]. St. Petersburg-Moscow, 2011 (in Russ.).
24. Tabynov K.K., Mamadaliev S.M., Azanbekova M.A., Mambetaliev M. *Aktual'nye voprosy veterinarnoi biologii*, 2009, 4(4): 8-11 (in Russ.).
25. Cai M., Huang J., Bu D., Yu Z., Fu X., Ji C., Zhou P., Zhang G. Molecular evolution of H1N1 swine influenza in Guangdong, China, 2016-2017. *Infection, Genetics and Evolution,* 2018, 60: 103-108 (doi: 10.1016/j.meegid.2018.02.029).