JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Low sensitivity of African Swine Fever active surveillance efforts in Serbia

Vesna Milićević[1], Klaas Dietze[2], Dimitrije Glišić[1], Sofija Šolaja[1], Ljubiša Veljović[1], Jelena Maksimović Zorić[1], Miroljub Dačić[3]

[1] *Virology Department, Institute of Veterinary Medicine of Serbia, Janisa Janulisa 14, Belgrade, Serbia*
[2] *Institute of International Animal Health, Friedrich Loeffler Institute, Südufer 10, Greifswald - Insel Riems, Germany*
[3] *Department of Epizootiology, Veterinary Specialized Institute "Jagodina", Jagodina, Serbia*

Abstract

Introduction: African Swine Fever (ASF) poses a significant threat to swine populations and the global pork industry. Effective surveillance is critical for early detection and control of the disease. However, active surveillance programs may face challenges in sensitivity, particularly in regions like Serbia, where ASF is an emerging concern.
Methodology: This study evaluated the sensitivity of active surveillance efforts for ASF in Serbia, focusing on sampling strategies, diagnostic methods, and data analysis. Surveillance activities included field sampling in high-risk areas, testing of domestic pigs and wild boars, and assessment of diagnostic accuracy for early detection.
Results: The analysis revealed low sensitivity in the current active surveillance framework, attributed to suboptimal sampling density, limited diagnostic reliability, and logistical constraints in high-risk regions. These limitations potentially delay ASF detection, increasing the risk of disease spread and complicating control measures.
Conclusions: Enhancing ASF surveillance in Serbia requires improving sampling strategies, deploying advanced diagnostic tools, and addressing logistical challenges to increase detection sensitivity and safeguard the swine industry.

Key words: African swine fever; active surveillance; sensitivity; domestic pigs; Serbia.

J Infect Dev Ctries 2025; 19(9):1419-1424. doi:10.3855/jidc.21119

(Received 27 November 2024 – Accepted 08 January 2025)

Copyright © 2025 Milićević *et al*. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

African swine fever (ASF) is a viral infectious disease of *Suidae* family members. Naturally, the virus is transmitted by *Ornithodoros* spp. ticks among African wild species. The incubation period ranges from 4 to 19 days, but it is strongly related to virus titer acquired, the route of infection, and the virus virulence. For the highly pathogenic Pol18_28298_O111 strain, the average incubation period was estimated at 9 ± 4 days [1]. Virus shedding begins 1–2 days prior to the onset of clinical signs and occurs through various excretions and secretions. While the highest viral concentration is found in the blood, oral fluid, nasal discharge, feces, and urine exhibit elevated viral loads during the acute phase [2]. In domestic pigs, the predominant route of infection is through close contact with infected animals, primarily via the nasal route. However, oral transmission becomes a significant concern, particularly when pigs consume contaminated swill. The primary route of infection in wild boars is through direct contact between an infected live animal and a susceptible one, similar to domestic pigs. Environmental exposure, particularly at sites where infected wild boars have died, plays a more prominent role than carcass-mediated transmission, as wild boars tend to avoid cannibalism but frequently investigate the area surrounding a carcass. Currently, no effective vaccine is available for ASF control. Consequently, the management of the disease relies on measures such as culling infected pigs, implementing movement controls, and maintaining rigorous biosecurity standards, which have proven effective in preventing the spread of ASF.

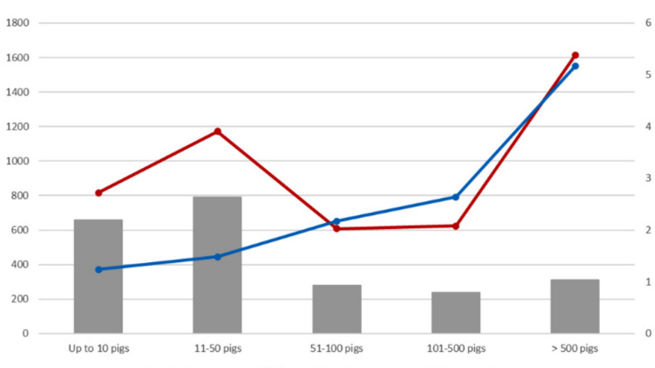
The effectiveness of these measures is closely associated with early diagnosis and rapid response, especially in ASF-free regions. Rapid responses are crucial in preventing the sale of infectious animals, a factor identified as contributing to the long-distance transmission of ASF [3,4]. Enhanced passive surveillance has been recommended to improve early detection, given the limited utility of active surveillance among clinically healthy animals for this purpose [5]. In addition, the degree of disease containment success is in inverse proportion to the number of backyard farms lacking adequate biosecurity measures, as this is a major factor contributing to the spread of ASFV and the

Table 1. Number of visited and sampled farms within active surveillance.

Farm classification based on the number of sows					Farm classification based on the number of pigs				
No sows (%)	1-2 sows (%)	3-10 sows (%)	11-50 sows (%)	> 50 sows (%)	Up to 10 pigs (%)	11-50 pigs (%)	51-100 pigs (%)	101-500 pigs (%)	> 500 pigs (%)
329 (14.46)	766 (33.66)	683 (30.01)	253 (11.12)	245 (10.75)	658 (28.91)	790 (34.71)	280 (12.30)	236 (10.37)	312 (13.71)

persistence of long-term outbreaks [6,7]. Since African Swine Fever caused by genotype II was detected for the first time in Serbia in 2019 [8], the number of outbreaks has progressively risen, reaching its peak in 2023, as reported by the EU Animal Diseases Information System (ADIS). In Serbia, both active and passive surveillance methods have been utilized in domestic pig populations to achieve early detection of diseases. In ASF-free regions, farms with fewer than 100 pigs undergo active surveillance every three months. Farms housing between 101 and 500 pigs are subject to active surveillance every two months, while those with over 501 animals are surveilled monthly. Monthly active surveillance is specifically conducted in regions where ASF is present only in wild boars. In areas where ASF is detected in domestic pigs, farms housing up to 100 pigs are scheduled for monthly active surveillance, while those with over 100 pigs undergo surveillance twice a month. The active surveillance involves clinical inspection and the sampling of every discovered deceased sow and boar, or at least two deceased animals from other categories, primarily those older than two months or a minimum of two ailing animals exhibiting hemorrhagic symptoms and fever, treated with antibiotics for more than three days. Pig production in Serbia is predominantly characterized by numerous backyard farms, typically housing up to 10 animals. These farms generally operate with minimal or no biosecurity measures, often lacking proper fencing or being only partially fenced. The animals are usually kept indoors without outdoor access, and there is little to no control over visitor access or quarantine procedures for newly purchased animals [9].

Figure 1. Overview on number of tested samples, the number of tested farms and average sample number per farm across farm-size categories.



Apart from the clear evidence of numerous backyard farms with low biosecurity measures [9] that are challenging to control, the inexplicable speed of disease spread raises questions. Consequently, the present study aimed to evaluate the sensitivity of the active surveillance system in detecting ASF in Serbia, with the objective of generating scientific evidence to support policy recommendations for optimizing surveillance efforts.

Methodology

The study did not involve the use of live animals and was based solely on the interpretation of data obtained through active surveillance. As only pre-existing data were analyzed, ethics approval was not required.

Data were collected during active surveillance of domestic pigs conducted in 20 districts from July to December 2022. The data included farm category, sample type, sampling date, sample reception date, and laboratory report date. According to the active surveillance protocol, clinical inspections were performed, and blood swab samples from clinically ill animals as well as organ samples (spleen, kidney) from deceased pigs were collected and tested using real-time PCR. The laboratory analyses were carried out in 9 regional laboratories and a national reference laboratory for ASF.

Data on ASF outbreaks in 2022 were retrieved from World Animal Health Information System (WAHIS). The number of pigs and holdings were acquired from The Statistical Office of the Republic of Serbia. Descriptive statistical methods were applied for data analysis.

The sensitivity of active surveillance was estimated using the formula $EDSSe = C_p \times C_t \times Se_d$ [10]. The population coverage (Cp) was defined as the ratio between the number of active surveillances conducted with sampling and the total number of farms. The temporal coverage (Ct) represented the relationship between the incubation time and the sampling intervals depending on the farm category. Detection sensitivity (Se_d) was assumed to be 100% considering primary testing of dead animals and sick ones with hemorrhagic symptoms or unresponsive to antibiotic therapy.

Results

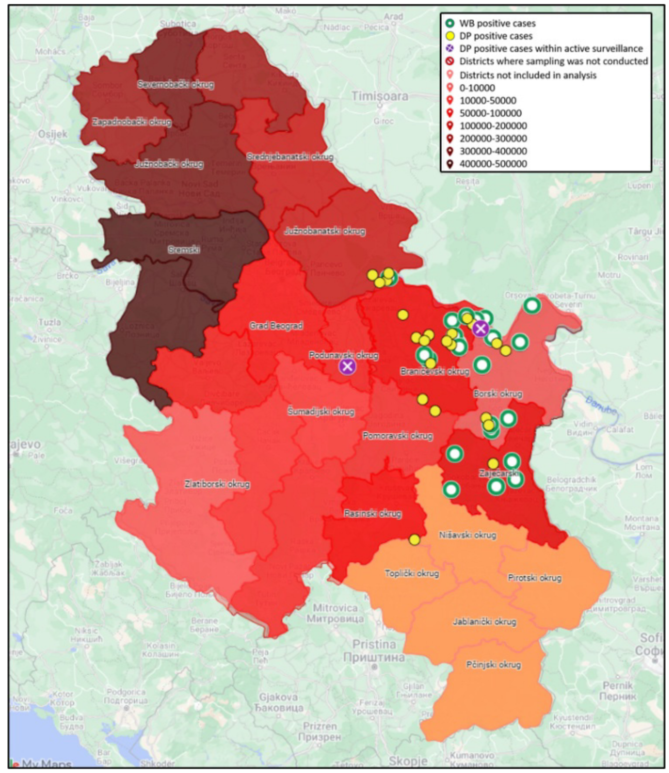
During the six months, active surveillance and sampling were conducted on 2,276 farms categorized according to the number of sows and the total pig count (Table 1) across 15 districts. In five districts, no samples were collected during active surveillance in 2022, as no animals met the sampling criteria defined in the surveillance plan.

Within the active surveillance, 4173 blood swab samples and 658 organ samples were collected and tested for ASFV genome presence using real-time PCR (Figure 1). Three blood swab samples (0.07%) and one organ sample (0.2%) were positive for ASFV DNA.

Official data from July to December 2022 reported 42 affected farms across the observed districts, involving 102 affected animals and 745 susceptible animals (Figure 2).

The estimated swine population in these districts was 2.812.031 pigs, distributed across 171.124 farms. The mean number of pigs per farm was 16.4 animals (Figure 3). In the same period, 14 wild boar cases were reported in these regions. Only 3 out of the 42 outbreaks were identified through active surveillance, occurring in 2 out of the 5 infected districts. ASF has been present in both districts in domestic pigs and wild boars since 2020. The population coverage (C_p) was calculated to be 1.3%, the C_t at 10% for farms with up to 100 pigs, 15% for farms with 101 to 500 pigs, and 30% for farms with more than 500 pigs, and the detection Se_d at 100%. Accordingly, the sensitivity of active surveillance was estimated at 0.13% for small farms (≤ 100 pigs), 0.19% for medium farms (101–500 pigs), and 0.39% for large farms (> 500 pigs). The highest sensitivity, at 0.73%, was calculated for farms with over 100 animals in regions where ASF was present in the domestic population. The median number of days from sampling to laboratory reception was 2.6 days (range 0-20). The median number of days from sample reception to the laboratory report was 9.28 days (range 0-42 days) (Figure 4). On average, the time from sampling to laboratory submission was 0.88 days for organ samples and 2.89 days for blood swabs. However, the duration from laboratory reception to report was 7.12 days for organs and 6.28 days for blood samples.

Figure 2. Map showing the domestic pig population toward the ASF cases in domestic pigs and wild boar.



(WB: wild boar; DP: domestic pig).

Figure 3. Number of farms, outbreaks, tested samples, and average days to diagnosis per district.

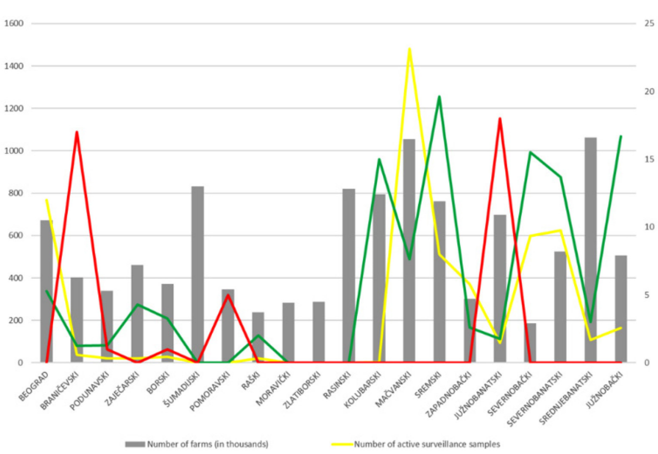
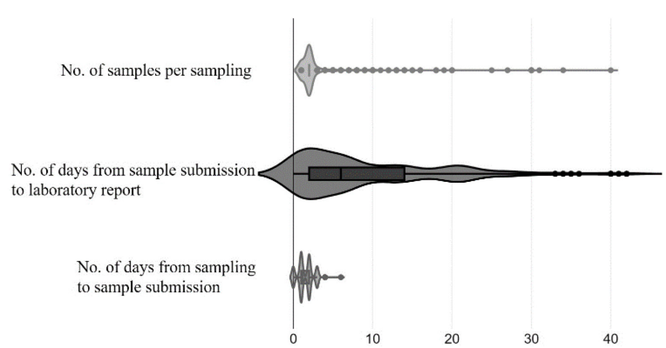


Figure 4. Violine-box plot showing the distribution of samples toward the time between sampling and sample submission and sample submission to laboratory report.



Discussion

Early detection surveillance is used for identifying disease appearance in a previously unaffected population which is of particular significance due to the severe consequences and expenses associated with delayed detection when a disease spreads to a new population.

This is especially critical in the case of African swine fever (ASF), where detection on large farms may take up to 23 days owing to the disease's slow progression and early mortality patterns that may appear clinically unremarkable [11]. Different systems for early disease detection exist, tailored to the characteristics of the diseases and the populations they affect.

As a result, the approach to detecting and managing ASF in wild boar and domestic swine populations differs. While active surveillance targeting virus detection in wild boar populations has shown limited effectiveness, identifying animals with positive antibody levels could become valuable or even preferable during the later stages of an endemic phase. However, this should be complemented by passive surveillance, mainly through discovering carcasses [12]. Hence, passive surveillance in wild boar and syndromic surveillance in domestic populations have been validated as the most effective strategies [13]. Nevertheless, active surveillance for ASF in domestic pigs remains in place in Serbia.

A surveillance system intended to detect diseases early should ideally be designed with high sensitivity. However, the active surveillance system in Serbia had a sensitivity of 0.13% to 0.39%, which is unsurprising, especially considering C_t and that sampling is infrequent compared to the incubation period. In Latvia, to facilitate the early detection of outbreaks, the sampling is conducted every week, which is shorter than the average incubation period [14]. The findings presented here are consistent with the sensitivity levels typically observed in periodic sampling surveys [10]. While official data on secondary outbreaks in Serbia remain unavailable, epidemiological investigations have proven to be the most effective means of identifying secondary spread, both within and outside restriction zones. In contrast, active surveillance was shown to be ineffective for this purpose in Latvia [15]. To achieve the necessary sensitivity for disease detection, such systems would need to continually monitor the entire population using a 100% sensitive detection system [10]. Given the epidemiological context in Serbia and surrounding countries, along with the prevailing production systems and limited relevance of export markets, it is unclear whether this objective is appropriate. The findings from analyzing the effectiveness of active surveillance reveal a concerning lack of awareness among the actors involved in conducting surveillance, with an average of 7.67 days from sampling to laboratory reporting and extreme values as high as 43 days. Organ samples from deceased animals were generally submitted more rapidly than blood samples, suggesting the inclusion of asymptomatic animals in blood sample submissions. The lack of urgency in submitting such samples may be attributed to the absence of clinical sign data on submission forms and the sample-based compensation model for field veterinarians. However, it is important to note that while viremia and clinical signs manifest simultaneously, pigs can become infectious via nasal or oral routes before clinical signs appear [16]. In light of the findings from the active surveillance evaluation, there was a possibility that sampled animals may have been sick and even died before laboratory analysis completion. Although the farmers are obliged, they are reluctant to report any health event due to the possible consequences.

Additionally, unrestricted sample submission for disease exclusion is neither common nor actively promoted in Serbia. The cascading impacts of the concerning performance of active surveillance are further exacerbated by the behavior of farmers towards sick animals. In an effort to minimize losses, the prevalent practice involves slaughtering sick animals and promptly selling those that appear to be healthy [8], a practice also observed in other countries [17].

In contrast to the veterinary service responsible for submitting samples, the laboratories, which operate under ISO17025 standards make no distinction between organ samples from deceased animals and blood samples when considering the average turnaround time from sample submission to the laboratory report. These standards ensure that lab staff are not aware of the owners or other identifying information about the samples and that the laboratory process is impartial to the sample type. This impartiality in the lab process means that staff cannot make informed decisions about when it might be acceptable to delay results.

One of the primary principles of ASF surveillance is minimizing the duration of the high-risk period through early detection of infected farms. In Serbia, the high-risk period was determined to be 21 days for backyard farms and 35 days for free-range farms [8]. This period is further supplemented by the time required for diagnosis in terms of active surveillance.

Another concerning aspect was that regions with the highest number of swine and a large number of samples experienced the longest reporting times, highlighting potential limitations in diagnostic system capacity. Therefore, alternative approaches such as clinical surveillance and syndromic surveillance, which demand fewer samples and offer highly accurate and sensitive surveillance systems, should be implemented. For larger, high-risk farms, the use of oral ropes for saliva sampling [18], could provide early detection although it also requires regular sampling. This approach could complement existing surveillance methods, enhancing the early detection and control of ASF. A critical evaluation of the current surveillance system is essential to identify performance gaps and inform necessary adaptations. Without these changes, the system risks becoming a burden on veterinary and laboratory resources, producing low-value results and potentially contributing to disease spread.

Conclusions

The findings indicate substantial limitations in the existing ASF active surveillance system in Serbia. These include low diagnostic sensitivity, delays in sample processing, and limited farmer cooperation, all of which hinder early detection efforts. Infrequent sampling relative to the ASF incubation period and delays in blood sample submission contribute to reduced system responsiveness. In addition, certain farmer practices such as underreporting and the unregulated slaughter of symptomatic animals further impair the effectiveness of surveillance. Although laboratory operations comply with ISO17025 standards, delays in reporting are exacerbated by system capacity limitations in high-risk regions. To enhance early detection and control of ASF, the implementation of alternative surveillance strategies such as clinical and syndromic surveillance is recommended. These approaches may achieve high sensitivity and accuracy while requiring fewer samples. The integration of innovative methods like oral rope saliva sampling on large farms could complement these approaches. A critical reassessment and adaptation of the current surveillance framework is essential to reduce the high-risk window and limit the further spread of the disease.

Funding

The study was funded by the Serbian Ministry of Science, Technological Development and Innovation (Contract No 451-03-66/2024-03/ 200030).

Data availability

The datasets generated during the current study are available from the corresponding author on request.

Authors contributions

VM conceived the study; KD reviewed and edited the manuscript; DG contributed to the methodology of the study; SŠ drafted the manuscript; LJV and JMZ performed formal analysis; MD carried out investigations. All authors approved the final manuscript, taking accountability for all aspects of the work.

Corresponding Author

Vesna Milićević, PhD, DVM
Institute of Veterinary Medicine of Serbia
Janisa Janulisa 14
11000 Belgrade, Serbia
Email: vesna.milicevic@nivs.rs
Tel: +381 11 2851096

Conflict of interests

No conflict of interests is declared.

References

1. Walczak M, Żmudzki J, Mazur-Panasiuk N, Juszkiewicz M, Woźniakowski G (2020) Analysis of the clinical course of experimental infection with highly pathogenic African swine fever strain, isolated from an outbreak in Poland. Aspects related to the disease suspicion at the farm level. Pathogens 9: 237-247. doi: 10.3390/pathogens9030237.
2. Davies K, Goatley LC, Guinat C, Netherton CL, Gubbins S, Dixon LK, Reis AL (2017) Survival of African swine fever virus in excretions from pigs experimentally infected with the Georgia 2007/1 Isolate. Transbound Emerg Dis 64: 425–431. doi: 10.1111/tbed.12381.
3. Clemmons EA, Alfson KJ, Dutton JW (2021) Transboundary animal diseases, an overview of 17 diseases with potential for global spread and serious consequences. Animals 11: 2039-2096. doi: 10.3390/ani11072039.
4. Costard S, Wieland B, De Glanville W, Jori F, Rowlands R, Vosloo W, Roger F, Pfeiffer DU, Dixon LK (2009) African swine fever: How can global spread be prevented? Philos Trans R Soc Lond B Biol Sci 364: 2683–2696. doi: 10.1098/rstb.2009.0098.
5. Ståhl K, Boklund A, Podgórski T, Vergne T, Abrahantes JC, Papanikolaou A, Zancanaro G, Mur L (2023) Epidemiological analysis of African swine fever in the European Union during 2022. EFSA Journal 21: 8016-8065. doi: 10.2903/j.efsa.2023.8016.
6. Zani L, Dietze K, Dimova Z, Forth JH, Denev D, Depner K, Alexandrov T (2019) African swine fever in a Bulgarian backyard farm-a case report. Vet Sci 6:94-102. doi: 10.3390/vetsci6040094.
7. Ardelean F, Globig A, Gârdan Năvălici AI, Blome S, Dietze K, Depner K, Zani L (2021) The course of African swine fever in

Romanian backyard holdings – a case report. Vet Med Sci 7: 2273–2279. doi: 10.1002/vms3.592.
8. Glišić D, Milićević V, Veljović L, Milovanović B, Kureljušić B, Đorđević I, Anđelković K, Petković J, Dačić M (2023) Patterns of ASFV transmission in domestic pigs in Serbia. Pathogens 12: 149-158. doi: 10.3390/pathogens12010149.
9. Prodanov-Radulović J, Đurđević B, Petrović J, Mirčeta J, Polaček V (2022) African swine fever: a biosecurity challenge for domestic pig production in Serbia. Arch Vet Med 15: 21–38. doi: 10.46784/e-avm.v15i2.310.
10. Cameron AR, Meyer A, Faverjon C, Mackenzie C (2020) Quantification of the sensitivity of early detection surveillance. Transbound Emerg Dis 67: 2532–2543. doi: 10.1111/tbed.13598.
11. Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Depner K, Drewe JA, Garin-Bastuji B, Gonzales Rojas JL, Gortázar Schmidt C, Herskin M, Michel V, Miranda Chueca MÁ, Pasquali P, Roberts HC, Sihvonen LH, Spoolder H, Ståhl K, Velarde A, Viltrop A, Winckler C, De Clercq K, Klement E, Stegeman JA, Gubbins S, Antoniou SE, Broglia A, Van der Stede Y, Zancanaro G, Aznar I (2021) Scientific opinion on the assessment of the control measures of the category A diseases of animal health law: African swine fever. EFSA J 19: 6402-6483. doi: 10.2903/j.efsa.2021.6402.
12. Gervasi V, Marcon A, Bellini S, Guberti V (2020) Evaluation of the efficiency of active and passive surveillance in the detection of African swine fever in wild boar. Vet Sci 7: 5-14. doi: 10.3390/vetsci7010005.
13. Guinat C, Vergne T, Jurado-Diaz C, Sánchez-Vizcaíno JM, Dixon L, Pfeiffer DU (2017) Effectiveness and practicality of control strategies for African swine fever: what do we really know? Vet Rec 180: 97-102. doi: 10.1136/vr.103992.
14. Lamberga K, Olševskis E, Seržants M, Berzinš A, Viltrop A, Depner K (2020) African swine fever in two large commercial pig farms in Latvia-estimation of the high-risk period and virus spread within the farm. Vet Sci 7: 105-115. doi: 10.3390/VETSCI7030105.
15. Lamberga K, Viltrop A, Nurmoja I, Masiulis M, Bušauskas P, Oļševskis E, Seržants M, Laddomada A, Ardelean F, Depner K (2024) The effectiveness of protection and surveillance zones in detecting further African swine fever outbreaks in domestic pigs—experience of the Baltic states. Viruses 16: 334-343. doi: 10.3390/v16030334.
16. Guinat C, Reis AL, Netherton CL, Goatley L, Pfeiffer DU, Dixon L (2014) Dynamics of African swine fever virus shedding and excretion in domestic pigs infected by intramuscular inoculation and contact transmission. Vet Res 45: 93-101. doi: 10.1186/s13567-014-0093-8.
17. Costard S, Zagmutt FJ, Porphyre T, Pfeiffer DU (2015) Small-scale pig farmers' behavior, silent release of African swine fever virus and consequences for disease spread. Sci Rep 5: 1704-17082. doi: 10.1038/srep17074.
18. Goonewardene KB, Chung CJ, Goolia M, Blakemore L, Fabian A, Mohamed F, Nfon C, Clavijo A, Dodd KA, Ambagala A (2021) Evaluation of oral fluid as an aggregate sample for early detection of African swine fever virus using four independent pen-based experimental studies. Transbound Emerg Dis 68: 2867–2877. doi: 10.1111/tbed.14175.