DOI: 10.29326/2304-196X-2021-10-4-285-294

Techniques of blood sampling for detection of African swine fever virus in wild boar and domestic pigs in the field conditions

A. S. Pershin[1], A. R. Shotin[2], E. O. Morozova[3], A. S. Igolkin[4], O. A. Manuylova[5], I. V. Shevchenko[6], A. A. Shevtsov[7], N. N. Vlasova[8]
FGBI "Federal Centre for Animal Health" (FGBI "ARRIAH"), Vladimir, Russia
[1] *https://orcid.org/0000-0002-5099-3050, e-mail: daredron@gmail.com*
[2] *https://orcid.org/0000-0001-9884-1841, e-mail: shotin@arriah.ru*
[3] *https://orcid.org/0000-0002-0955-9586, e-mail: morozova_eo@arriah.ru*
[4] *https://orcid.org/0000-0002-5438-8026, e-mail: igolkin_as@arriah.ru*
[5] *https://orcid.org/0000-0002-4616-5316, e-mail: o.manuylova@list.ru*
[6] *https://orcid.org/0000-0001-6482-7814, e-mail: shevchenko@arriah.ru*
[7] *https://orcid.org/0000-0002-2555-6043, email: shevcov@arriah.ru*
[8] *https://orcid.org/0000-0001-8707-7710, e-mail: vlasova_nn@arriah.ru*

SUMMARY

It is thought that due to the high virulence of the African swine fever virus its circulation in the Russian Federation is accompanied by a low seroprevalence. However taking into account a long-term ASF unfavourable situation, the introduction of the virus into the wild boar population, and the occurrence of attenuated viral variants, the significance of serological testing aimed at the detection of viral antibodies is increasing. To collect field samples of biological material from animals for molecular genetic, virological, and serological tests, filter paper, as well as swabs, can be used. The specificity and sensitivity of enzyme-linked immunosorbent assay when testing blood absorbed by filter paper are worse than those shown when testing sera, but they allow effective detection of African swine fever virus antibodies. It was demonstrated that blood absorbed on filter paper can be used for the immunoblot analysis, but the optimum performance could be achieved when the immunoperoxidase technique in combination with samples, taken by swabs was used. When comparing results of enzyme-linked immunosorbent assay performed on sera collected from domestic pigs (infected with ASFV isolates Antonovo 07/14 and Sobinka 07/15), and blood from ear veins absorbed on filter paper the sensitivity was 88.9%, specificity – 90.6%. However, the use of the immunoperoxidase technique for testing blood from swabs showed 100% coincidence with ELISA, while testing of sera with immunoperoxidase technique was superior to ELISA in sensitivity. This means blood sampling using swabs may be recommended for tests after proper validation. This technique can be especially useful for collecting data about infected wild boars because effective eradication strategies are impossible without such data.

Keywords: African swine fever, serological diagnosis, enzyme-linked immunosorbent assay, immunoperoxidase technique, immunoblot analysis, filter paper, swabs

Acknowledgements: The study was funded by the FGBI "ARRIAH" within the framework of "Veterinary Welfare" research work.

For citation: Pershin A. S., Shotin A. R., Morozova E. O., Igolkin A. S., Manuylova O. A., Shevchenko I. V., Shevtsov A. A., Vlasova N. N. Techniques of blood sampling for detection of African swine fever virus in wild boar and domestic pigs in the field conditions. *Veterinary Science Today*. 2021; 10 (4): 285–294. DOI: 10.29326/2304-196X-2021-10-4-285-294.

Conflict of interest: The authors declare no conflict of interest.

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

УДК 619:619.98:578.842.1:637.661:612.1:616-097:616-078

Методы отбора крови для выявления антител к вирусу африканской чумы свиней у диких кабанов и домашних свиней в полевых условиях

А. С. Першин[1], А. Р. Шотин[2], Е. О. Морозова[3], А. С. Иголкин[4], О. А. Мануйлова[5], И. В. Шевченко[6], А. А. Шевцов[7], Н. Н. Власова[8]
ФГБУ «Федеральный центр охраны здоровья животных» (ФГБУ «ВНИИЗЖ»), г. Владимир, Россия

[1] *https://orcid.org/0000-0002-5099-3050, e-mail: daredron@gmail.com*
[2] *https://orcid.org/0000-0001-9884-1841, e-mail: shotin@arriah.ru*
[3] *https://orcid.org/0000-0002-0955-9586, e-mail: morozova_eo@arriah.ru*
[4] *https://orcid.org/0000-0002-5438-8026, e-mail: igolkin_as@arriah.ru*
[5] *https://orcid.org/0000-0002-4616-5316, e-mail: o.manuylova@list.ru*
[6] *https://orcid.org/0000-0001-6482-7814, e-mail: shevchenko@arriah.ru*
[7] *https://orcid.org/0000-0002-2555-6043, e-mail: shevcov@arriah.ru*
[8] *https://orcid.org/0000-0001-8707-7710, e-mail: vlasova_nn@arriah.ru*

РЕЗЮМЕ

Считается, что вследствие высокой вирулентности вируса африканской чумы свиней его циркуляция на территории Российской Федерации сопровождается низкой серопревалентностью. Однако ввиду длительного неблагополучия, внедрения в популяцию дикого кабана и появления ослабленных вариантов вируса повышается значимость серологических исследований, направленных на выявление антител к возбудителю болезни. Для сбора полевых образцов биологического материала от животных с целью проведения молекулярно-генетических, вирусологических и серологических исследований можно использовать фильтровальную бумагу, а также зонд-тампоны. Специфичность и чувствительность твердофазного иммуноферментного анализа при исследовании образцов крови, нанесенной на фильтровальную бумагу, уступают результатам, получаемым при исследовании сыворотки крови, но тем не менее позволяют с успехом выявлять специфические антитела к вирусу африканской чумы свиней. Показана возможность использования фильтровальной бумаги, пропитанной кровью животных, для исследования методом иммуноблоттинга, однако оптимального результата удалось добиться при использовании иммунопероксидазного метода в сочетании с пробами, отобранными зонд-тампонами. При сравнении результатов твердофазного иммуноферментного анализа сывороток крови, полученных от домашних свиней (зараженных вирусом африканской чумы свиней изолятов Антоново 07/14 и Собинка 07/15), и крови, отобранной на фильтровальную бумагу при скарификации ушных вен, чувствительность составила 88,9%, специфичность – 90,6%. Однако использование иммунопероксидазного метода при исследовании образцов высушенной на зонд-тампоне крови показало 100%-е совпадение с иммуноферментным анализом, в то время как при исследовании сыворотки крови иммунопероксидазный метод превзошел иммуноферментный анализ по чувствительности. Следовательно, отбор крови с применением зонд-тампонов может быть рекомендован для проведения исследований после соответствующей валидации. Данный метод может быть особенно полезен для сбора информации об инфицированных диких кабанах, так как ее отсутствие делает невозможным применение эффективных стратегий эрадикации.

Ключевые слова: африканская чума свиней, серологическая диагностика, твердофазный иммуноферментный анализ, иммунопероксидазный метод, иммуноблоттинг, фильтровальная бумага, зонд-тампоны

Благодарности: Работа выполнена за счет средств ФГБУ «ВНИИЗЖ» в рамках тематики научно-исследовательских работ «Ветеринарное благополучие».

Для цитирования: Першин А. С., Шотин А. Р., Морозова Е. О., Иголкин А. С., Мануйлова О. А., Шевченко И. В., Шевцов А. А., Власова Н. Н. Методы отбора крови для выявления антител к вирусу африканской чумы свиней у диких кабанов и домашних свиней в полевых условиях. *Ветеринария сегодня*. 2021; 10 (4): 285–294. DOI: 10.29326/2304-196X-2021-10-4-285-294.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

Для корреспонденции: Шотин Андрей Романович, аспирант, ведущий биолог 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 contagious septic disease of domestic, including ornamental, pigs and wild boars. The disease can be hyperacute to asymptomatic [1–3]. The global ASF situation continues to deteriorate, threatening global food security. At the moment, more than 50 countries are infected with the disease. Dramatic losses are reported in Asian countries. For example, more than 100 million pigs have already been killed and died in China alone [4].

To prevent ASF spread and eradicate the disease in the population of domestic pigs, strict biological safety measures, stamping out policy, and the suppression of illegal trade in pigs and pig products are required. It is more complicated to take similar measures in the wild boar population due to the necessity to stick to biosafety principles in animal habitats and the tendency of the disease to become endemic if suboptimal eradication strategies are used. At the same time, evaluating the ASF control effectiveness is impossible without a properly functioning system of epizootological surveillance [5].

The presence of the virus in the wild boar population creates a risk of infection for domestic pigs. After the ASF virus introduction into the wild boar population, there is a long-term challenge growing in space and time. In most countries of the European Union, since the infection was detected in wild boar, attempts have been made to contain the infection by reducing the population density and, consequently, the number of contacts between infected and susceptible animals in order to prevent transmission of the virus. However, the results of this strategy at an early stage of epizootics in the infected area were unsatisfactory [6].

It is believed that the so-called human factor associated with the violation of veterinary and sanitary rules by people plays an important role in the spread of ASF [7]. At the same time, I. Iglesias et al. using the spatiotemporal analysis, established that in 2007–2013 in the Russian Federation, wild boars could be a source of infection in the populations of domestic pigs in 32.23% of cases and in wild pigs in 28.77% of cases [8].

The presence of infected wild boars increases the complexity of ASF eradication in areas with a large number of low biosecurity backyards. Wild boars are capable of forceful invasion into farm ecosystems, which involves them in the anthropogenic cycle of ASF transmission and allows them to participate in the spread and persistence of the virus in previously free areas [9]. Various biotechnical measures, such as hunting predators and winter feeding of animals, can lead to an increase in the population of wild boars and create conditions for the rapid spread of the infection [10]. Thus, in the system of ASF epizootological surveillance and in the complex of measures taken to eradicate outbreaks, it is necessary to take into account the presence of a susceptible wild boar population in Russia. In addition, according to the requirements of the World Organization for Animal Health (OIE) Terrestrial Code, it is necessary to regularly monitor the population of wild animals for the purposes of ASF and CSF status recognition.

At the moment, serological diagnostics is less effective for the early identification of infected animals than methods for viral antigen or genetic material detection. Thus, from the moment of registration of the first outbreak in 2007 to the present, only a small number of animals have been found to have antibodies to the ASF virus in the tests of swine serum samples using enzyme-linked immunoassay (ELISA) [11].

However, the possibility is not excluded and there is evidence of the occurrence of attenuated ASFV variants, and infection with them may not lead to the rapid death of pigs. Various studies show that in ASF-endemic areas, mortality decreases and the number of asymptomatic and chronic cases grows up, which in the future may require adjustment of the applied diagnosis and control strategies [12, 13].

In addition, at large pig-breeding establishments, where the so-called "naturally occurring death losses" occur, a mild or asymptomatic course of infection may remain unnoticed for up to several weeks [12]. This explains the importance of diagnostic tests aimed at detecting antibodies to the ASF virus, which should help reduce the risk of spreading the disease.

Therefore, in order to determine the real ASF situation, a fast, effective and reliable laboratory diagnosis is necessary. At the same time, in addition to the viral genome identification by polymerase chain reaction (PCR), it is necessary to conduct serological tests for antibodies. It was found that if at the beginning of the epizootic, a greater number of ASF seropositive animals are identified among young animals, then by the late phases of the epizootic process, seroprevalence is higher in animals of older age groups. This fact is probably related to the presence of infected, not convalescent boars [14].

It is believed that due to the high virulence of the ASFV, its circulation in the Russian Federation is associated with low seroprevalence [15]. Nevertheless, serological tests can contribute to the early detection of the disease in wild boar and increase the effectiveness of diagnostic tests used in parallel with virological (molecular biological) diagnostic methods, which can reduce the potentially dramatic consequences of ASF spread [5].

These methods require an appropriate infrastructure and technology for the rapid detection of seropositive wild boars [16]. One of the possible solutions may be the use of immunochromatographic methods [15, 17]. However, they are expensive for large-scale screening tests and are not always applicable [18]. An alternative solution is to detect antibodies in the tissues of internal organs (spleen, lungs, liver) or in the saliva of infected animals [19]. Of all the sample pools tested by researchers in 2008–2012, the percentage of antibody detections in pig tissues using indirect immunofluorescence assay was 45% [20]. However, the concentration of antibodies in such samples, as a rule, is not comparable with their concentration in serum.

By itself, the technology currently used for sampling serum into test tubes with a coagulation activator has shortcomings. Thus, hemolysis is a big challenge, since it is difficult to comply with the technology of sample preparation and transportation in the field, which can lead to false positive results during laboratory diagnostics.

There are a large number of disposable consumables for blood collection available on the market, containing components that accelerate coagulation and facilitate the process of separating serum from a clot. However, the instructions for use do not always provide the necessary information about the composition and properties of all additives. As a result, there may be errors in the results of immunoassay when using blood sampling kits, mainly when testing fragile analytes (cytokines, etc.), which do not include antibodies. On the other hand, some additives are able to interact with antibodies and induce their conformational changes regardless of the type of the solid phase used [21], which can lead to a decrease in the immunospecific activity of antibodies [22].

In the Russian Federation, it is not uncommon for hunters not to send biological material from shot wild boars for testing or not to report detected fallen animals, despite the fact that this is prescribed by the current veterinary regulations [23]. A simple explanation for this is the lack of consumables and the difficulty to take blood samples from dead animals. In order to expand ASF surveillance in the wild, to increase the probability of detecting infected animals and increase the sampling coverage, a simple and inexpensive method of blood sampling on filter paper (FP) can be used. The simplicity of sampling and transportation of samples using FP can help hunters in solving logistical problems of delivering samples to the laboratory [24]. Blood samples can be taken without any special equipment and special training, including from recently shot animals. This approach will allow agent identification and antibody testing, using a single sample easily obtained in the field [25].

At the same time, swabs are successfully used for virological studies, which is especially important when carcasses of wild boars are found, since their destruction is an important link in anti-epizootic measures, and the natural decomposition of the remains can take from several days in the summer to several years under appropriate conditions [26].

The use of FP samples and swabs for serological studies. The method of drying adsorbed blood on FP with subsequent elution and testing of various analytes has been successfully used in diagnostic tests since 1927 [27].

Currently, FP is an affordable option for collecting biological samples to diagnose various infectious diseases by serological and molecular biological methods. At the same time, the sensitivity and specificity of solid-phase enzyme-linked immunoabsorbent assay (SP-ELISA) when using FP decrease compared to the use of blood serum, although in most cases it remains at an acceptably high level [25, 28–30]. However, reduced sensitivity is a potential limitation of FP samples in protocols that require undiluted serum [28].

Modern FP can be divided into two main types. The first is carriers specially designed for the storage and isolation of nucleic acids. This type of FP is impregnated with substances that lyse cells, denature proteins, inactivate biological material and protect nucleic acids from the nuclease action. Carriers of the second type do not contain an inactivation component and can be used for virus isolation and serological studies [31].

When comparing the effectiveness of antibody detection by the ELISA method for blood samples adsorbed on FP and SP-ELISA serum samples obtained from pigs, the results of the studies showed comparable sensitivity [31]. It has also been shown that the ASF virus is isolated from the blood-soaked FP after 9 months of storage at 37 °C on a sensitive cell culture and can be genotyped. Nevertheless, the use of FP for the detection of genetic material goes along with the decrease in the sensitivity [32].

Blood sera can be stored frozen for a long time. However, their subsequent testing shows a progressive decrease in the number of detected antibodies by most of the diagnostic methods used [28]. Various studies have shown that samples adsorbed on FP can be stored for a relatively long period with a slight decrease in the titer due to antibody destruction. Using immunoblotting, it was found that a decrease in the titer of antibodies is observed in all detectable protein epitopes [33].

Temperature and humidity play an important role in the degradation of the analyte [29]. For example, the antibody titer to the human immunodeficiency virus decreases by approximately 15% during 30 days of storage at room temperature and uncontrolled humidity. At higher temperatures, titer loss increases. Samples stored under controlled humidity conditions (with a dissectant) at room temperature remain stable up to 190 days. At low temperature and humidity, antibodies to the human immunodeficiency virus on FP are stable for at least 56 months [33].

In the experiments of P. S. Curry et al. when comparing samples on FP after one year, compared to frozen serum stored for the same period, sensitivity was > 88% (for all but two assays), and specificity remained > 90% [28].

General recommendations for increasing the storage period is the need for complete drying of the blood applied to the FP, storage in a place protected from direct sunlight and at a humidity of no more than 30%. For short-term storage (up to two years) at a temperature of 4 °C it is recommended to use re-sealable zipper storage bags (ziplocks) with a dissector and a humidity indicator. Long-term storage is recommended at minus 70 °C [28, 33].

Thus, an important link in the diagnosis of various infectious diseases is the observance of temperature conditions during the transportation of samples to the laboratory and their storage. For example, according to the World Health Organization, more than 50% of the vaccines produced in the world are lost. At the same time, vaccine losses in unopened vials are usually associated with cold chain and logistics problems [34]. Twenty-five percent of the vaccines produced in the world are delivered to the destination in various stages of degradation due to violation of conditions during delivery [35]. Moreover, according to the Global Alliance for Vaccines and Immunization, half of the health care institutions in the poorest countries do not have access to electricity at all, and only 10% have uninterrupted power supplies [36].

At the same time, the practice of using swabs by many laboratories for sampling and testing by various methods (PCR, commercial ELISA kits) demonstrates the versatility of the samples obtained, as well as the simplicity of their adaptation to the conditions of a particular laboratory.

Therefore, swabs and FP are a good option for serum sampling to perform tests for ASF. They have a number of advantages: the samples adsorbed on swabs or FP are easy to handle, can be stored for long periods, can be separated if repeated or additional diagnostic tests are required. Thus, it is a practical, inexpensive and simple approach for ASF epizootological surveillance in wild boar [30].

The aim of the work was to experimentally confirm the possibility of using filter paper and swabs in sampling for serological testing aimed at detecting ASFV specific antibodies, as well as comparing various methods of testing and sampling.

MATERIALS AND METHODS

The following materials were used for studies and experiments:

– domestic pigs (crossbreeds of large white, landrace, duroc breeds), delivered from a farm, free from major infectious diseases of pigs of the Vladimir Oblast;

– wild European wild boars (8 animals, age 3–4 months), imported from the ASF free farm of the Kostroma Oblast;

– ASFV isolates: Shikhobalovo 10/13, isolated from a dead wild boar in the territory of the Yuryev-Polsky Raion of the Vladimir Oblast; Antonovo 07/14, isolated from a domestic pig in the village of Lobok, Nevelsky Raion, Pskov Oblast; Sobinka 07/15, isolated from a wild boar in the hunting farm of the Vladimir Oblast; low virulent ASF virus of the 60th passage, obtained in cell culture CV-1 – ASF/ARRIAH/CV-1/60;

– Class 3 filter paper (Whatman®, UK);

– swab (cotton swab) with a viscose tip (Ningbo Greetmede Medical Instruments Co., Ltd, China).

All animal experiments were carried out in strict accordance with the interstate standards for the care and use of laboratory animals adopted by the Interstate Council for Standardization, Metrology and Certification, as well as in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22.09.2010 on the protection of animals used for scientific purposes.

Enzyme-linked immunosorbent assay was performed using the INgezim PPA Compac kit (Ingenasa, Spain) in accordance with the kit instructions.

Immunoblotting was performed using commercial reagents (CISAINIA, Spain) in accordance with the manufacturer's instructions.

The immunoperoxidase technique (IPT) for detecting antibodies to the ASF virus was carried out in accordance with the guidelines of the FGBI "ARRIAH"[1].

RESULTS AND DISCUSSION

During the bioassay on wild boars and domestic pigs infected with the ASFV Shikhobalovo 10/13 isolate [3], the possibility of using FP for blood sampling from dead wild boars and subsequent testing for antibodies was evaluated. For this purpose, a few hours after the death of the animals the filter paper was soaked in the unclotted blood flowing out the dissected vessels in the process of the partial evisceration during the autopsy, mainly in the thoracic cavity. Since systemic biochemical processes take place in the blood after the death of the animal, blood must be sampled as soon as possible. It is advisable to use whole blood, since, despite the fact that the serosanguinous fluid contains antibodies, it is not equivalent to whole blood [28]. Paired blood samples were taken from domestic pigs into test tubes with a coagulation activator and on FP.

The filter paper was dried at room temperature in the animal facilities and placed in separate re-sealable bags. About 0.5 cm^3 of blood was required to impregnate 1 cm^2 of FP. ELISA was performed 3 days after sampling. The ASF ID Screen® African Swine Fever Indirect (IDvet, France) kit for the detection of ASFV antibodies is commercially available, which has a protocol for testing blood filter paper samples [24]. There are also specially manufactured versions of commercial kits for testing FP samples [31]. However, in this experiment, the possibility of using the Ingezim PPA Compac kit (Ingenasa, Spain) followed by immunoblotting confirmation was tested.

During immunoblotting testing of a sample taken from a wild boar, the several slightly colored stripes were observed on the nitrocellulose membrane, which indicates the formation of an immune complex in areas containing ASF virus proteins and, consequently, the presence of antibodies in the sample. Stripes are not formed when testing negative FP samples. When comparing samples obtained from a hyperimmune piglet by the conventional method and using FP, it is seen that the intensity of staining decreases in all areas of the nitrocellulose membrane coated with a specific antigen. However, the samples remain positive.

Various methods of using FP were studied. In the first case, the paper was placed in the sample buffer in a volume of 5 cm^3 on 5 cm^2 of the FP area, which is comparable to the ratio prescribed in the instructions for the serum kit, and was kept at room temperature for 5 minutes for elution. The resulting eluate was introduced in a volume of 0.1 cm^3 per well as test samples.

In the second case, the FP placed in the sample buffer was kept for 3 minutes in a homogenizer with active stirring. Before being introduced into the wells of the microplate, the eluate was centrifuged to precipitate the formed blood elements and FP particles for 3 minutes at 1000 g.

In the third case, circles with a diameter of about 3 mm were cut out of the dry FP and placed in the well of a microplate with a buffer added. According to V. G. Pomelova et al., within one hour, more than 90% of antibodies are extracted from a disk with a 3.2 mm diameter placed in the well of a coated microplate [25]. However, taking into account the average FP impregnation capacity, the blood:buffer ratio was approximately 1:30, which is lower than the serum working dilution of the kit used (1:2).

For a reliable comparison of test methods for samples obtained by different methods, it is necessary to compare paired samples of blood applied to the FP and blood serum taken from live animals. However, in our experiment, due to the high viral virulence, which leads to rapid death, the animals did not have time to develop antibodies. In blood samples taken 2 or 3 days before death (6–12 days after infection, according to the sampling schedule – once every 3 days), antibodies were not detected in serum. Therefore, the blood applied on the FP during autopsy was tested using SP-ELISA and compared with the results of immunoblotting, since this method is highly specific and highly sensitive.

When comparing the SP-ELISA results of sera obtained from domestic pigs (infected with ASFV isolates Antonovo 07/14 and Sobinka 07/15), and blood sampled on FP during scarification of the ear veins, sensitivity was 88.9% (with a 95% confidence interval – from 65.3 to 98.6%), specificity – 90.6% (79.3–96.9%).

The sensitivity of the method using FP circles cut out by the well diameter, placed directly into the well of a microplate with a buffer, was low when tested by this kit. Probably, this method can be recommended only for use in kits that allow high dilution of samples or using staining enhancers. The method without centrifugation showed a large number of false positive results. The method with homogenization and centrifugation of the sample showed the best sensitivity and specificity (Table 1).

When studying the results presented in Table 1, it can be seen that the use of FP leads to both false positive and false negative results. When comparing the data obtained by testing of FP blood samples using immunoblot analysis and SP-ELISA, it was found that one negative sample also showed a false positive result and SP-ELISA.

The results obtained in this experiment are consistent with the data published by T. Randriamparany et al. [31], who tested ASF FP blood samples. In the mentioned work, slight decrease in sensitivity was also observed. At the same time, the authors concluded that the method of blood sampling using FP is a suitable method for collecting and storing samples.

In the experiments conducted by J. Carlson et al., testing blood swab samples by commercial ELISA kit when compared to serum samples showed sensitivity of 93.1% (95% confidence interval, 83.3–98.1%), and 100% specificity (95.9–100.0%). The authors, therefore, concluded, that the swabs are suitable for sampling and subsequent testing for ASFV antibodies by ELISA. In addition, field tests of swabs during sampling from decomposed carcasses of wild boars in an endemic area of Estonia by PCR also showed high accuracy of the results [26].

[1] Metodicheskie rekomendatsii po vyyavleniyu antitel k virusu afrikanskoi chumy svinei immunoperoksidaznym metodom = Methodological recommendations for the detection of antibodies to the African swine fever virus by the immunoperoxidase method: approved by FGBI "ARRIAH" on 19.11.2020 No. 68-20. Vladimir: FGBI "ARRIAH"; 2019. 12 p. (in Russ.)

Table 1
Comparison of sensitivity and specificity of SP-ELISA and immunoblot analysis when testing sera and blood absorbed on filter paper

SP-ELISA				Immunoblotting		
Filter paper blood sample	Serum					
	Pos.	Neg.	Total	Pos.	Neg.	Total
Pos.	16	5	21	3	1	4
Neg.	2	48	50	0	4	4
Total	18	53	71	3	5	8

pos. – positive result;
neg. – negative result.

In the current conditions, when there is practically no testing for ASFV antibodies performed in wild boar samples, the use of methods that simplify the sampling procedure from wild animals, even taking into account some decrease in sensitivity, will enable to collect valuable information about the dynamics of ASF spread. Therefore, the possibility of detecting antibodies using a more sensitive immunoperoxidase technique (IPT) was tested.

To compare the methods of blood sampling, IPT and ELISA were used. The samples were represented by blood collected by ear vein scarification using swabs (Fig. 1), as well as serum taken from domestic pigs on the 10th and 31st days after infection with ASF virus (ASF isolate/ARRIAH/CV-1/60). The results of the tests are presented in Table 2.

Thus, the use of IPT for testing dried blood swab samples (Fig. 2) showed a 100% match with ELISA, while IPT used for testing serum even surpassed the sensitivity of ELISA. Therefore, blood sampling using swabs can be recommended for testing after appropriate validation. This method will be especially useful for collecting information about infected wild boars, since the lack of data makes it impossible to use effective eradication strategies.

Wild boar play an important role in the ASFV spread. Disease surveillance can be based both on testing of dead boar carcasses (passive surveillance) and on the detection of virus or antibodies in shot/caught wild boars (active surveillance). Wild boars can be a reservoir for the infection, regardless of the virus circulation in the population of domestic pigs. At the same time, passive surveillance provides a higher probability of early detection of ASF, especially during the first year in the early stages of the epizootics.

The use of serological testing methods has a low diagnostic value at the initial stages of the epizootics. Nevertheless, active surveillance aimed at the identification of seropositive wild boars may be preferable at later stages (in the endemic phase) of the epizootics [5].

Consequently, in order to increase the effectiveness of ASF epizootological surveillance in wild boar, especially at

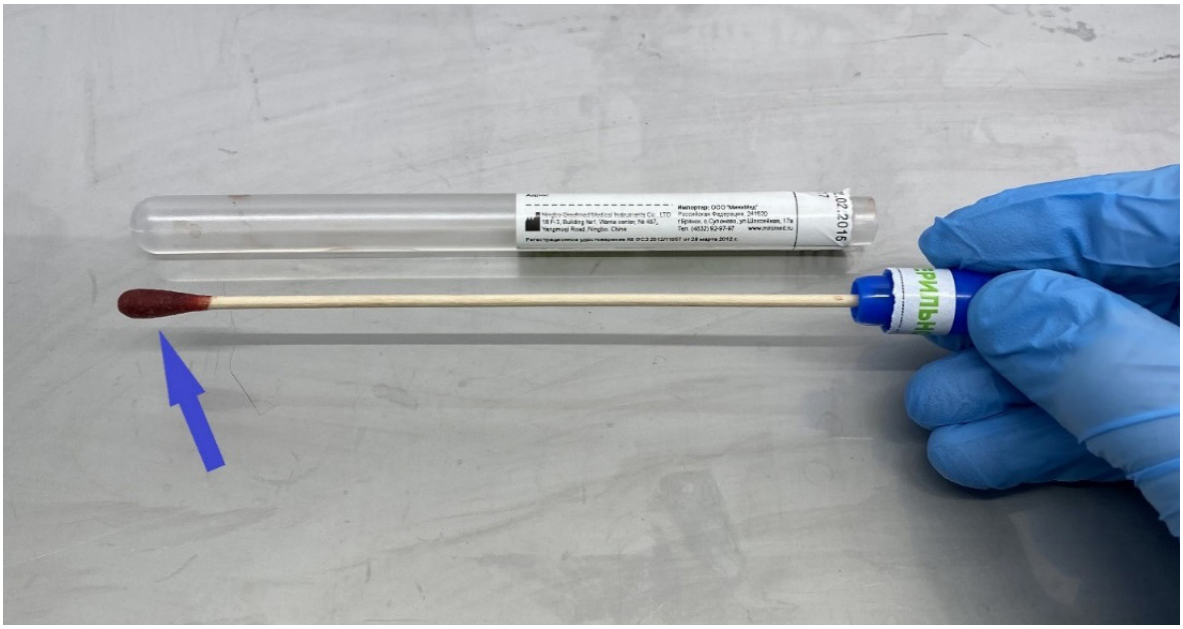

Fig. 1. Blood sample absorbed on the swab

Table 2
Comparison of results of serum and blood swab testing by ELISA and immunoperoxidase technique

Days after infection	ELISA (serum)	IPT (swabs)	IPT (serum)
10	+	1:20	1:320
10	+	1:5160	1:81920
10	–	–	1:20
10	+	1:640	1:640
31	+	1:20	1:320
31	+	1:1280	1:20480
31	+	1:1280	1:10240
31	+	1:80	1:5160
31	+	1:160	1:40960
31	+	1:160	1:81920

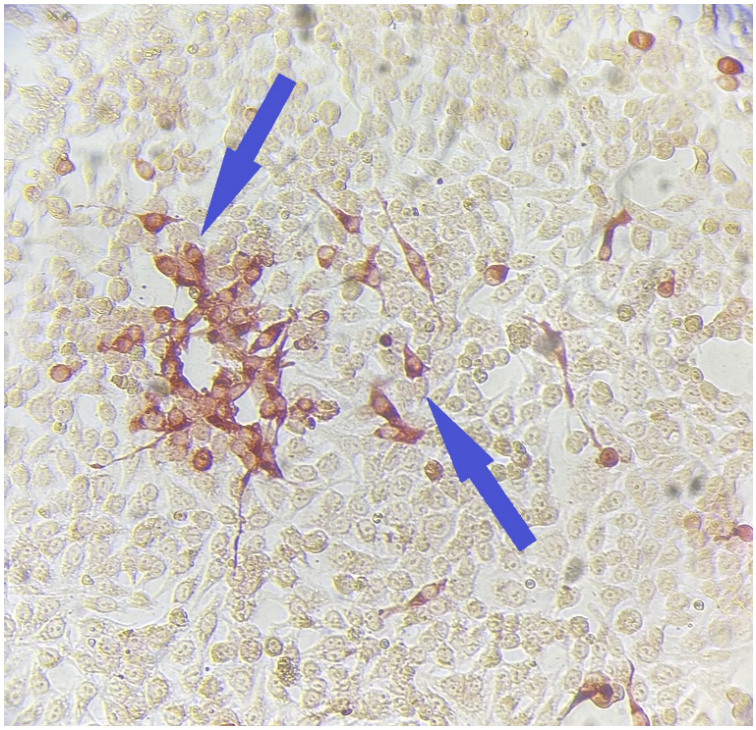

Fig. 2. Stained cytoplasm of infected cells when testing blood samples from swabs (magnification 400×)

the late stages of epizootic, serological tests are necessary, while the use of alternative sampling methods is justified.

CONCLUSION

The use of even the most highly accurate diagnostic methods may be limited by the quality of the delivered samples. And since it is required to detect not only the ASFV antigen or genetic material, but also antibodies to it, an alternative may be blood sampling using FP or swabs. Thus, it is possible to take samples from recently died and shot animals. In domestic pigs, blood samples can be collected by simple scarification of the ear vein without the use of test tubes, which reduces the risk of environmental contamination when they are destroyed or unintentionally opened, since the sample is not fluid. The use of FP as a sample carrier can reduce the amount of space required for long-term storage of the sample in a frozen state. The disadvantages of such sample preparation include a decrease in the sensitivity of the diagnostic methods used, however, the use of FP allows increasing the mass coverage of tests for the main purposes of the surveillance (early detection, prevalence determination,

and evidence of the population freedom). Therefore, the concept of using swabs and IPT is very promising, and in some cases there is no alternative, especially after appropriate validation and, possibly, the selection of swabs that provide more effective adsorption of samples, and can be recommended for the detection of ASFV antibodies in wild boar and domestic pigs. This approach combines the advantages of using FP with the high sensitivity and specificity of this diagnostic method.

The use of FP or swabs is most feasible when conducting mass screenings of target animal populations within the ASF surveillance (including during state monitoring). Blood samples can be collected using this method anywhere by minimally trained personnel, while the cold chain is not required to be maintained.

There are various special types of FP and swabs, therefore, it is necessary to select a material that ensures effective adsorption of the sample, optimal cost of collection, transportation and storage of samples. Before use, calibration will be required for the corresponding method, or a diagnostic kit.

REFERENCES

1. Gruzdev K. N., Igolkin A. S., Rakhmanov A. M., Shevtsov A. A. African swine fever in Russia: spread, clinical and anatomical manifestations. *Veterinary Science Today*. 2014; 4: 10–24. Available at: https://www.elibrary.ru/item.asp?id=22445544.

2. Remyga S. G., Pershin A. S., Shevchenko I. V., Igolkin A. S., Shevtsov A. A. Clinical and post-mortem signs in European wild boars and domestic pigs infected with African swine fever virus. *Veterinary Science Today*. 2016; (3): 46–51. Available at: https://veterinary.arriah.ru/jour/article/view/260. (in Russ.)

3. Shevchenko I. V., Remyga S. G., Pershin A. S., Igolkin A. S., Rakhmanov A. M. Kliniko-anatomicheskoe proyavlenie afrikanskoi chumy svinei pri zarazhenii raznymi metodami virusom, vydelennym ot dikogo kabana = Clinical and anatomical manifestations of African swine fever when challenged with the wild boar virus using different routes. *Sovremennye problemy patologicheskoi anatomii, patogeneza i diagnostiki boleznei zhivotnykh: materialy 18-i Mezhdunarodnoi nauchno-metodicheskoi konferentsii = Current challenges of animal disease pathological anatomy, pathogenesis and diagnostics: Proceedings of the 18th International Scientific and Practical Conference*. Moscow: Moscow SAVMB; 2014; 82–84. eLIBRARY ID: 22496854. (in Russ.)

4. Schoder M. E., Tignon M., Linden A., Vervaeke M., Cay A. B. Evaluation of seven commercial African swine fever virus detection kits and three Taq polymerases on 300 well-characterized field samples. *J. Virol. Methods*. 2020; 280:113874. DOI: 10.1016/j.jviromet.2020.113874.

5. Chenais E., Depner K., Guberti V., Dietze K., Viltrop A., Ståhl K. Epidemiological considerations on African swine fever in Europe 2014–2018. *Porcine Health Manag*. 2019; 5:6. DOI: 10.1186/s40813-018-0109-2.

6. Marcon A., Linden A., Satran P., Gervasi V., Licoppe A., Guberti V. R_0 estimation for the African swine fever epidemics in wild boar of Czech Republic and Belgium. *Vet. Sci*. 2019; 7 (1):2. DOI: 10.3390/vetsci7010002.

7. Gruzdev K. N., Zakutsky N. I., Diev V. I. African swine fever: current state and epizootological features and prospects of prevention and control (review). *Veterinarian*. 2017; 5: 3–8. Available at: http://vetvrach-vnivi.ru/attachments/5ac1ea7624c56.pdf. (in Russ.)

8. Iglesias I., Montes F., Jesús Muñoz M., Arias M., Gogin A., Kolbasov D., de la Torre A. Role of wild boars and domestic pigs in the spread of African swine fever in the Russian Federation (2007–2013). *8th Annual EPIZONE Meeting, 23–25 September 2014 Copenhagen, Denmark*. Available at: https://www.epizone-eu.net/en/Home/Sitemap/Annual-meeting-1/Abstract-book-and-poster-abstracts.htm.

9. Makarov V. V., Gusev A. A., Guseva E. V., Sukharev O. I., Kolomytsev A. A. Prirodnaya ochagovost' afrikanskoi chumy svinei = Natural nidality of African swine disease. *Veterinarnaya patologiya*. 2011; 3: 9–18. Available at: https://fsvps.gov.ru/fsvps-docs/ru/news/asf/asf_makarov/public2.pdf. (in Russ.)

10. Fertikov V. I., Egorov A. N., Tikhonov A. N., Kolbasov D. V., Khripunov E. M., Kurinnov V. V., et al. Classical and African swine fever: about strategy for its prophylaxy on hunting territories in Russia. *Agricultural Biology*. 2010; 4: 7–12. Available at: https://www.agriscience.ru/pdf/0131-6397/2010/4/7-12.pdf. (in Russ.)

11. Pershin A. S., Shevchenko I. V., Igolkin A. S., Zhukov I. Yu., Vlasova N. N., Manuylova O. A. Dynamics of antibodies production after experimental challenge with ASF virus. *Veterinaria Kubani*. . 2019; 4: 3–7. DOI: 10.33861/2071-8020-2019-4-3-7. (in Russ.)

12. Nurmoja I., Petrov A., Breidenstein C., Zani L., Forth J. H., Beer M., et al. Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. *Transbound. Emerg. Dis*. 2017; 64 (6): 2034–2041. DOI: 10.1111/tbed.12614.

13. Pershin A., Shevchenko I., Igolkin A., Zhukov I., Mazloum A., Aronova E., et al. Long-term study of the biological properties of ASF virus isolates originating from various regions of the Russian Federation in 2013–2018. *Vet. Sci*. 2019; 6 (4):99. DOI: 10.3390/vetsci6040099.

14. Cerbule A. R. Prevalence and age structure of African swine fever survivor animals in the wild boar population during the epidemic in Estonia: Final Thesis Curriculum in Veterinary Medicine. Tartu; 2020. Available at: http://hdl.handle.net/10492/5775.

15. Mur L., Igolkin A., Varentsova A., Pershin A., Remyga S., Shevchenko I., et al. Detection of African swine fever antibodies in experimental and field samples from the Russian Federation: implications for control. *Transbound. Emerg. Dis*. 2016; 63 (5):e436–440. DOI: 10.1111/tbed.12304.

16. Rodríguez-Prieto V., Vicente-Rubiano M., Sánchez-Matamoros A., Rubio-Guerri C., Melero M., Martínez-López B., et al. Systematic review of surveillance systems and methods for early detection of exotic, new and re-emerging diseases in animal populations. *Epidemiol. Infect*. 2015; 143 (10): 2018–2042. DOI: 10.1017/S095026881400212X.

17. Tsibezov V. V., Terekhova Yu. O., Verkhovsky O. A., Pershin A. S., Vlasova N. N., Igolkin A. S., et al. Efficacy of the immunochromatographic method for the rapid detection of African swine fever virus. *Veterinariya*. 2015; 5: 58–64. eLIBRARY ID: 23527048. (in Russ.)

18. Novikov A. A., Isaev D. V., Pavlov P. M., Dezhkin A. V., Panteleeva O. A. Approbation of express-methods for detection of pathogens of hunting resources in the field (ASF).

Sostoyanie sredy obitaniya i fauna okhotnich'ikh zhivotnykh Rossii i sopredel'nykh territorii: materialy II Mezhdunarodnoi, VII Vserossiiskoi nauchno-prakticheskoi konferentsii (10–11 marta 2016 g.) = Conditions of game habitat and fauna in Russia and neighboring regions: Proceedings of II International, VII All-Russian Scientific and Practical Conference (March 10–11, 2016). Balashikha; 2016; 381–386. (in Russ.)

19. Sereda A. D., Dubrovskaya O. A., Imatdinov A. R., Strizhakova O. M., Vasil'ev A. P., Sindryakova I. P., Lunitsin A. V. Laboratory diagnostics of chronic and asymptomatic forms of African swine fever. *Agricultural Biology.* 2016; 51 (4): 459–466. DOI: 10.15389/agrobiology.2016.4.459eng.

20. Kurinnov V. V., Belyanin S. A., Vasllyev A. P., Strizhakova O. M., Lyska V. M., Nogina I. V., et al. Experimental and field researches of specific antibodies in tissues of the house pigs infected with virus ASF and wild boars with a sharp clinical course. *Veterinaria Kubani.* 2012; 4: 9–11. Available at: http://vetkuban.com/num4_20124.html. (in Russ.)

21. Pendergraph G. E., Pendergraph C. B. Handbook of Phlebotomy and Patient Service Techniques. 4th ed. Baltimore: Williams&Wilkins; 1998; 245.

22. Vlasov G. S., Ponomareva A. M., Polyntsev D. G., Golovachenko V. A., Fadin D. V. Sistemy zabora krovi i interferentsiya v immunoanalize = Blood sampling tools and interference during the immunoassay. *Klinicheskaya laboratornaya diagnostika.* 2007; 4: 24–29. (in Russ.)

23. Vergne T., Guinat C., Petkova P., Gogin A., Kolbasov D., Blome S., et al. Attitudes and beliefs of pig farmers and wild boar hunters towards reporting of African swine fever in Bulgaria, Germany and the Western Part of the Russian Federation. *Transbound. Emerg. Dis*. 2016; 63 (2):e194–204. DOI: 10.1111/tbed.12254.

24. Blome S., Goller K. V., Petrov A., Dräger C., Pietschmann J., Beer M. Alternative sampling strategies for passive classical and African swine fever surveillance in wild boar – extension towards African swine fever virus antibody detection. *Vet. Microbiol*. 2014; 174 (3–4): 607–608. DOI: 10.1016/j.vetmic.2014.09.018.

25. Pomelova V. G., Bychenkova T. A., Bekman N. I., Kozlova E. G., Vorobyeva N. N., Frizen V. I. Testing for antibodies to tick-borne encephalitis virus using blood dried on filter paper. *Journal of Microbiology, Epidemiology and Immunobiology.* 2008; 1: 50–55. eLIBRARY ID: 9943075. (in Russ.)

26. Carlson J., Zani L., Schwaiger T., Nurmoja I., Viltrop A., Vilem A., et al. Simplifying sampling for African swine fever surveillance: Assessment of antibody and pathogen detection from blood swabs. *Transbound. Emerg. Dis.* 2018; 65 (1):e165–e172. DOI: 10.1111/tbed.12706.

27. Bang I. Mikromethoden zur Blutuntersuchung. *In: Mikromethoden zur Blutuntersuchung. Ed. by G. Blix.* Munich: J. F. Bergmann-Verlag; 1922; 9–54. DOI: 10.1007/978-3-662-34421-7_1.

28. Curry P. S., Ribble C., Sears W. C., Orsel K., Hutchins W., Godson D., et al. Blood collected on filter paper for wildlife serology: evaluating storage and temperature challenges of field collections. *J. Wildl. Dis*. 2014; 50 (2): 308–321. DOI: 10.7589/2012-06-150.

29. Duncombe L., Commander N. J., Erdenlig S., McGiven J. A., Stack J. Investigating the use of protein saver cards for storage and subsequent detection of bovine anti-*Brucella abortus* smooth lipopolysaccharide antibodies and gamma interferon. *Clin. Vaccine Immunol*. 2013; 20 (11): 1669–1674. DOI: 10.1128/CVI.00033-13.

30. Wasniewski M., Barrat J., Combes B., Guiot A. L., Cliquet F. Use of filter paper blood samples for rabies antibody detection in foxes and raccoon dogs. *J. Virol. Methods*. 2014; 204: 11–16. DOI: 10.1016/j.jviromet.2014.04.005.

31. Randriamparany T., Kouakou K. V., Michaud V., Fernández-Pinero J., Gallardo C., Le Potier M. F., et al. African swine fever diagnosis adapted to tropical conditions by the use of dried-blood filter papers. *Transbound. Emerg. Dis*. 2016; 63 (4): 379–388. DOI: 10.1111/tbed.12295.

32. Katorkin S. A., Gazaev I. Kh., Kudryashov D. A., Aronova E. V., Burdinskaya O. N., Lunitsin A. V., et al. Ispol'zovanie «sukhikh pyaten krovi» dlya vyyavleniya DNK virusa afrikanskoi chumy svinei s pomoshch'yu polimeraznoi tsepnoi reaktsii v rezhime real'nogo vremeni = Use of "dry blood dots" for ASFV DNA detection using real-time polymerase reaction. *Molekulyarnaya diagnostika* – 2014: *materialy VIII Vserossiiskoi nauchno-prakticheskoi konferentsii s mezhdunarodnym uchastiem (18–20 marta 2014 g.) = Molecular Diagnostics – 2014: Proceedings of VIII All-Russian Scientific and Practical Conference with international participation (March 18–20, 2014).* Vol. 2. Moscow: MVA; 452–453. eLIBRARY ID: 21410394. (in Russ.)

33. Arya S. C. Stability of human immunodeficiency virus type 1 antibodies in whole blood-impregnated filter papers under various tropical conditions. *J. Clin. Microbiol*. 1993; 31 (3): 765–766. DOI: 10.1128/jcm.31.3.765-766.1993.

34. World Health Organization. Monitoring vaccine wastage at country level: guidelines for programme managers. WHO; 2005. Available at: https://apps.who.int/iris/handle/10665/68463.

35. CEIV Farma. How to become CEIV Pharma Certified. Available at:https://www.iata.org/contentassets/494bc14afd934b0193735e9a47091d72/iata_ceiv-pharma_how20to20become20ceiv20pharma20certified.pdf.

36. GAVI Alliance. Report to the Programme and Policy Committee: GAVI's supply chain strategy framework, 9–10 October 2013.

Received 13.05.2021
Revised 02.07.2021
Accepted 05.08.2021

INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Andrey S. Pershin, Candidate of Science (Veterinary Medicine), Senior Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Andrey R. Shotin, Post-Graduate Student, Leading Biologist, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

Першин Андрей Сергеевич, кандидат ветеринарных наук, старший научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

Шотин Андрей Романович, аспирант, ведущий биолог референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

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

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

Olga A. Manuylova, Biologist, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

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

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

Natalia N. Vlasova, Doctor of Science (Biology), Chief Researcher, Reference Laboratory for African Swine Fever, FGBI "ARRIAH", Vladimir, Russia.

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

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

Мануйлова Ольга Анатольевна, биолог референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.

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

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

Власова Наталья Никифоровна, доктор биологических наук, главный научный сотрудник референтной лаборатории по африканской чуме свиней ФГБУ «ВНИИЗЖ», г. Владимир, Россия.