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The detection and monitorization of the African Swine Fe-ver Virus infection in domestic pigs and wild boars

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Abstract

In recent years, African Swine Fever (ASF), a deadly pig disease, has spread throughout Europe, America, and Asia, devastating pork production all over the world. The ASF is an acute viral haem-orrhagic disease of pigs and wild boars. ASF outbreaks have been reported in Romania since 2017. Routine surveillance is done in Constanta County by constantly checking for clinical and anato-mopathological conditions of the swine heads and by PRC testing to help with early detection of the disease and ELISA testing for detection of antibodies to the virus. The laboratory diagnostic results of ASF virus conducted from 2020 to 2023 in Constanta County confirmed, using the PCR method, four positive cases of ASF infection in wild boars out of 510 analysed samples and in domestic pigs from private households, 50 positive cases of ASF infection out of 171 analysed samples. We did not detect, using the PCR method, the ASF virus in 3626 samples analysed from commercial farms. With ELISA, we confirmed ten positive cases in wild boar out of 511 analysed samples and 16 posi-tive cases in domestic pigs from private households out of 742 analysed samples. We did not detect, using the ELISA method, the ASF virus in 967 samples analysed from commercial farms. We con-clude that laboratory testing can be used as an indicator to investigate the dynamic of ASF infection in case of positive results identified by genome detection. 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.

Keywords: African Swine Fever, PCR, ELISA, molecular diagnostic

1.Introduction

African Swine Fever (ASF) is a viral disease described for the first time in Kenya in 1921 by Montgomery [1]. The agent responsible for the onset of the disease is the ASF virus, characterized as a double-stranded, icosahedral, enveloped arbovirus belonging to the *Asfarviridae* family [2]. Through complete genome sequencing, approximately 160 genes have been identified within the virus, organized like that of poxviruses [3]. The incubation period for the ASF virus spans from four days to about three weeks, with the duration influenced by factors such as the virulence of the virus, host-specific characteristics, the viral load, and the route of infection [2].

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ASF shows several clinical presentations: acute, subacute, and asymptomatic and affects only porcine species of all ages [4]. Domestic pigs can contract the disease through exposure to infected wild boars to which the disease is endemic, and through close contact, nose to nose, or bodily secretion can transmit the disease. Once a pig is infected, symptoms appear and include high fever, skin hemorrhage on the ears, abdomen, legs, dyspnoea, nasal discharge, and death in 7-10 days [5].

ASF virus exhibits diverse transmission cycles, primarily categorized into three types. The sylvatic cycle involves the circulation of the ASF virus in wild reservoirs such as bush pigs, warthogs, and soft ticks (*Ornithodoros spp*.), which serve as the natural hosts for the virus [3]. In the domestic cycle, ASF virus spreads among domestic pigs through various biological vectors (*Ornithodoros spp*.) (the tick–pig cycle), direct pig-to-pig contact in the pig–pig cycle, fomites, contaminated food or water, and less common routes like artificial insemination with contaminated semen or mechanical vectors like insects [6]. Notably, the ongoing ASF epidemic in Central and Eastern Europe has revealed an additional epidemiological cycle, the wild boar–habitat cycle. In this cycle, the Eurasian wild boar (*Sus scrofa*), habitat, and carcasses play pivotal roles in maintaining the infection [7]. Understanding these distinct transmission cycles is essential for developing effective control and prevention measures, given the significant economic consequences and implications for the swine industry associated with ASF outbreaks.

The symptoms of ASF can mimic other diseases such as classical swine fever, salmonella, or septicemia, so it is important to contact the local veterinarian who will investigate and to make sure to send the correct samples for PCR are spleen, tonsils, kidney, and blood on EDTA and for ELISA bold with cloth activator. Robust surveillance programs are in place for diagnostic testing of wild boars and domestic pigs using PCR and ELISA methods.

2. Materials and methods

2.1 Materials

The samples were collected following the National Legislation Order 35, published on the 30th 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 the spleen, kidney, lymph nodes, 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 was used to detect antivirus antibodies ASF by immunoassay technique. Plasma and serum samples were analysed using the ID VET kit and ELISA reader (Ledetect 96 Led Based & Channel Microplate Reader Austria) [10].

3. Results and discussion

The results obtained from samples collected from 2020 to 2023 highlighted exciting results. Using PCR, we confirmed 4 cases of ASF infection in wild boars out of 510 analysed samples and 50 in domestic pigs from private households out of 171 analysed samples (Table 1). However, we did not detect the African Swine Fever virus using the PCR method in 3626 samples analysed from commercial farms (Table 1). Using the ELISA test, we confirmed ten wild boar-positive cases out of 511 analysed samples (Table 2) and 16 domestic pig-positive cases from private households out of 742 analysed samples (Table 2). However, we did not detect the African Swine Fever virus using the ELISA method in 967 samples analysed from commercial farms (Table 2).

Both methods are used to establish 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 ongoing or past infection. The investigated cases show that only one wild boar case was confirmed by a positive result using both analysis methods (Table 1). These animals were still viraemic, with clinical signs, and in the seroconversion period (> 10 days).

The results obtained from the total analysed samples of domestic pigs from private households showed that 16 tested positive for antibodies, and from this group, none of them tested positive for genome detection. (Table 2).

Table 1. Samples collected and analysed using RT-PCR in 2020-2023

Category	Year	Matrix	Total no.	Positive	Negative
Wild boars	2020	Organs	155	1	154
	2021		96	0	96
	2022		157	1	156
	2023		102	2	100
Private households	2020	Blood on EDTA	24	4	20
		Organs	5	0	5
	2021	Blood on EDTA	17	12	5
		Organs	7	7	0
		Cadaver	1	1	0
	2022	Blood on EDTA	29	1	28
		Organs	43	1	42
	2023	Blood on EDTA	25	18	7
		Organs	20	13	7
Commer-cial holdings	2020	Blood on EDTA	461	0	461
		Organs	522	0	522
	2021	Blood on EDTA	494	0	494
		Organs	512	0	512
	2022	Blood on EDTA	573	0	573
		Organs	330	0	330
	2023	Blood on EDTA	417	0	417
		Organs	314	0	314

Table 2. Samples collected and analysed using ELISA method in 2020-2023

Category	Year	Matrix	Total no.	Positive	Negative
Wild boars	2020	Blood serum	155	4	151
	2021		96	3	93
	2022		157	3	154
	2023		102	0	102
Private households	2020	Blood serum	110	0	110
	2021		439	16	423
	2022		15	0	15
	2023		178	0	178
Commercial holdings	2020	Blood serum	-	-	-
	2021		681	0	681
	2022		233	0	233
	2023		53	0	53

4. Conclusions

In this study, the ELISA assay was used to detect antibodies against the ASF virus by using high antigenic viral proteins p32, p62, and p72 and a Real-Time PCR assay to detect the specific ASF genome. We concluded that a positive Real-Time PCR test identifies pigs undergoing viral replication within the cell and early detection of infection and virus transmission. The serological test using the recombinant proteins of ASF as a reagent helped determine ASF and antecedents of previous infection with ASF. However, the specific recombinant proteins showed very little relationship with viral replication regarding active and recurrent infection since using the ELISA assay, only one wild boar case tested positive, and the same cases tested positive using the Real-Time PCR assay for ASF. Furthermore, there is a time lag between primary infection and ASF antibody production as it can remain undetected because of delayed seroconversion due to immunosuppression, or it can persist long after infection and not show clinical signs. Similarly, to our results Gallardo and collaborators reported that the accuracy of the serological tests for diagnosing ASF infection was lower than the Real-Time PCR [11].

The objective of our current work was to present the diagnostic results of the ASF virus in pigs and wild boars obtained from 2020 until 2023 using Real-Time PCR and ELISA assay in Constanta County. We concluded that the Real-Time PCR method allows a rapid detection of ASF 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. The ELISA can help statistically record the percentage of pigs who became immune to the disease.

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Compliance with Ethic 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 exists) respect the specific regulation and standards.

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