JAP&T
TPA-Timișoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2023, *29*(1), 30-33

Journal of Agroalimentary Processes and Technologies

The use of PCR and Elisa method to detect and monitor the infection of domestic pigs and wild boars with African Swine Fever Virus

Larisa Anghel (Cireașa)[1,5*], Maria-Virginia Tanasa (Acretei)[1], Corneliu Ovidiu Vrancianu[2], Natalia Roșoiu[1,3,4]

[1]*Ovidiu's University Constanta, Institute of PhD Studies, Doctoral School of Applied Sciences, 900573, Ion Vodă, 58, Constanta, Romania*
[2]*University of Bucharest, Faculty of Biology, 050095, Splaiul Independenței, 91–95, Bucharest, Romania*
[3]*Ovidiu's University, Faculty of Medicine, 900470, Aleea Universității, 1, Constanta, Romania*
[4]*Academy of Romanian Scientists, 50085, Ilfov, 3, Bucharest, Ramnia*
[5]*D.S.V.S.A. Constanta, Molecular Biology Laboratory, Veterinary Health and Food Safety Department, 900111, Mangaliei, 78, Constanta, Romania*

Abstract

African Swine Fever (ASF) is an infectious disease affecting domestic pigs, wild boars, and other related species. It is caused by a double-stranded DNA virus from the Asfarviridae family. Currently, 24 African Swine Fever genotypes have been identified; genotype I is circulating in Eu-rope, South America, and Western Africa. The infection causes high mortality rates in animals, mainly due to hemorrhagic fever. The first signaling of the ASF was in 1921 in Kenya. Subsequent-ly, the virus was reported in 1957 in Portugal and afterward in Spain, but it was eradicated from the Iberian Peninsula in the '80s. In Romania, the ASF virus entered probably via Danube Delta, causing a major outbreak in 2018. Currently, the outbreak is under control in Romania due to the implemen-tation of a molecular diagnostic program and a slaughter policy. The objective of our current work was to present the diagnostic results of the ASF virus in pigs and wild boars obtained in 2019 by PCR and ELISA in Constanta County. We used blood samples collected on a clot activator to evalu-ate the presence of post-infection antibodies with ELISA. In addition, we collected blood or differ-ent organs to detect active African Swine Fever virus infection by Real-Time PCR. Using PCR, we confirmed 28 cases of ASF infection in wild boars out of 256 analyzed samples and 15 in domestic pigs from private households out of 64 analyzed samples. However, we did not detect the African Swine Fever virus in 1370 samples analyzed from commercial farms. Performing the ELISA method, we confirmed 18 wild boars out of 199 analyzed samples and positive cases and five domestic pigs from private households out of 879 analyzed samples. We concluded that the Real-Time PCR meth-od allows rapid detection of the African Swine Fever virus a few days after infection. At the same time, ELISA can monitor the post-infection immunological status of pigs / wild boar populations in response to the ASF virus.

Keywords: African Swine Fever, PCR, ELISA, molecular diagnosis.

1. Introduction

African Swine Fever (ASF) is a complex disease caused by a double-stranded DNA virus from *the Asfarviridae* family, which affects domestic pigs, wild boars, and other related species [1]. To understand how this large (170-190 kbp) double-stranded DNA virus causes disease, we must understand how it infects its host.

The infection can occur *via* direct contact of healthy animals with other infected pigs, feces or body fluids, consumption of infected pigs, or by indirect contact *via* fomites such as equipment and vehicles.

In addition, there were cases of infection *via* biological vectors such as soft ticks belonging *Ornthodoros* genus. The virus spreads within the host, damaging their target tissues represented by monocytes and macrophage cells.

It is vehiculated through secretions and faces into the environment and can infect a new host. It can be passed congenitally from the mother to the offspring

Corresponding authors: cireasa.larisa-ct@ansvsa.ro

[2]. That viral infection does not result in clinical disease unless it reaches a certain degree, where the number of viruses inside the body overwhelms the immune responses [3]. Therefore, the outcome of the host and virus encounter is decided by the virus's virulence and susceptibility to the host. The virus uses the immune defense reaction to help infect the monocyte cells of the pigs, which are macrophage cells and dendritic cells. Those immune cells are found inside pig lymph nodes [4]. Another unique feature of this virus is that the pig does not produce neutralizing antibodies against it, which is one of the reasons why it is so deadly [5]. The body produces neutralizing antibodies (NAb) as part of the immune response triggered by both infections and vaccinations against infections. Neutralizing antibodies can result in lifelong immunity to certain infections and can be used to see if pigs developed immunity to an infection after recovering [6]. Neutralizing antibodies can be confused with binding antibodies. These are responsible for binding to a pathogen and signalling the immune system of its presence, which triggers the white blood cells reaction [7].

The aim of this work was to present the diagnostic results of the ASF virus in pigs and wild boars obtained in 2019 by PCR and ELISA in Constanta County.

2. Materials and methods

2.1 Materials

Samples were collected following the National Legislation Order 35, published on the 30th of March 2016, by the National Veterinary and Food Safety Authority. This contains detailed rules for the implementation program of surveillance, prevention, control, and eradication of animal diseases [8].

For the PCR experiments, 8 ml EDTA tubs were used to collect blood. Different organs, such as a mix of organs (spleen, kidney, lymph nodes, or liver), bone marrow, and cadavers, were brought to the laboratory as samples. For the ELISA experiments, 6 ml vials with cloth activator (plasma and serum).

2.2 Methods

The Real-Time PCR method was used to identify the specific genome targets of the ASF virus with specific primers and probes following the WOAH Terrestrial Manual Chapter 3.9.1. [9]. The DNA samples were purified using a commercial kit IndiSpin Pathogen (Indical Bioscience). All samples were amplified using 7900HT Fast Real-Time PCR Systems (Applied Biosystems Singapore) [10].

The ELISA method detected antivirus antibodies in ASF by immunoassay technique. Plasma and serum samples were analyzed using the ID VET kit and ELISA reader (Ledetect 96 Led Based & Channel Microplate Reader Austria) [10].

3. Results and discussion

Evaluating the results obtained from samples collected during 2019 highlighted exciting results. Using PCR, we confirmed 28 cases of ASF infection in wild boars out of 256 analyzed samples and 15 in domestic pigs from private households out of 64 analyzed samples (Tabel 1,2).

However, we did not detect the African Swine Fever virus in 1370 samples analyzed from commercial farms (Tabel 3).

Using the ELISA test, we confirmed 18 wild boars out of 199 analyzed samples (Tabel 4), positive cases, and six domestic pigs from private households out of 879 analyzed samples (Tabel 5).

According to the European Union Reference Laboratory for ASF virus, both methods should be used for establishing the dynamic of infection in case of positive results of genome detection and antibodies showing that the animals were infected at the time of sampling where a positive result on antibody test in the absence of genome detection indicates an ongo-ing or past infection [1]. The investigated cases show that only six wild boar cases were con-firmed by a positive result using both analysis methods (Table 1,4). These animals were still viraemic, with clinical signs, and in the period of seroconversion (> ten days) [1]. Also, one wild boar had a weak positive result using the genome detection method.

The same animal tested negative at the antibody test, which demonstrates that this animal was recently infected with ASF. It had not yet seroconverted (< 7 days after contact), clinical signs were not obvi-ous, and antibodies were not yet produced. One wild boar tested weakly positive for genome detection and positive for antibody detection. In these particular cases, the animal had passed the infection, and it may not present clinical signs. This with boar might have been infected with an attenuated strain [1].

Table 1. Results obtained from wild boar samples analysed using Real Time PCR method.

Matrix	Wild boars	Positive	Weak Positive	Negative
Blood on EDTA	6	0	0	6
Organs	229	5	2	222
Bone Marrow	21	21	0	0

Table 2. Results obtained from pig samples from private households using Real Time PCR method.

Matrix	Pigs from private households	Positive	Weak Positive	Negative
Blood on EDTA	50	7	0	43
Organs	11	5	0	6
Bone Marrow	0	0	0	0
Cadaver	3	3	0	0

Table 3. Results obtained from pig samples from commercial holdings using Real Time PCR method.

Matrix	Pigs from commercial holdings	Positive	Weak Positive	Negative
Blood on EDTA	326	0	0	326
Organs	1044	0	0	1044
Bone Marrow	0	0	0	0

Table 4. Samples collected form wild boars and analysed using ELISA method.

Matrix	Wild boars	Positive Results	Weak Positive Results	Negative Results
Blood serum	199	17	1	181

Table 5. Results obtained from pig samples from private households using ELISA method.

Matrix	Pigs from private households	Positive	Weak Positive	Negative
Blood serum	879	5	1	873

Table 6. Results obtained from pig samples from commercial holdings using ELISA method

Matrix	Pigs from commercial holdings	Positive	Weak Positive	Negative
Blood serum	967	0	0	967

The results obtained from the total analyzed samples of domestic pigs from private households showed that 18 tested positive for antibodies, and from this group, three tested positive for genome detection. These pigs were still viraemic with clinical signs and had seroconverted (> 10 days), and 2 tested weak positive these animals had passed the infection and recovered clin-ical signs may not be present (Table 2,6).

4. Conclusions

The objective of our current work was to present the diagnostic results of the ASF vi-rus in pigs and wild boars obtained in 2019 by PCR and ELISA in Constanta County. We con-cluded that the Real-Time PCR method allows rapid detection of the African Swine Fever virus a few days after infection.

ELISA test is more suitable to detect the presence of specific antibodies to ASF from the 14th day after contact with the virus. Therefore, it is not reliable to identify if the animals have recently been infected with the ASF virus. However, it can be used to monitor the post-infection immunological status of pigs / wild boar populations in response to the ASF virus. In addition, the ELISA can help statistically to record the percentage of pigs who became immune to the disease.

Author contributions

L.A.(C). and N.R. conceived, revised, and corrected the manuscript; L.A.(C), M.-V.T.(A.) and M.C.C. drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

References

1. Wang, N.; Zhao, D.; Wang, J.; Zhang, Y.; Wang, M.; Gao, Y.; Li, F.; Wang, J.; Bu, Z.; Rao, Z.; Wang, X. Architecture of African swine fever virus and implications for viral assembly, Science 2019, 366(6465), 640-644, doi: 10.1126/science.aaz1439.
2. Jia, N.; Ou, Y.; Pejsak, Z.; Zhang, Y.; Zhang, J. Roles of African swine fever virus structural proteins in viral infection, Journal of Veterinary Research, 2017, 61(2), 135, doi: 10.1515/jvetres-2017-0017.
3. Njau, E.P.; Machuka, E.M.; Cleaveland, S.; Shirima, G.M.; Kusiluka, L.J.; Okoth, E.A.; Pelle, R. African Swine Fever Virus (ASFV): Biology, genomics and genotypes circulating in Sub-Saharan Africa, Viruses, 2021, 13(11), 2285, doi: 10.3390/v13112285.
4. Wu, L.; Yang, B.; Yuan, X.; Hong, J.; Peng, M.; Chen, J. L.; Song, Z. (2021). Regulation and evasion of host immune response by African swine fever virus Frontiers in Microbiology, 2021, 12, 698001, doi: 10.3389/fmicb.2021.698001.
5. Zheng, X.; Nie, S.; Feng, W.H. Regulation of antiviral immune response by African swine fever virus (ASFV), Virologica Sinica, 2022, 37(2), 157–167, doi: 10.1016/j.virs.2022.03.006.
6. Neilan, J. G.; Zsak, L.; Lu, Z.; Burrage, T.G.; Kutish, G.F.; Rock, D.L. (2004). Neutralizing antibodies to Afri-can swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection, Virology, 2004, 319(2), 337–342, doi: 10.1016/j.virol.2003.11.011.
7. Carmina, G.; Nieto, R.; Arias, M. African swine fever virus (ASFV) indirect ELISA test based on the use of the soluble cytoplasmic semi-purified antigen (ASFV CP-Ag), Methods Mol Biol. 2022, 2503, 133-145. doi: 10.1007/978-1-0716-2333-6_9.
8. The Official Monitor of Romania. Order no. 35 from 30 March 2016. http://www.ansvsa.ro/download/legislatie/legislatie_sanatate_animala/ORDIN-Nr35-2016.pdf (ac-cessed April 26, 2022).
9. Terrestrial Code Online Access - WOAH - World Organisation for Animal Health. https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/.(accessed May 3, 2023).
10. Anghel, L.; Tanasa, M.-V.; Roșoiu, N. (2022). African swine fever virus genome detection using Real Time Q PCR polymerase chain reaction method-comparison of two sample specimen (blood and organs), Academy of Romanian Scientists, Annals-Series on Biological Sciences 2022, 11(1), 81–90.