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African swine fever in the Primorsky Krai: disease situation and molecular and biological properties of the isolate recovered from a wild boar long bone

A. R. Shotin[1], A. S. Igolkin[2], Ali Mazloum[3], I. V. Shevchenko[4], N. S. Bardina[5], E. O. Morozova[6], A. A. Shevtsov[7]

FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia

[1] *https://orcid.org/0000-0001-9884-1841, e-mail: shotin@arriah.ru*

[2] *https://orcid.org/0000-0002-5438-8026, e-mail: igolkin_as@arriah.ru*

[3] *https://orcid.org/0000-0002-5982-8393, e-mail: mazlum@arriah.ru*

[4] *https://orcid.org/0000-0001-6482-7814, e-mail: shevchenko@arriah.ru*

[5] *https://orcid.org/0000-0002-6620-8838, e-mail: bardina@arriah.ru*

[6] *https://orcid.org/0000-0002-0955-9586, e-mail: morozova_eo@arriah.ru*

[7] *https://orcid.org/0000-0002-2555-6043, e-mail: shevcov@arriah.ru*

SUMMARY

It is necessary to continue the analysis of the situation and molecular and biological properties of the current African swine fever virus isolates, recovered in the Russian border territories to cover the following tasks: eradication of African swine fever; development of effective disease surveillance and control programs; search for promising genome markers for the vaccine development; implementation of the differentiation strategy between vaccinated and non-vaccinated animals; and clustering of the isolates. The post-hoc analysis of some ASF epidemiological data and comparative genetic analysis of isolates circulating in the Far East Federal District suggested the agent introduction and spread routes, as well as the seasonality of the infection occurrence in the Primorsky Krai. It was established, that two ASFV subgenotypes (IGR-I и IGR-II), differentiated by intergenic region I73R/I329L, circulated in the region under study during the first months post infection. Analysis of biological properties of ASFV/Primorsky 19/WB-6723 isolate recovered from the long bone of a dead wild boar in the Primorsky Krai suggested that the isolate is highly virulent, able to cause peracute to subacute disease and up to 100% mortality among infected animals. The incubation period and duration of the disease course in experimentally infected pigs were 4–6 and 3–5 days post infection, respectively. The ASFV genome was detected in blood samples collected from infected pigs on 5–8 days post infection by real-time polymerase chain reaction. Specific antibodies in blood samples were not detected. The need in further research of molecular and biological properties of current ASFV isolates was reaffirmed. To prevent the continuation of the epizooty and deterioration of the current situation the approaches to the disease surveillance and control need to be modified.

Keywords: African swine fever, Primorsky Krai, epizootology, molecular and genetic analysis, bioassay

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For correspondence: Andrey R. Shotin, Junior Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", 600901, Russia, Vladimir, Yur'evets, *e-mail: shotin@arriah.ru.*

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Африканская чума свиней в Приморском крае: эпизоотическая ситуация и молекулярно-биологические свойства изолята, выделенного из трубчатой кости от дикого кабана

А. Р. Шотин[1], А. С. Иголкин[2], Али Мазлум[3], И. В. Шевченко[4], Н. С. Бардина[5], Е. О. Морозова[6], А. А. Шевцов[7]

ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

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[1] *https://orcid.org/0000-0001-9884-1841, e-mail: shotin@arriah.ru*
[2] *https://orcid.org/0000-0002-5438-8026, e-mail: igolkin_as@arriah.ru*
[3] *https://orcid.org/0000-0002-5982-8393, e-mail: mazlum@arriah.ru*
[4] *https://orcid.org/0000-0001-6482-7814, e-mail: shevchenko@arriah.ru*
[5] *https://orcid.org/0000-0002-6620-8838, e-mail: bardina@arriah.ru*
[6] *https://orcid.org/0000-0002-0955-9586, e-mail: morozova_eo@arriah.ru*
[7] *https://orcid.org/0000-0002-2555-6043, e-mail: shevcov@arriah.ru*

РЕЗЮМЕ

Для успешного искоренения африканской чумы свиней, построения эффективных программ надзора и контроля за болезнью, поиска перспективных геномных маркеров для создания профилактических препаратов, реализации стратегии по дифференциации инфицированных и вакцинированных животных, а также кластеризации изолятов необходимо продолжать изучение эпизоотической ситуации и молекулярно-биологических свойств современных изолятов вируса африканской чумы свиней, выделенных на приграничных территориях Российской Федерации. Ретроспективный анализ некоторых эпизоотологических данных в отношении эпизоотии африканской чумы свиней и сравнительный генетический анализ изолятов, циркулирующих на территории Дальневосточного федерального округа, позволили предположить пути заноса и распространения возбудителя, а также определить сезонность регистрации инфекции в Приморском крае. Отмечена циркуляция двух субгенотипов вируса африканской чумы свиней (IGR-I и IGR-II), определенных по интергенному региону I73R/I329L, на территории изучаемого края в течение первых месяцев неблагополучия по заболеванию. Исследования по изучению биологических свойств изолята вируса африканской чумы свиней ASFV/Primorsky 19/WB-6723, выделенного из трубчатой кости от павшего дикого кабана на территории Приморского края, позволили охарактеризовать его как высоковирулентный, способный вызывать от сверхострой до подострой формы течения инфекции с гибелью до 100% зараженных животных. Инкубационный период и длительность течения болезни у экспериментально инфицированных свиней составили от 4 до 6 и от 3 до 5 дней после заражения соответственно. Геном вируса африканской чумы свиней при использовании метода полимеразной цепной реакции в реальном времени детектировали в пробах крови, полученной от зараженных животных на 5–8-й день после инфицирования. Специфические антитела в образцах сыворотки крови обнаружены не были. Подтверждена необходимость проведения дальнейшего изучения молекулярно-биологических свойств современных изолятов вируса африканской чумы свиней. Во избежание продолжения эпизоотии и ухудшения сложившейся ситуации требуется корректировка применяемых подходов к осуществлению эпизоотического надзора и контроля болезни.

Ключевые слова: африканская чума свиней, Приморский край, эпизоотология, молекулярно-генетический анализ, биологическая проба

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Для корреспонденции: Шотин Андрей Романович, младший научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», 600901, Россия, г. Владимир, мкр. Юрьевец, *e-mail: shotin@arriah.ru.*

INTRODUCTION

African swine fever (ASF) is a highly contagious and deadly viral disease affecting both domestic and wild swine of all ages and breeds [1, 2].

The ASF causative agent belongs to a separate family *Asfarviridae*, has 10 serotypes identified by hemadsorption inhibition test, and 24 genotypes identified based on sequencing of B646L variable C-terminal regions encoding the vp72 capsid protein [3].

Due to the wide spread of the disease in the countries and regions, there is a need to differentiate closely related isolates, which can be done by using other, less conservative viral genes and intergenic regions (IGR), like IGR I73R/I329L.

Since the introduction of ASF in Georgia in 2007, the ASF epidemic has insidiously and progressively extended across the European continent and it continues in the present time, causing severe economic damage to the pig production and related sectors, including indirect impacts associated with trade restrictions [4].

To date, ASF II genotype virus variants are widespread in the infected countries, which cause mainly an acute disease with near 100% mortality [4, 5].

ASF epidemics in the Far Eastern Federal District of the Russian Federation began a year after the first officially notified outbreak in the People's Republic of China (PRC) in 2018 [6]. The first ASF cases in Primorsky Krai were reported in the China border area. At the same time, over the following month, new cases were also reported by the Amur Oblast and Jewish Autonomous Oblast (JAO) bordering China [6].

According to publications of S. V. Terebova et al. [7, 8] and N. V. Momot et al. [9, 10], dedicated to ASF spread in the Primorsky Krai, the migration of infected wild boars from China is the reason for the disease introduction into the region, while the anthropogenic factor

contributed to the widespread infection within the region, which is confirmed by the ASF spread along motorways, the virus introduction into the population of domestic pigs in most cases occurred due to violations of the animal management rules (feeding with non-decontaminated slaughter waste, free ranging, etc.). However, O. I. Zakharova et al. [11] confirmed the hypothesis of multiple ways of ASF spreading in the region, and the spatial-temporal analysis by the authors did not reveal patterns of the infection transmission from one population to another.

Since the introduction of the ASF virus into the Eurasian continent in 2007 Russian scientists have isolated and studied many viral isolates different in their biological properties from domestic and wild pigs from various RF regions. To date, both highly virulent and low virulent viral variants are known, including those causing an asymptomatic disease [12–19].

The results of the analysis of the epidemic situation and molecular and biological properties of current ASFV isolates isolated in the border territories of the Russian Federation can be used in the development of effective disease surveillance and control programs, as well as in the search for promising genomic markers for the vaccine development, DIVA strategy implementation and clustering of isolates. This suggests that for successful eradication of ASF, it is necessary to continue a comprehensive study of the circulating viral variants.

The aim of the work was to conduct a retrospective analysis of some epidemiological data on ASF circulation in the Primorsky Krai, as well as to study the molecular and biological properties of the ASFV/Primorsky 19/WB-6723 isolate recovered from a long bone of a dead wild boar in the region under study.

MATERIALS AND METHODS

Epidemiological analysis. Data on the epidemic situation and results of laboratory tests were obtained from official sources (the World Organization for Animal Health, the FGBI "Veterinary Center", the Information and Analysis Center of the FGBI "ARRIAH"). The experimental findings and results of the epidemic situation analysis in other countries and Russian regions was obtained from public sources.

The systematized data and the retrospective analysis of the epidemic development in the Primorsky Krai were mapped and expressed graphically.

Molecular genetic analysis. The sequencing library was constructed for the ASFV/Primorsky 19/WB-6723 using the Nextera XT DNA Library Preparation Kit (Illumina, USA). New generation sequencing (NGS) was performed using MiSeq Reagent Kits v2 (with 2 × 250 bp paired-end sequencing) using MiSeq system (Illumina, USA). Analysis and alignment based on the genome of the reference strain Georgia 2007/1 (FR682468.2_ASFV/Georgia 2007/1) was performed using the QIAGEN CLC Genomics Workbench v9 program (Qiagen, www.qiagen.com). The open reading frames were determined using GATU. The genome sequence of the ASFV/Primorsky 19/WB-6723 isolate is published in the GenBank international database with number MW306191. Determination of single nucleotide polymorphisms and comparison of isolates were performed using the BioEdit 7.1 program.

The gene sequences of ASFV isolates from other countries and regions used in the work were obtained from the GenBank database, as well as from the publications of A. K. Sibgatullova, M. E. Vlasov and D. A. Lunina [20, 21].

Biological assay. The biological assay was done on domestic pigs, brought from the Vladimir Oblast farm, free from major porcine infectious diseases, strictly following the interstate standards for the maintenance and care of laboratory animals adopted by the Interstate Council for Standardization, Metrology and Certification, as well as the requirements of Directive 2010/63/EU of the European Parliament and the Council of the European Union of 22.09.2010 on protection of animals used for scientific purposes.

Seven 15–20 kg Large White pigs were used in the experiment. Six of them (No. 1–6) were infected intramuscularly with a reconstituted virus-containing suspension of ASFV/Primorsky 19/WB-6723 isolate sample at a dose of 10 HAd/animal, and one animal (No. 7) was co-kept with infected pigs to assess potential contact infection.

The ASFV/Primorsky 19/WB-6723 isolate used for infection was recovered from a long bone of a dead wild boar found on August 06, 2019 in a forest area in the Pogranichny Raion of the Primorsky Krai.

The animals were kept in isolation in the animal facilities of the FGBI "ARRIAH" intended for II–IV pathogenicity group pathogen handling.

Biological assay, as well as the assessment of clinical signs and post-mortem lesions were performed pursuant to the guidelines and instructions of the FGBI "ARRIAH" [22, 23].

The animals were monitored daily, including observation of clinical signs and measuring (rectally) the body temperature of each pig. Blood was sampled from animals on the 0; 5; 8; 11 and 14 days post infection (DPI). The obtained samples were tested in parallel by real-time polymerase chain reaction (real-time PCR), solid-phase enzyme-linked immunosorbent assay (SP-ELISA) and immunoperoxidase technique (IPT).

ASFV specific antibodies were identified using SP-ELISA kits: INgezim PPA Compac (Ingenasa, Spain) and ID Screen® African Swine Fever Indirect screening test (IDvet, France) in accordance with the manufacturer's instructions, as well as using IPT in accordance with the FGBI "ARRIAH" guidelines [24].

The ASFV genome was detected by real-time PCR-RV using the ASF test kit (FBIS "Central Research Institute of Epidemiology" of Rospotrebnadzor, Russia) according to the manufacturer's instructions.

Data processing and plotting were performed using Statistica software (http://statsoft.ru), GraphPad Prism 8.0 (https://www.graphpad.com) and Microsoft Excel packages (https://www.microsoft.com/ru).

TEST RESULTS

Epidemiological analysis. Until mid-2019, the ASF-infected zone on the territory of the Russian Federation was limited to the European part of the country (with the exception of sporadic cases).

The first ASF outbreak in the Far Eastern Federal District was reported on July 30, 2019 in the Primorsky Krai (Fig. 1). The outbreak occurred among the pigs in a farm of the Pogranichny settlement, Pogranichny Raion,

Primorsky Krai, located 14 km from the state border of the Russian Federation with China, and 90 km and 490 km from the nearest outbreaks among domestic (11.12.2018) and wild (14.11.2018) pigs in China, respectively. The Amur Region (05.08.2019) and the JAO (28.08.2019) became the next infected regions in the Far Eastern Federal District after the aforementioned Primorsky Krai.

During the first month of infection in the Primorsky Krai, eight new outbreaks of the disease were reported (5 among domestic pigs and 3 in the wild boar population) on the territory of three Raions of the region at a distance of 315 km from the site of the primary detection. The first cases of ASF in wild fauna (01–06.08.2019) were reported after testing of samples from dead wild boars at a distance of 10 to 60 km from the state border with the PRC. At the same time, on August 07, 2019, at a distance of 30 km from the nearest place of ASF detection in the wild boar population (August 06, 2019), the pathogen was found in the sample from a shot wild boar.

Since the beginning of the epidemics in the region and until September 2021, 122 outbreaks were officially reported in the Primorsky Krai (66 among domestic pigs, 56 among wild boars). All cases were reported on the basis of PCR positive results. At the same time, laboratory tests of samples from wild boars for specific antibodies were not performed in the region.

As can be seen in Figure 2, the dynamics of ASF registration in the territory of the Krai during the study period had a wave-like pattern. It can be divided into three time periods (No. 1: 07.2019–02.2020; No. 2: 06.2020–03.2021; No. 3: 04.2021–09.2021), each of them had a following pattern: it started with the predominance and increase in the number of cases among domestic pigs, then the number of cases declined and outbreaks in the wild boar population started to be reported.

According to urgent reports and publications, outbreaks among domestic pigs occurred mainly in small farms (backyards), in which up to 100 pigs are kept. The causes of the disease include: feeding with virus-contaminated swill and slaughter waste, used as the basic part of the diet and not properly heat treated; free ranging in the forests, and, as a result, their contact with wild boars; as well as unintentional mechanical introduction of the pathogen by humans [7, 8, 10].

During the above-mentioned periods (No. 1, 2 and 3), ASF was reported in 10, 14 and 1 previously free Raions of the Primorsky Krai, respectively.

The data presented in Figure 3 demonstrate that the maximum number of new areas was covered by the epizootics at the beginning of the first (07.2019–12.2019) and second (06.2020–12.2020) periods, when ASF was initially reported in 12 Raions in domestic pigs, in 2 Raions in wild pigs and once in both populations simultaneously. During the second parts of periods No. 1 and 2, eight cases were initially notified in the wild boar population and only two cases among domestic pigs.

In total, 25 Raions of the region became ASF-infected during the study period (including 22 among domestic pigs and 22 in the wild boar population). On average, 4.88 ± 1.203 cases of the disease were notified in each of the Raions (95% confidence interval), while in 19 of them ASF was reported in both susceptible populations and

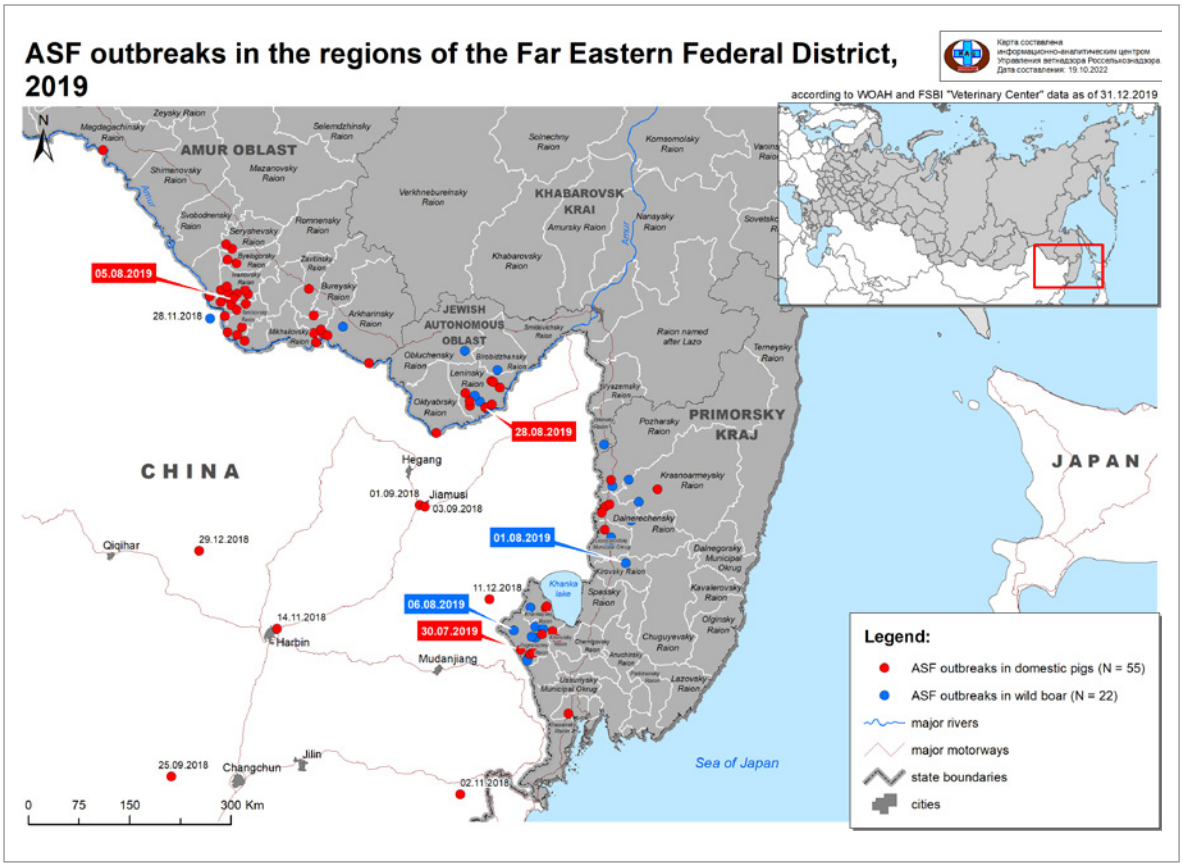

Fig. 1. ASF outbreaks in the Subjects of the Far East Federal District as of December 31, 2019

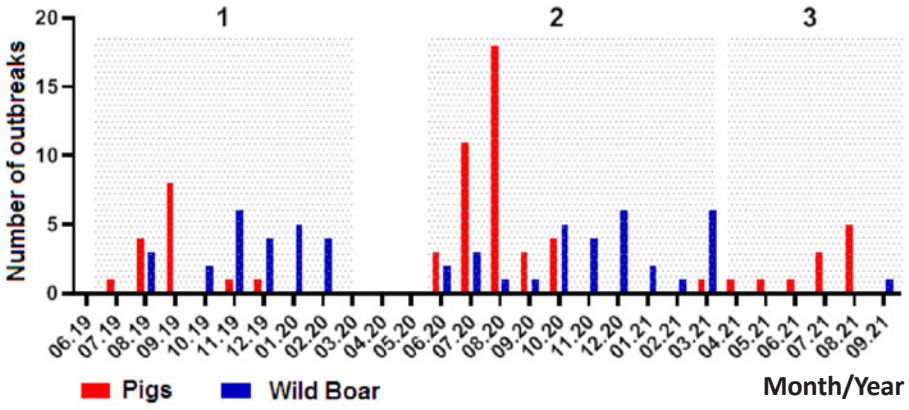

Fig. 2. ASF reporting in the Primorsky Krai from July 2019 to September 2021.
Figures 1, 2 and 3 mean three epizootic periods (n = 122)

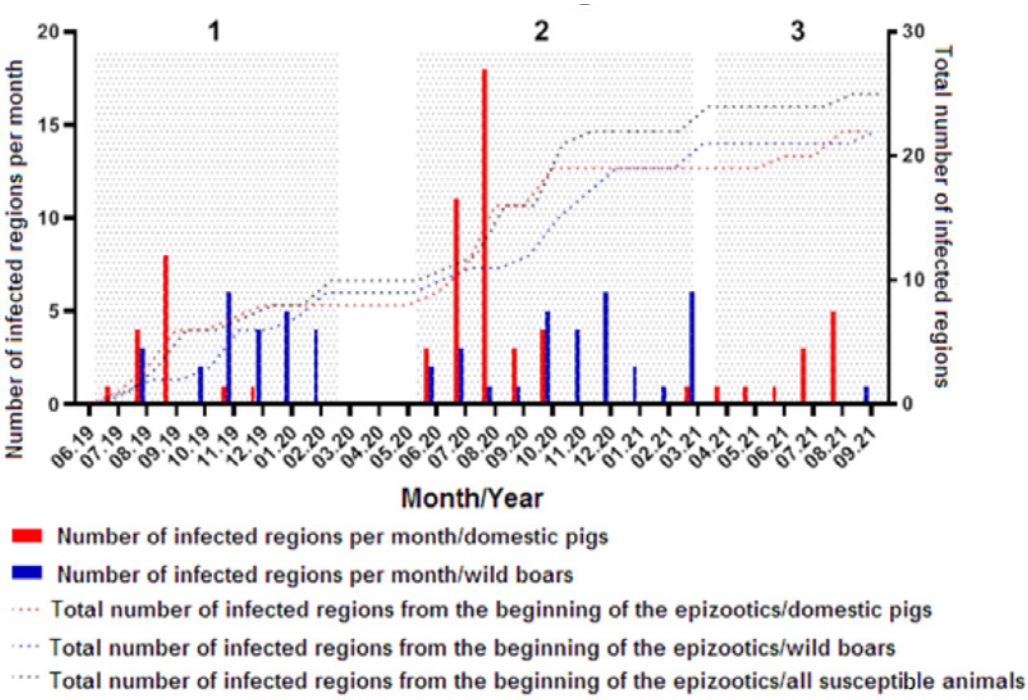

Fig. 3. ASF infected Raions of the Primorsky Krai from July 2019 to September 2021.
Figures 1, 2 and 3 mean three epizootic periods

only in six Raions in one of the populations (Nadezhdinsky, Spassky Raions and Arsenyevsky Urban Okrug – in domestic pigs; Dalnegorsky Urban Okrug, Olginsky and Chernigovsky Raions – among wild boars). The maximum number of outbreaks was reported in the Pogranichny Raion (*n* = 12).

On the territory of eight Raions, the disease was registered both among the dead and among the shot wild boars, in 11 Raions only among the dead animals, in three Raions exclusively among the shot animals. In 72% of cases, a positive ASF result was obtained by laboratory testing of samples from dead wild boars and in 28% from shot ones.

Among the dead wild boars, ASF was reported during 10 months of the year (with the exception of April and May), reporting peaks were noted in July – August and November, while in samples from shot wild boars during 7 months of the year (with the exception of the period from April to July and September), and peaks occurred in December – January and March.

It is worth noting that in the period from June to November, the infection was registered mainly among dead wild boars, then from December to March there was an increase in the number of detected cases of the disease among the shot animals.

Among domestic pigs, the peak of ASF registration occurred in the summer and autumn (July – September) with the maximum number of outbreaks in August.

Molecular genetic analysis. In a previous publication by A. Mazloum et al. [25], the results of the ASFV/Primorsky 19/WB-6723 genetic analysis were presented, which showed that its 189,263 bp genome encodes 189 open reading frames. The analysis of the B646L gene C-terminal region indicates that the virus belongs

Fig. 4. Comparative analysis of IGR I73R/I329L nucleotide sequences of isolates from Europe, Asia and Russia.

Red dots mark ASFV isolates, recovered from the biological material of domestic pigs; blue dots – from wild boars

to genotype II, and analysis of intergenic region I73R/I329L clusters it into IGR-II (Fig. 4). Phylogenetic analysis of full genome sequencing grouped the isolate under study into the same group with isolates from China and Poland, while isolates from Georgia (Georgia 2007/1 – reference isolate), Tanzania (LR813622_Rukwa_Tanzania_2017), the Central Federal District of the Russian Federation (ASFV/Ulyanovsk 19/WB-5699 and ASFV/Kabardino-Balkaria 19/WB-964) and Lithuania (MK628478) were clustered into the IGR-I group.

Analysis of IGR I73R/I329L shows that in addition to the ASFV/Primorsky 19/WB-6723, Primorskiy 8954/19 (Primorsky Krai), MT840353 Russia 2019 Amur/19.08.19 (Amur Obalst) and Ev. avt. reg 8951/19 (JAO) and various isolates from Poland, Europe and China belong to subgenotype II (IGR-II), having an insertion of 10 bp (GAATATATAG). However, in the same regions of the Russian Federation, ASFV isolates belonging to subgenotype I (IGR-I) were found within the same time period: Primorsky Krai 26.09.2019 DP (Primorsky Krai), Amurskaya 21.08.2019 DP and ASFV/Amur 19/WB-6905 (Amur Oblast). At the same time, the above-mentioned ASFV variants are prevalent not only in the territory of the Russian Federation, but also in China.

Biological assay. ASFV/Primorsky 19/WB-6723 isolate was selected to analyze the biological properties of the ASFV circulating in the Primorsky Krai. The experimental design is presented in the section "Materials and methods" of this publication.

The results of thermometry and parallel tests of animal blood samples by real-time PCR and SP-ELISA are given in the section Additional Materials at https://doi.org/10.29326/2304-196X-2022-11-4-347-358.

It was found that body temperature above 40.0 °C was recorded in infected animals starting from 4–6 DPI, while in contact animals starting from 7 DPI.

On 5 DPI 67% (4) of infected animals showed positive results in real-time PCR. At the same time, inconclusive results were obtained by this method in 33% (2) of infected and contact animals. On 8 DPI, all the surviving individuals were positive for the virus genome in blood. The minimum *Ct* value was 8.02 cycles and was detected in infected pig No. 4.

ASFV specific antibodies were not detected in sera from experimental animals using commercial SP-ELISA kits, as well as by IPT. The percent inhibitions did not exceed 25.7% for the INgezim PPA Compac kit and 12.1% for the ID Screen® African Swine Fever Indirect Screening Test.

To assess the severity of the clinical signs of ASF, a scoring system was used.

As can be seen from Table 1, the duration of the disease course was 3–5 days in the group of infected animals and 8 days for a contact animal, which is typical for the peracute and acute forms of ASF. The death of animals was recorded no later than 8 days after the first ASF clinical signs.

The following clinical signs were recorded in animals: an increase in body temperature above the physiological norm (40.0 °C) to 42.0 °C, a change in appetite (from

decrease to anorexia), weight loss (from slight to cachexia), cyanotic skin (from 1 to 20% of the skin) and nectrotic skin, digestive disorders (from mild to severe diarrhea, developing dehydration signs), disorders of the nervous (from lethargy to posterior paralysis) and respiratory systems (from mild to moderate dyspnea). The severity of clinical signs depended on the duration of the disease. For example the severity of ASF in pig No. 3, which died from the peracute disease (the duration of the disease is 3 days), was scored 11, while in animals with an acute ASF (the duration of the disease is 4–8 days), the score ranged from 12 to 18.

After the death the animals were autopsied. The severity of post-mortem lesions was also scored.

Table 2 shows that, similar to assessing of clinical signs, the severity of post-mortem lesions depended on the form of the disease. A lower total score was registered in pig No. 3 (12 points, peracute disease), while more intense changes were observed in contact pig No. 7 (36 points, subacute form). The typical post-mortem lesions included: minimal to moderate blood filling of the spleen (total 12 points); slight to moderate (up to 25% of the norm) hyperplasia of spleen (12 points) and lymph nodes (8–12 points); pneumonia (10 points); single to few hemorrhages under the epicardium (10 points) and others.

During post-mortem examination samples from spleen were taken and tested by real-time PCR.

Spleens from all animals were positive for ASFV genome. The *Ct* value averaged 9.7 (n = 7) – 8.5 for infected, n = 6. Testing of the spleen sample from pig No. 5, who died first (8 DPI) gave the minimum *Ct* value (6.56), and, conversely, the highest *Ct* value was obtained when testing the spleen from pig No. 7, who died last (14 DPI).

Table 1
Assessment of clinical signs

Group	Animal	Days post infection														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Contact	7	·	·	·	·	·	·	·	5	4	6	7	4	8	12	18 †
Infected	1	ϟ	·	·	·	·	4	6	9	12	†					
	2	ϟ	·	·	·	5	6	7	14	18	†					
	3	ϟ	·	·	·	·	·	5	8	11	†					
	4	ϟ	·	·	·	·	4	5	5	10	14	†				
	5	ϟ	·	·	·	·	5	8	13	†						
	6	ϟ	·	·	·	·	·	6	6	7	13	16	†			

† – death date; ϟ – infection date.

Table 2
Scoring of post-mortem lesions

Animal	Lungs			Heart		Spleen		Lymph nodes			Liver		Kidneys			Bladder	Transudate		Score
	edema	pneumonia	pleural hemorrhages	hemorrhagic diathesis, dystrophy	transudate in the pericardial cavity	blood filling	splenomegaly	submandibular	mesenteric	inguinal	hepatopathy	biliary tract	hemorrhagic diathesis in the cortical and medullary substance	subcapsular hemorrhages	pleural renal	hemorrhagic diathesis in the mucous membrane	thorax cavity	abdominal cavity	
7	2	3	1	3	2	2	3	2	3	1	2	2	2	2	2	1	1	2	**36**
1	0	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	0	0	**18**
2	1	2	1	1	2	2	2	2	2	2	2	1	1	2	1	1	0	1	**26**
3	0	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	0	0	**12**
4	1	1	0	1	0	2	1	2	1	1	1	1	1	1	1	0	0	1	**16**
5	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	**14**
6	1	2	1	2	2	2	2	2	1	1	2	1	2	1	2	1	0	1	**26**
Score	**6**	**10**	**6**	**10**	**9**	**12**	**12**	**12**	**10**	**8**	**9**	**7**	**9**	**8**	**8**	**6**	**1**	**5**	**148**

DISCUSSION

The first case of ASF in China was reported on August 3, 2018 in domestic pigs (Shenyang). In the next 260 days, the disease spread to 31 provinces [26]. As of April 21, the proportional rate of outbreaks in small farms (livestock ≤ 500, 127/168) was significantly higher than that of medium (501 ≤ livestock < 2,000, 14/168; 2001 ≤ livestock ≤ 5,000, 9/168) and large farms (livestock ≥ 5,001, 18/168). Six outbreaks in total were notified in the wild boar population during the above-mentioned period [27].

It is believed that the major contributing factors of the disease spread in China are the pork turnover and swill feeding for small farms while mechanical dissemination with vehicles and personnel is the major contributing factor for large farms [27]. Herewith, J. Yang [26], L. Gao [27], L. K. Dixon [28] indicate the illegal transportation and sale of pigs and pig products, high density of domestic pigs and wild boars, a large number of small and medium farms (95% of the pig industry), the difficulty of controlling trans-regional transportation, diagnostic difficulties and ineffective surveillance and control programs at the beginning of the epizootics as aggravating factors [27, 28].

Potentially unnoticed cases of infection on some farms and underreporting of outbreaks at pork production enterprises arise concerns. For instance, 2 months before the first case of ASF in the PRC, a disease with consistent clinical and post-mortem signs was reported in animals on two farms in Shenyang suburbs; pigs had been illegally brought to one of those farms from a neighboring province [28]. The publication of S. You [29] provides information about a large gap between the officially stated and actual data on the number of pigs that had died or been culled because of ASF. The above does not exclude the wide spread of the disease in the wild boar population, which was never officially reported.

In 2019, in the Far Eastern Federal District, in a short period of time (one month), the disease was first reported in the Primorsky Krai, the JAO and the Amur Oblast bordering China. The first outbreaks of the disease in the above-mentioned regions were registered in domestic pigs. However, if in the Amur Oblast and the JAO outbreaks occurred exclusively among domestic pigs and spread mainly in the livestock during 4 and 1 months, respectively, then in the territory of the Primorsky Krai, the disease was also recorded in wild boars (3 outbreaks) during the first month after infection.

During the first month after primary infection in the Primorsky Krai, ASF outbreaks were registered at a distance of about 90 km from the nearest officially notified domestic case in China (December 11, 2018) and 490 km from a wild boar case (November 14, 2018). However, given the high density of susceptible populations, the low level of farm biosecurity, weak movement control, as well as illegal transportations, ineffective disease control policies, as well as possible underreporting of cases (including in territories bordering the Russian Federation) and delayed detection of ASF [26, 27, 29], it can be assumed that the virus circulates in the immediate vicinity of the Russian borders.

The open press criticizes the official veterinary service of the Primorsky Krai, which is accused of mistakes in the organization and implementation of ASF prevention measures [30]; it is indicated that the speed of infection spread in the wild boar population was 12 to 36 km per year [31, 32]. The above, as well as the fact of simultaneous detection of the pathogen in samples from dead wild boars (with outbreaks among domestic pigs) and, given the long-term survival of the virus in animal carcasses, does not allow for an unambiguous conclusion regarding the route of ASF introduction into the Primorsky Krai.

A number of researchers hypothesize that the ASFV was introduced from the PRC to the Russian Federation during the migration of infected wild boars. They justify their assumption by a significant density of wild boar in the region with a high probability of underreporting of wild boar cases in China [7–10]. However, the existing trade relations with China and the official notification data also suggest the introduction of the virus with transportations of infected pigs and pig products.

Registration of the first outbreaks at a considerable distance from each other (up to 315 km from the first detection) and from the state border with the PRC (from 10 to 60 km) in both domestic and wild pigs can be a consequence of the viral long-lasting circulation in wild fauna and/or underreporting of cases and/or multiple routes of the virus introduction to the region. O. I. Zakharova et al. [11] came also to this opinion.

According to the literature, the leading role in ASF spread in the Russian Federation is played by the anthropogenic factor [33, 34]. The analyzis of the epizootics spread in the Far Eastern Federal District, conducted by O. I. Zakharova et al., concluded that ASF outbreak clusters in domestic pigs were formed in the JAO, the Amur Oblast and the Khabarovsk Krai, while mixed clusters (wild boar + domestic pigs) were formed in the Primorsky Krai. The authors also analyzed the median spread of infection in the region, which showed the movement of the disease from the west (Amur Oblast) to the east and from the south (Primorsky Krai) to the north with the concentration of outbreaks in the Khabarovsk Krai [11].

Analysis of the laboratory testing of the ASFV/Primorsky 19/WB-6723 (Primorsky Krai) in comparison with other isolates isolated in the same year in the territories of the Primorsky Krai, Amur Oblast and China shows that they belong to Groups I and II based on IGR, and it is not possible to find an association between them. Thus, it is believed that the repeated introduction of the infection into the territory of the Far Eastern Federal District and/or the circulation of a non-homologous population of the ASF virus are possible.

The use of a limited number of molecular markers, such as IGR I73R/I329L, is currently becoming uninformative, which confirms the relevance and necessity of searching for new markers with higher resolution. Thus, one of the ways to solve this problem is to conduct full genome sequencing of circulating ASFV isolates and their comparative analysis [25].

During the period under study (07.2019–09.2021), ASF epizootics in the region can be divided into 3 periods; each of them has similar temporal and epidemiological (registration of cases in both susceptible populations and the number of first outbreaks in previously free areas of the region) parameters. So, from June to September, the disease was reported increasingly mainly in the population of domestic pigs, then from October to March – mainly among wild boars.

The disease was initially reported in the population of domestic pigs in 14 infected areas (56% of cases), 12 of which occurred in the first half of periods No. 1 and 2. In 10 infected areas (40% of cases) ASF was diagnosed first in wild boars, 8 of them occurred in the second half of all three periods.

It is also worth noting that the registration of outbreaks mainly in backyards and small farms, with low compartment levels (hence biosecurity), was associated with violations of veterinary rules for pig management. The above-mentioned, as well as the spread of the disease along motorways, may be a consequence of the disease underreporting by owners, which is possible when ineffective strategies of the disease monitoring and the movements of susceptible animals, as well as inappropriate preventive and quarantine measures are involved. Therefore, only strict quarantine measures and strict compliance with their requirements can prevent further spread of the infection [8].

The seasonality of ASF registration in domestic pigs turned out to be apparent and typical for the entire infected territory of the Russian Federation. The notification of the disease in the summer-autumn period (July – September) with a peak in August is most likely due to the peculiarities of the pig farming practice (free ranging, feeding with non heat-treated swills, grouping of animals) and production cycles, including in backyards where up to 100 pigs are kept, especially considering the predominant registration of the disease in small farms along motorways [8, 10, 33].

The predominant number of reported ASF cases in dead wild boars (72% of cases on the territory of 19 Raions) against shot animals (28% of cases on the territory of 11 Raions) may indicate a high mortality, which is typical for the disease (up to 100% of infected animals). However, on the territory of three Raions, ASF outbreaks in wild boars were reported exclusively during the testing of shot animals, which suggests a low effectiveness of the ASF surveillance strategy in the studied region, and/or delayed detection of infection in the above-mentioned areas, and/or circulation of low-virulent isolates. Thus, V. Gervasi et al. concluded that at the beginning of the epizootics, passive surveillance (search for animal carcasses and testing 100% of the samples) makes it possible to detect infected animals at an earlier time from the moment of introduction of the virus into the population and in greater numbers compared with active surveillance (testing of shot animals) [35].

Comparing the seasonality of ASF reporting in dead and shot wild boars, it is worth noting that the previous seasonal peak of the disease registration (July, November) in dead animals and the subsequent one (December – February) among the shot ones also confirms the great importance of the passive surveillance for an early detection of the disease in the region [35].

However, while the ASF reporting data in domestic pigs and shot wild boars can be demonstrative for the seasonality of the disease spread in these groups, then reports on the disease in dead animals, due to unknown dates of death and long-term survival of the pathogen in carcasses, do not allow to conclude about the true seasonality of ASF infection in this group.

The lack of tests aimed at specific antibodies in samples from wild boars can distort the real picture of the epizootic situation in the region and does not allow detecting all infected animals [36]. This fact may be related to the difficulties of wild boar serum sampling and transportation, as well as the possible lack of test kits and/or methods that allow testing of samples other than serum (organs, meat juice and/or blood, including dried on filter paper) for antibodies to the virus, such as commercial SP-ELISA ID Screen® African Swine Fever Indirect (IDvet, France) test-kits and such reference methods as IPT, immunoblotting and/or indirect immunofluorescence assay. At the same time, it is worth noting that commercial immunochromatographic rapid tests, for example INgezim PPA CROM Anticuerpo (Ingenasa, Spain) [37] for detection of specific antibodies that can be used in the field, are available, which is especially important for monitoring in wild boar populations.

Clinical and post-mortem disease picture in animals infected with ASFV/Primorsky 19/WB-6723 was typical for the isolates recovered in Russia in 2007–2018, which caused 100% mortality in pigs [12–15, 17–19].

The testing of blood samples from animals infected with this isolate gave positive results exclusively when they were tested by direct methods (for example, by real-time PCR), while the use of indirect diagnostic methods (for specific antibodies) turned out to be ineffective (all serum samples were negative). The latter may be a consequence of the short disease course and the death of infected animals before the antibodies were generated at detectable levels.

It is worth noting that this study describes biological properties related exclusively to the studied ASFV isolate using the selected infection model (intramuscular), which was isolated at the beginning of the epizootics in the Primorsky Krai, and they may differ from those of modern circulating isolates. This means that with the development of the epizootics both molecular genetic and biological properties can change, which is confirmed by the increase in the number of seropositive animals in the EU countries, as well as by detection of genetically modified and less virulent viral isolates, including China [38, 39]. Moreover, some animals can survive when infected with highly virulent isolates. For example after oral infection of wild boars with ASFV isolate recovered in Estonia, nine animals out of ten died on 7–13 DPI from acute disease, but one animal survived and was euthanized on 96 DPI [40]. A. Pershin et al. also noted that the data on viral biological properties obtained by the experimental infection may differ from those, obtained in the field, which may be associated with the species, age, physiological conditions if the experimental animals and the selected infection route and sampling method [17].

CONCLUSION

The ASFV incursion into the territory of the Far Eastern Federal District has probably occurred from China. The conclusion made by O. I. Zakharova et al. [11] regarding multiple routes of pathogen incursion into the region, including Primorsky Krai is confirmed. Thus, both anthropogenic factors (economic relations) and cross-border migration of wild boars could serve as infection transmission factors [7–10, 29].

Simultaneous reporting of outbreaks in both populations of susceptible animals (domestic pigs and wild boars) during the first month after primary infection

and the detection of two different genetic groups based on IGR I73R/I329L (Groups I and II) also confirm the hypothesis of multiple routes of introducing infection into the region and/or may be a consequence of late detection of the pathogen in the region due to improper ASF preventive measures, which, in particular, was reported by the Rosselkhoznadzor [29].

The widespread infection and the long-term infection of the studied region were probably caused by the introduction of the pathogen into the wild fauna, the high density of the wild boar population in the region, low level biosecurity of pig farms, as well as the uncontrolled movement of infected pigs and pig products [7–10, 29].

The cyclical (annual) reporting of the disease was noted, the corresponding periods were determined. The seasonality of infection among domestic pigs is defined as typical for the Russian Federation. The seasonal peak of ASF detection in dead wild boars was revealed, it preceded the reporting of outbreaks in shot wild boars. At the same time, the revealed indicators may most likely be due to pig management and movement, the species characteristics of wild boars (breeding period, herd regrouping) and their seasonal feeding, as well as the specific features of ASF surveillance measures (including uneven distribution of diagnostic tests) in both susceptible populations than due to the true seasonality of the disease [33, 41].

In several Raions of the Primorsky Krai, the disease was reported exclusively among shot wild boars, which may suggest the lack of measures aimed at searching for and destruction of animal carcasses and further long-term infection of these territories.

For the first time, the biological properties of the ASFV/Primorsky 19/WB-6723 isolated from a long bone of a dead wild boar in the Primorsky Krai were tested and analyzed. The selected isolate is characterized as highly virulent, capable of causing peracute to subacute forms of infection with the death of up to 100% of infected animals.

The fundamental importance of passive epidemiological surveillance with regular sampling and testing of samples from all animals with ASF consistent signs (case diagnostics among suspicious and dead animals by direct methods for the virus, its antigen, genome) was confirmed [35].

In cases when the ASF virus circulates in a susceptible population for a long time (for example, more than six months), it is rational to complement direct research methods with indirect ones (for antibodies, taking into account the possibility of testing both blood samples and samples of internal organs), primarily when testing samples from shot wild boars.

In order to avoid the epizootics persistence and the deterioration of the current situation both in the Primorsky Krai and throughout the Russian Federation, it is necessary to adjust the approaches applied to the disease surveillance and control, including strengthening of animal health monitoring, as well as their identification, traceability of pigs and products thereof, proper protection of all types of pig farms from the ASFV incursion, timely and strict implementation of all infection control measures pursuant to regulations.

In order to differentiate and study the evolution of the ASF virus circulating in the Russian Federation (including the Primorsky Krai), it is necessary to send samples tested positive or inconclusive by regional laboratories to the ASF Reference Laboratory of the FGBI "ARRIAH" for confirmation and research, including full genome sequencing of virus isolates and search for new relevant genetic markers.

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INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Andrey R. Shotin, Junior Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Шотин Андрей Романович, младший научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Alexey S. Igolkin, Candidate of Science (Veterinary Medicine), Head of Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Иголкин Алексей Сергеевич, кандидат ветеринарных наук, заведующий референтной лабораторией по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Ali Mazloum, Candidate of Science (Biology), Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Мазлум Али, кандидат биологических наук, научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Ivan V. Shevchenko, Candidate of Science (Biology), Senior Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Шевченко Иван Вячеславович, кандидат биологических наук, старший научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Natalia S. Bardina, Junior Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Бардина Наталья Сергеевна, младший научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Elizaveta O. Morozova, Post-Graduate Student, Biologist, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Морозова Елизавета Олеговна, аспирант, биолог референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Alexander A. Shevtsov, Candidate of Science (Veterinary Medicine), Leading Researcher, Information and Analysis Centre, FGBI "ARRIAH", Vladimir, Russia.

Шевцов Александр Анатольевич, кандидат ветеринарных наук, ведущий научный сотрудник информационно-аналитического центра ФГБУ «ВНИИЗЖ», г. Владимир, Россия.