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African swine fever clinical scoring system

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ABSTRACT
African swine fever (ASF) is a hemorrhagic viral disease that brings serious implications for animal health and economy due to high mortality rate, quarantine measures and restrictions on international trade in pig products. Only domestic and wild species of the *Suidae* family of all breeds and ages are susceptible to infection with ASF virus. To date, no safe and effective ASF vaccines have been developed, but in recent years some progress has been made in development of ASF modified live virus first-generation vaccine candidates, which have been used only in some countries of Southeast Asia. The expansion of their use is hindered, among other things, due to the lack of international and state recommendations (requirements) for the evaluation of purity, activity, safety and effectiveness of ASF vaccine candidates. Clinical signs of the disease are one of the main indicators of safety and effectiveness of ASF modified live virus vaccine candidates. The purpose of this work was to develop a clinical symptom-based scoring system to be used for characterizing of newly recovered ASFV isolates causing various forms of the disease, as well as for the determination of safety and effectiveness of ASF modified live virus vaccine candidates. It is proposed to take into account 7 major clinical manifestations: an increase in body temperature, reduced liveliness, loss of appetite, skin lesions, joint swelling, laboured breathing, neurological disorders, each scored from 0 to 3 or 4. The study of twelve ASFV strains of various virulence revealed that acute and subacute ASF produce the maximum clinical scores ranged from 13 to 22, chronic form gives 6–18 points, subclinical form is scored 0–8.

Keywords: African swine fever, clinical signs, vaccine candidates

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Балльная система оценки клинических признаков при африканской чуме свиней

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РЕЗЮМЕ
Африканская чума свиней (АЧС) – это геморрагическая вирусная болезнь, которая вызывает серьезные санитарные и экономические последствия из-за высокого уровня смертности животных, карантинных мероприятий и ограничений международной торговли продукцией свиноводства. Вирус АЧС поражает исключительно домашних и диких свиней семейства *Suidae* всех пород и возрастных групп. До настоящего времени безопасные и эффективные средства специфической защиты против АЧС не разработаны, но в последние годы достигнут определенный прогресс в исследованиях по разработке вакцин первого поколения на основе модифицированного живого вируса, которые ограниченно использовали в некоторых странах Юго-Восточной Азии. Расширение их применения сдерживается в том числе из-за отсутствия международных и государственных рекомендаций (требований) по оценке чистоты, активности, безопасности и эффективности кандидатных вакцин против АЧС. Клинические признаки болезни являются одним из основных показателей безопасности и эффективности кандидатных вакцин против АЧС на основе модифицированного живого вируса. Целью данного исследования являлась разработка системы балльной оценки клинических признаков, пригодной для использования при характеристике вновь выделенных изолятов вируса АЧС, вызывающих различные формы течения болезни, а также при определении безопасности и эффективности кандидатных вакцин, изготовленных на основе модифицированного живого вируса. Предложено учитывать 7 преобладающих клинических признаков: повышение температуры тела, снижение активности, снижение аппетита, поражение кожных покровов, поражение суставов, нарушение дыхания, поражение центральной нервной системы, – каждый из которых оценивается от 0 до 3 или 4 баллов. В результате исследования двенадцати штаммов вируса АЧС различной вирулентности установлено, что при острой и подострой формах АЧС максимальные суммы баллов клинических признаков составляли от 13 до 22, при хронической форме – от 6 до 18, при субклинической – от 0 до 8.

Ключевые слова: африканская чума свиней, клинические признаки, кандидатные вакцины

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INTRODUCTION

African swine fever (ASF) is an infectious disease of domestic and wild pigs of all breeds and ages caused by a virus of *Asfivirus* genus, *Asfarviridae* family. Since its introduction in 2007 to Georgia ASF outbreaks have been reported in more than 50 countries of Europe, Asia, Africa and the Caribbean Basin region [1, 2, 3].

As regards the degree of virulence, ASFV strains can be divided into high, moderate, low virulence or non-virulence. Depending on the strain /isolate virulence the clinical course of ASF can be hyperacute, acute (highly virulent isolates/strains), subacute (highly and moderately virulent strains), chronic (moderate and low virulent strains), subclinical and asymptomatic (non-virulent strains) [4]. Superacute ASF kills pigs on days 3–5 post infection, either without clinical manifestations, or with short-term hyperthermia (> 41.0 °C), without loss of activity and appetite. Acute ASF is manifested by an early increase in body temperature (> 41.0 °C), loss of appetite, dullness (animals are recumbent most of time), rapid breathing, cyanosis of ear, underbelly, hind limbs and perineum skin, laboured breathing, leg paresis and paralysis, sometimes constipation or bloody diarrhea may be present. Death occurs on days 6–14 post infection. Hyperacute and acute forms of ASF result in 100% mortality rate. Pigs infected with subacute forms of ASF demonstrate clinical signs similar to the acute form, but they are less pronounced. Recurrent hyperthermia, depression, loss of appetite are reported, joint swelling and severe respiratory disorders occur at later stages of the disease. Most animals (about 70%) die within 15–30 days post infection [3, 5, 6]. Chronic form lasts for more than 30 days with periodic relapses. Animals demonstrate intermittent hyperthermia, exhaustion, stunting, arthritis of varying severity, respiratory disorders, and necrotic skin ulcers. The mortality rate is about 30% [7, 8]. Subclinical and asymptomatic forms of ASF are observed in wild indigenous pigs (warthogs, giant forest hogs, bushpig) in African endemic countries, in wild boars in Sardinia, as well as in domestic pigs and wild boars experimentally infected with some ASFV attenuated strains [9, 10, 11].

In recent years, some progress has been made in the research and development of ASF modified live virus (MLV) first-generation vaccine candidates, which are used in some Southeast Asian countries (Vietnam, Philippines) [12]. A serious obstacle to the possible use of already developed and future ASF MLV vaccine candidates is the lack of internationally and nationally agreed parameters (requirements) for the evaluation of their purity, activity, safety and efficacy. In the world's leading laboratories, various methods are used to evaluate the safety and efficacy of the vaccine candidates, which hinders the scientific community from side-by-side comparison and assessment of the results obtained, and government agencies and institutions authorized to issue permits for their use do not have such grounds.

Currently, the Biological Standards Commission of the World Organization for Animal Health is approving the updated Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, which includes harmonized standards and recommendations for ASF MLV vaccine candidates. Similar work is being done in the Russian Federation.

The main parameters of the safety and efficacy of ASF MLV vaccine candidates are: clinical signs, viremia, survival rate after immunization and virulent 'homologous' challenge [13]. To assess the clinical signs in pigs infected with ASF virus of various pathogenicity, a number of authors proposed using clinical scoring systems that cover thirteen signs (Table 1). The least number of clinical signs (4) was covered by the scoring system proposed by E. B. Howey et al. [14]; the most extensive scoring system (11) was created by A. S. Olesen et al. [15], which totaled 12 and 42 points, respectively.

The purpose of this work was to develop a clinical symptom-based scoring system to be used for characterizing of newly recovered ASFV isolates causing various forms of the disease, as well as for the determination of safety and efficacy of ASF MLV vaccine candidates.

MATERIALS AND METHODS

The results of our own experiments conducted with ASF virus strains of various virulence were used in the study:

– highly virulent strains: Stavropol 01/08, Vladimir-Vyazniki/2017, Bryansk-21 (VIII serotype, II genotype), Lisbon-57 (I, I), Mozambique-78 (III, V), France-32 (IV, I);

– moderately virulent: Novgorod-2019 (VIII, II), PSA-1-NH (IV immunotype, I);

– non-virulent (attenuated): Katanga-350 (I, I), MK-200 (III, V), FC-32/135 (IV, I), Stavropol 71/2017 (VIII, II) [22, 23, 24, 25, 26, 27, 28, 29].

Twenty-five to thirty kilograms large white pigs were received from the Unit of experimental animal

preparation of the Federal Research Center for Virology and Microbiology. Experiments on pigs were performed in accordance with the "Guidelines for keeping and use of laboratory animals" [30]. The observed ASF clinical signs were recorded daily. Body temperature was measured rectally using mercury thermometers, other signs were determined visually.

A detailed description of porcine leukocyte (PL) cell culture preparation and determination of ASFV infectivity were performed as described earlier [22, 31]. The results were evaluated by hemadsorption or cytopathogenic effect during 7 days. Virus titers were calculated using Kärber method, modified by I. P. Ashmarin, and expressed as 50% hemadsorbing units (HAE_{50}/cm^3) or tissue culture infectious doses ($TCID_{50}/cm^3$) [32].

RESULTS AND DISCUSSION

Based on our tests using 144 animals and the published criteria for evaluation of ASF clinical signs, seven major clinical signs that were observed in infected pigs were selected: increased temperature, dullness, loss of appetite, skin lesions, joint involvement, respiratory disorders, neurological disorders. Each symptom, depending on the severity, was scored 3 or 4 points (Table 2). Such ASF clinical signs as eye discharges (conjunctivitis), diarrhea, bloody feces, bloody urine, vomiting, were not demonstrated by most infected animals, and they were not included in the scoring system. The system is based on the following principles: a) inclusion of the most characteristic clinical signs; b) increasing score representing a greater severity of clinical manifestation; c) the characteristic of a specific score does not exclude the simultaneous presence of those rated with lower scores.

Hyperthermia, loss of activity and appetite were observed in all pigs with acute, subacute and chronic forms of ASF, and redness and cyanotic skin were noted in 80–95% of the animals. These signs were scored from 0 to 4 points (Table 2). Less often (60–70%) the above mentioned forms of ASF induced incoordination and breathing problems. Joint involvement was observed in 35–50% of pigs with subacute and chronic disease. These three signs were scored from 0 to 3 points. Constipation, diarrhea, and bloody feces were reported only in some animals with acute and sub-acute ASF.

The developed ASF clinical scoring system was evaluated by the experiments on pigs infected with 12 strains of various virulence (Table 3) [22, 23, 24, 25, 26, 27, 28, 29].

It should be noted that the minimum and maximum total scores given in Table 3 are not the sum of the minimum and maximum points for each of the seven clinical

Table 1
Published ASF clinical sign scoring systems

Clinical signs	Corresponding clinical scores							
	[14]	[15]	[16]	[17]	[18]	[19]	[20]	[21]
Temperature	0–4	0–5	–*	0–5	1–3	–	0–5	–
Appetite/anorexia	–	0–6	0–3	1–6	1–3	1–3	1–6	0–3
Behaviour/liveliness	0–4	0–6	0–3	1–6	1–3	0–3	1–6	0–3
Skin lesions, cyanosis	–	0–3	0–3	0–3	1–3	1–3	1–3	0–3
Joint swelling	–	0–4	0–3	1–4	–	–	1–4	0–3
Respiration	–	0–3	0–3	1–3	1–3	1–3	1–3	0–3
Ocular discharges	–	0–2	0–3	1–2	–	1–3	1	0–3
Defecation	0–2	0–4	0–3	1–3	1–3	1–3	1–4	0–3
Urination	–		–	4	–	–	4	–
Vomiting	–	–	–	1–3	–	1–3	4	–
Neurology	0–2	–	–	0–6	–	1–3	–	0–3
Posture	–	0–6	–	–	–	1–3	–	0–3
Body condition	–	–	–	–	1–3	1–3	–	
Maximum score	12	42	21	42	18	30	40	27

*(–) – the parameter was not taken into account;
clinical signs in the colored cells are considered to be one parameter.

Table 2
African swine fever clinical sign scoring

Clinical signs	Points				
	0	1	2	3	4
Increase in body temperature, °C	38.0–40.0; no	40.1–40.5	40.6–41.0	41.1–41.5	41.6–42.0
Decreased activity	no	slight dullness	recumbent, if touched rises quickly	after a few touches rises with difficulty	recumbent, can't rise
Loss of appetite (feed consumption rate, %)	no	about 10% of the feed remains in the feeder	about 50% of the feed remains in the feeder	comes up to the feeder, but does not eat	loss of interest in food (complete refusal)
Skin lesions	no	erythema, redness the ear tip skin	cyanosis of the ear tips and tail	limited cyanosis in various parts of the body with sporadic necrotic lesions	extensive cyanosis of the skin, multiple necrotic skin lesions
Joint involvement	no	joint swelling	joint swelling and slight lameness	joint swelling and evident painful lameness	–
Respiratory disorders	no	Shortness of breath	laboured breathing, nasal discharge	painful breathing, wheezing, coughing	–
Neurology	no	unsteady walk	paresis of one or more limbs	convulsions, paralysis, muscle tremor	–

signs considered; they correspond to individual scores of the test animals in each of these groups.

Highly virulent strains (Stavropol 01/08, Vladimir-Vyazniki/2017, Lisbon-57, Mozambique-78, France-32, Bryansk-21) caused acute and less often subacute disease regardless of the infection routes and doses used. Acute ASF produced hyperthermia (41.0–42.0) °C, partial or complete loss of activity and appetite, redness or cyanosis of the ear, abdominal wall, tail and perineum, severe painful breathing with nasal discharge, leg paresis and paralysis, convulsions were recorded. Bloody diarrhea was observed in only a few animals. Before death, on days 6–14 post infection, the body temperature in pigs decreased to 38.0 °C. The subacute ASF produced the following clinical signs: hyperthermia, loss of activity and appetite, minor redness or cyanosis (tips of the ears, tail). Acute and subacute ASF, regardless of the infection routes or doses, gave the clinical scores in different individuals ranging from 13 to 22.

Chronic ASF was observed in pigs infected intramuscularly with Novgorod-2019 or PSA-1-NH strains. The disease was characterized by intermittent hyperthermia (40.5–41.5) °C, loss of activity and appetite. In 10–14 days, the animals demonstrated cachexia, stunting, different respiratory disorders and joint problems (arthritis). Some animals demonstrated spotted skin redness, which subsequently could become cyanotic and sporadically necrotic (PSA-1-NH strain). The duration of the disease could be different and lasted for more than 30 days. "Clinical recovery" was recorded in some animals with subsequent recurrence, manifested by an in increase in body temperature. The ASF clinical scores in such animals ranged from 6 to 18.

Less pronounced signs of chronic ASF were recorded in 40–60% of pigs after intranasal inoculation of PSA-1-NH strain, as well as in 7% of pigs inoculated with attenuated Katanga-350 strain. Recurrent hyperthermia was observed (from 40.1 to 40.7 °C), a slight decrease in activity (got up quickly when touched) and appetite (50–90% of feed consumed) during 1–4 days, in some cases shortness of breath and slight joint swelling were observed. The maximum ASF clinical scores in these animals reached 6–10.

Most pigs infected intramuscularly with attenuated Katanga-350 strain, attenuated but not protective Stavropol 71/2017 strain, as well as intranasally with a low dose (10^3 $TCID_{50}$) of naturally attenuated PSA-1-NH strain demonstrated subclinical ASF: body temperature in 40–60% of animals not exceeded 40.5 °C during 1–4 days, 90–100% of the feed was consumed. No other changes were recorded. The total clinical score ranged from 0 to 5.

The asymptomatic ASF was recorded in 100% of pigs inoculated intramuscularly with strain FK-32/135, in 75–90% of pigs inoculated with strain MK-200, in 40–60% with strain Katanga-350. ASF clinical signs in such animals were not recorded, the total scores were 0.

It should be noted that the ASF clinical scoring system is not applicable for isolates causing peracute disease, since the death of pigs occurs 3–5 days after infection without most clinical signs manifested due to intramuscular administration of virulent virus isolates in large doses.

CONCLUSION

Over the past 5–7 years, circulation of ASF genotype II virus isolates of various virulence, from highly virulent

Table 3
Clinical sign scores of pigs infected with various ASFV strains

Name of the strain	Infection route	Dose*	Survivors/total	Clinical signs and their scores							Total score
				Increased temperature	Decreased activity	Loss of appetite	Skin lesions	Joint involvement	Respiratory disorders	Neurology	
Stavropol 01/08	i/m	$10^{2–3}$	0/15	4	4	4	3–4	0	2–3	2–3	19–22
	nas.	$10^{2–3}$	0/4	4	3–4	4	3–4	0	2–3	1–3	17–22
	oral	10^{7}	0/3	3–4	3	3–4	2–3	0	2	2	15–18
Vladimir-Vyazniki/2017	i/m	$10^{2–3}$	0/6	3–4	3–4	3	2–3	0	1–2	1–3	13–19
	nas.	$10^{2–3}$	0/6	3–4	3–4	3–4	2–3	0	1–2	1–3	13–20
	oral	$10^{4–7}$	0/6	3	3–4	3–4	1–2	0	1–3	1–2	12–18
	cont.	–	0/4	3–4	3	3–4	1–3	0	1–2	1–2	14–17
Lisbon-57	i/m	10^{3}	0/5	4	4	3–4	3–4	0	2–3	2–3	18–22
Mozambique-78	i/m	10^{3}	0/5	4	4	4	2–4	0	2–3	2–3	18–22
France-32	i/m	10^{3}	0/5	3–4	3–4	3–4	2–3	0	1–2	1–3	13–17
Bryansk-21	i/m	10^{3}	0/4	3–4	3	3	2–3	0	1–2	1–2	13–15
Novgorod-2019	i/m	10^{3}	1/5	3	3	3–4	1–3	0	1–2	1–3	13–18
				2–3	2	1–2	0	1–2	1–2	0	7–11
PSA-1-NH	i/m	10^{3}	4/5	1–4	1–4	1–4	0	0	1–3	0	1–15
	i/m	10^{5}	7/15	2–4	3–4	2–4	1–4	1–3	1–3	1–2	12–18
	nas.	10^{3}	5/5	1–3	1–2	1–2	0	0	1–2	0	1–8
	nas.	10^{5}	4/5	3–4	2–3	2–3	1	1–2	2–3	1–2	8–15
Katanga-350	i/m	10^{6}	15/15	0–2	0–2	0–2	0	1	1	0	0–8
MK-200	i/m	10^{6}	16/16	0–3	0–2	0–2	0	0	1	0	0–8
FK-32/135	i/m	$10^{6–7}$	16/16	0	0	0	0	0	0	0	0
Stavropol 71/2017	i/m	10^{5}	9/9	1	0–1	0–1	0	0	0	0	1–3

i/m – intramuscular, nas. – nasal, cont. – contact;
* $TCID_{50}$ for PSA-1-NH strain, HAU_{50} for other strains.

to naturally attenuated, have been evidenced in Europe and Asia [2, 3, 6, 9, 11, 33].

According to our observations, the dynamics of ASF clinical signs varies depending on the virulence of the isolate/strain, to a lesser extent on the dose and method of the virus inoculation [26, 27, 28]. The developed and tested ASF clinical scoring system is suitable both for the characterization of newly recovered isolates and for the evaluation of MLVs and MLV vaccine candidates. Based on our experience, for candidate strains, the total clinical score should not exceed 4–5 points. Ideally, when the score is 0, as for example after vaccination of pigs with ASFV FK-32/135 strain [26].

It should be noted that for the evaluation of MLV vaccine safety, in addition to clinical signs, it is proposed to study the levels and duration of viremia, virus shedding, post-mortem changes, persistence of the vaccine and virulent (infecting) viruses in tissues, potential transmission and reversion to virulence, safety in the field and some others [13]. However, if, after the inoculation of a candidate vaccine strain to pigs, the total clinical score exceeds 5, it is not reasonable to conduct other studies.

REFERENCES

1. Shotin A. R., Igolkin A. S., Mazloum A., Shevchenko I. V., Aronova E. V., Gruzdev K. N. Biological properties of African swine fever virus ASFV/Kaliningrad 17/WB-13869. *Agricultural Biology*. 2023; 58 (4): 773–783. https://doi.org/10.15389/agrobiology.2023.4.773eng

2. Schambow R., Reyes R., Morales J., Diaz A., Perez A. M. A qualitative assessment of alternative eradication strategies for African swine fever in the Dominican Republic. *Frontiers in Veterinary Science*. 2022; 9:1054271. https://doi.org/10.3389/fvets.2022.1054271

3. Gallardo C., Nurmoja I., Soler A., Delicado V., Simón A., Martin E., et al. Evolution in Europe of African swine fever genotype II viruses from highly to moderately virulent. *Veterinary Microbiology*. 2018; 219: 70–79. https://doi.org/10.1016/j.vetmic.2018.04.001

4. Mebus C. A., Dardiri A. H. Western hemisphere isolates of African swine fever virus: asymptomatic carriers and resistance to challenge inoculation. *American Journal of Veterinary Research*. 1980; 41 (11): 1867–1869. https://pubmed.ncbi.nlm.nih.gov/7212418

5. 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. https://elibrary.ru/wwrlmf (in Russ.)

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

7. Boinas F. S., Hutchings G. H., Dixon L. K., Wilkinson P. J. Characterization of pathogenic and non-pathogenic African swine fever virus isolates from *Ornithodoros erraticus* inhabiting pig premises in Portugal. *Journal of General Virology*. 2004; 85 (8): 2177–2187. https://doi.org/10.1099/vir.0.80058-0

8. Sun E., Huang L., Zhang X., Zhang J., Shen D., Zhang Z., et al. Genotype I African swine fever viruses emerged in domestic pigs in China and caused chronic infection. *Emerging Microbes & Infections*. 2021; 10 (1): 2183–2193. https://doi.org/10.1080/22221751.2021.1999779

9. Gallardo C., Soler A., Rodze I., Nieto R., Cano-Gómez C., Fernandez-Pinero J., Arias M. Attenuated and non-haemadsorbing (non-HAD) genotype II African swine fever virus (ASFV) isolated in Europe, Latvia 2017. *Transboundary and Emerging Diseases*. 2019; 66 (3): 1399–1404. https://doi.org/10.1111/tbed.13132

10. Havas K. A., Gogin A. E., Basalaeva J. V., Sindryakova I. P., Kolbasova O. L., Titov I. A., et al. An assessment of diagnostic assays and sample types in the detection of an attenuated genotype 5 African swine fever virus in European pigs over a 3-month period. *Pathogens*. 2022; 11 (4):404. https://doi.org/10.3390/pathogens11040404

11. Zani L., Forth J. H., Forth L., Nurmoja I., Leidenberger S., Henke J., et al. Deletion at the 5′-end of Estonian ASFV strains associated with an attenuated phenotype. *Scientific Reports*. 2018; 8:6510. https://doi.org/10.1038/s41598-018-24740-1

12. Borca M. V., Ramirez-Medina E., Silva E., Vuono E., Rai A., Pruitt S., et al. ASFV-G-ΔI177L as an effective oral nasal vaccine against the Eurasia strain of Africa swine fever. *Viruses*. 2021; 13 (5):765. https://doi.org/10.3390/v13050765

13. Brake D. A. African swine fever modified live vaccine candidates: transitioning from discovery to product development through harmonized standards and guidelines. *Viruses*. 2022; 14 (12):2619. https://doi.org/10.3390/v14122619

14. Howey E. B., O'Donnell V., de Carvalho Ferreira H. C., Borca M. V., Arzt J. Pathogenesis of highly virulent African swine fever virus in domestic pigs exposed via intraoropharyngeal, intranasopharyngeal, and intramuscular inoculation, and by direct contact with infected pigs. *Virus Research*. 2013; 178 (2): 328–339. https://doi.org/10.1016/j.virusres.2013.09.024

15. Olesen A. S., Lohse L., Boklund A., Halasa T., Gallardo C., Pejsak Z., et al. Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes. *Veterinary Microbiology*. 2017; 211: 92–102. https://doi.org/10.1016/j.vetmic.2017.10.004

16. Gallardo C., Soler A., Nieto R., Sánchez M. A., Martins C., Pelayo V., et al. Experimental transmission of African swine fever (ASF) low virulent isolate NH/P68 by surviving pigs. *Transboundary and Emerging Diseases*. 2015; 62 (6): 612–622. https://doi.org/10.1111/tbed.12431

17. Pietschmann J., Guinat C., Beer M., Pronin V., Tauscher K., Petrov A., et al. Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. *Archives of Virology*. 2015; 160 (7): 1657–1667. https://doi.org/10.1007/s00705-015-2430-2

18. Galindo-Cardiel I., Ballester M., Solanes D., Nofrarías M., López-Soria S., Argilaguet J. M., et al. Standardization of pathological investigations in the framework of experimental ASFV infections. *Virus Research*. 2013; 173 (1): 180–190. https://doi.org/10.1016/j.virusres.2012.12.018

19. De Carvalho Ferreira H. C., Weesendorp E., Elbers A. R. W., Bouma A., Quak S., Stegeman J. A., Loeffen W. L. African swine fever virus excretion patterns in persistently infected animals: a quantitative approach. *Veterinary Microbiology*. 2012; 160 (3–4): 327–340. https://doi.org/10.1016/j.vetmic.2012.06.025

20. King K., Chapman D., Argilaguet J. M., Fishbourne E., Hutet E., Cariolet R., et al. Protection of European domestic pigs from virulent African isolates of African swine fever virus by experimental immunisation. *Vaccine*. 2011; 29 (28): 4593–4600. https://doi.org/10.1016/j.vaccine.2011.04.052

21. Mittelholzer C., Moser C., Tratschin J.-D., Hofmann M. A. Analysis of classical swine fever virus replication kinetics allows differentiation of highly virulent from avirulent strains. *Veterinary Microbiology*. 2000; 74 (4): 293–308. https://doi.org/10.1016/S0378-1135(00)00195-4

22. Balyshev V. M., Vlasov M. E., Imatdinov A. R., Titov I. A., Morgunov S. Yu., Malogolovkin A. S. Biological properties and molecular genetic characteristics of the African swine fever virus, isolated in 2016–2017 in various regions of the Russian Federation. *Russian Agricultural Sciences*. 2018; 44 (5): 469–473. https://doi.org/10.3103/S106836741805004X

23. Balyshev V. M., Vlasov M. E. Pathogenicity of African swine fever virus isolated from wild boar with consecutive passages on pig. *Veterinariya*. 2022; (1): 28–32. https://doi.org/10.30896/0042-4846.2022.25.1.28-32 (in Russ.)

24. Vlasov M. E., Kudryashov D. A., Sindryakova I. P., Sevskikh T. A., Pivova E. Yu., Lyska V. M., et al. Comparative assessment of the pathogenicity of the African swine fever virus circulating in the Russian Federation since 2007. *Veterinariya*. 2024; (4): 28–35. https://doi.org/10.30896/0042-4846.2024.27.4.28-35 (in Russ.)

25. Vlasov M. E., Sibgatullova A. K., Balyshev V. M. The course of disease in pigs infected with ASF virus isolates, obtained in different regions of the Russian Federation. *Veterinariya*. 2019; (4): 15–19. https://doi.org/10.30896/0042-4846.2019.22.4.15-19 (in Russ.)

26. Sereda A. D., Kazakova A. S., Namsrayn S. G., Vlasov M. E., Kolbasov D. V. The attenuated ASFV strains MK-200 and FK-32/135 as possible models for investigation of protective immunity by ASFV infection. *PLoS ONE*. 2022; 17 (7):e0270641. https://doi.org/10.1371/journal.pone.0270641

27. Vlasov M., Sindryakova I., Kudryashov D., Morgunov S., Kolbasova O., Lyska V., et al. Administration routes and doses of the attenuated African swine fever virus strain PSA-1NH influence cross-protection of pigs against heterologous challenge. *Animals*. 2024; 14 (9):1277. https://doi.org/10.3390/ani14091277

28. Vlasov M. E., Sindryakova I. P., Kudrjashov D. A., Morgunov S. Y., Kolbasova O. L., Lyska V. M., et al. Inoculation with ASFV-Katanga-350 partially protects pigs from death during subsequent infection with heterologous type ASFV-Stavropol 01/08. *Viruses*. 2023; 15 (2):430. https://doi.org/10.3390/v15020430

29. Zakutskii N. I., Shirokova T. G., Yurkov S. G., Balyshev V. M. Immunobiological features of an attenuated African swine fever virus strain FC-135 grown in a porcine bone marrow cell suspension. *Agricultural Biology*. 2014; (4): 70–74. https://doi.org/10.15389/agrobiology.2014.4.70eng (in Russ.)

30. National Research Council. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington, DC: National Academies Press; 2011. 246 p. https://doi.org/10.17226/12910

31. Sereda A. D., Namsrayn S., Balyshev V. M., Vlasov M. E., Sindryakova I. P., Koltsova G., Kolbasov D. V. Seroimmunotyping of African swine fever virus. *Frontiers in Microbiology*. 2023; 14:1225587. https://doi.org/10.3389/fmicb.2023.1225587

32. Ashmarin I. P., Vasiliev N. N., Ambrosov V. A. Fast Methods of Statistical Processing and Experiment Planning. Leningrad: Publishing House of the Leningrad University; 1975. 78 p. (in Russ.)

33. Sun E., Zhang Z., Wang Z., He X., Zhang X., Wang L., et al. Emergence and prevalence of naturally occurring lower virulent African swine fever viruses in domestic pigs in China in 2020. *Science China Life Sciences*. 2021; 64 (5): 752–765. https://doi.org/10.1007/s11427-021-1904-4

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