STATISTICAL ASPECTS OF DATA COLLECTED FROM AFRICAN SWINE FEVER VIRUS OUTBREAK'S IN CONSTANTA COUNTY

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Abstract. African swine fever (ASF) is a highly contagious and deadly swine disease, causing a lot of damage to farmers and smallholder village farms, as well as pork production worldwide. Unfortunately, the disease has spread significantly in recent years and is now a major concern in many countries. ASF was first identified in a Black Sea harbour in Georgia in 2007, and since then, it has spread to the European Union (EU), including Romania. In Romania, the disease was first diagnosed in Satu Mare County in 2017 and then in Constanta County in July 2018. Since then, ASF has been reported among pig farms with generally low biosecurity and in wild boar populations. Considering the role of wild boars in the maintenance and transmission of ASF virus, the occurrence of ASF in wild boar should not be underestimated. The study involved surveillance actions carried out by official veterinarians and hunters who collected a total of 6820 samples for PCR analysis and 4248 samples were analysed using ELISA method, from 2018-2013. The data obtained from these tests were statistically analysed using IBM SPSS Statistics for Windows, version 29.0 emphasizing the advantage of using reliable and advanced statistical tools that can lead to a better understanding and management of ASF disease. This extensive collection of data improves the robustness of the study and allows for a more thorough analysis of health trends over time. The detailed breakdown of samples collected each year on each species in which the disease was confirmed, the number of susceptible animals or showing clinical signs of the disease provides valuable information on temporal changes in ASF disease status data. The methodology and findings presented can serve as a reference for future studies that increase understanding of trends and can lay the foundations for future efforts that can influence decisions and interventions in the field.

Keywords: African Swine Fever transmission; contagious; biosecurity; pig; wild boar; statistical analyse of the disease evolution

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Introduction

African Swine Fever (ASF) is a highly contagious and deadly swine disease, devastating the livelihood of farmers and smallholder village farms and impacting pork production worldwide [1]. ASF virus is a DNA virus of icosahedral form and large size belonging to the genus *Asfavirus*, the only member of the *Asfaviridae* family [2]. ASF evolved as a disease endemic to the African continent, spreading to Europe, Asia, America, and Oceania [3].

ASF was initially documented in Kenya in 1921, marking the first confirmed case. Initially confined to sub-Saharan African nations, it has persisted as an endemic presence, impacting as many as 35 African countries. The virus is upheld within an ancient sylvatic cycle, with African wild pigs, primarily warthogs (Phacochoerus africanus) and argasid ticks (Ornithodoros spp.), serving as the natural vector of the virus [4]. From the late 1950s to the early 1980s, the ASF virus (ASFV) genotype I emerged in various regions across the globe, including Europe, Russia, the Caribbean, and South America. The virus's presence in Europe was first identified in Portugal in 1957, and it reappeared in 1960, subsequently spreading rapidly to Spain, France, Malta, Belgium, Italy, and the Netherlands [5]. In 1978, a new outbreak of ASF occurred in Sardinia, Italy. Russia reported ASFV in 1977, and during the late 1970s, the virus surfaced in Brazil, Cuba, and the Caribbean Islands. By the mid-1990s, ASFV was successfully eradicated outside of Africa, except for an isolated outbreak in Portugal in 1999, attributed to its introduction into a pig shelter infested with Ornithodoros erraticus ticks and the enduring endemic presence on the island of Sardinia (Italy) [6].

Direct contact between sick and healthy animals is one of the most evident ways of virus transmission. Another source of ASFV incursion in ASF-free countries is the import of pork products derived from infected animals. If Local Authorities do not detect the dis-ease at the farm or abattoir level, infected pigs can be slaughtered, and their contaminated carcasses can be used for fresh or processed pork products. Even though swill feeding is illegal in most countries worldwide, including the EU, some pigs raised in backyard or free-ranging small farms are fed with untreated food leftovers or catering waste [7]. Bellini and collaborators conducted a meta-analysis involving 52 studies to identify risk factors involved in introducing and spreading ASF. They identified several factors, including contaminated objects and meat products, pig transportation in contaminated vehicles, using feed or bedding from areas where wild boars have access, and the possibility of farm employees or visitors participating in activities related to wild boars, such as hunting [8].

The clinical forms of the disease range from acute, characterized by sudden death and without clinical signs or with minor clinical signs, to sub-clinic infection (asymptomatic) and chronic form. In hyper-acute and acute forms, clinical signs are dominated by anorexia, lethargy, weakness, decubitus, hyperthermia, marked leukopenia, and cyanosis of the extremities and abdominal region. Erythema, skin hemorrhage, melena, epistaxis, morbidity, and high mortality are characteristic. Death occurs in 1-5 days in hyper-acute forms and 7-10 days in acute forms, with a mortality rate of 100%. In the acute form, severe leukopenia, dyspnea, vomiting, and epiphora are found. Some experience recurrent hyperthermia, lack of appetite, and emaciation. The chronic form is characterized by fever, loss of appetite, thickening of joints, occasional diarrhea, and vomiting [9; 10]. In addition, a recent study found that pregnant sows experienced the earliest disease onset, which led to abortion [11].

Technical difficulties, such as the lack of stable cell lines, gaps in knowledge concerning ASFV infection and immunity, ASFV genetic complexity, and the lack of development of neutralizing antibodies, have hindered vaccine development [12]. In 2019, a research group published the first report on the oral immunization of wild boars with a non-hem adsorbing, attenuated ASF virus of genotype II isolated in Latvia in 2017 [13]. However, further studies should assess the safety of repeated administration and overdose, characterize long-term shedding, and verify the genetic stability of the vaccine virus to confirm its suitability for free-ranging wild boars in ASF control programs. In June 2022, the National Veterinary Medicine Joint Stock Company (Navetco, Vietnam) approved the first ASF vaccine. This recombinant attenuated ASF vaccine was developed from the ASFV-G-ΔI177L strain in porcine peripheral blood mononuclear cells and has been reported to be safe and efficacious in two pig breeds grown in Vietnam [14; 15]. Recent studies have moved forward, being a step closer to a future vaccine using attenuated strain HLj/18-7GD with the deletion of seven genes. This vaccine has been fully evaluated and proven safe and effective against ASF [16; 17].

Materials and methods

Epidemiological investigation

In order to identify the ASF virus, the host and the environmental factors that cause this disease, an entire team, represented by veterinarians and engineers from the Animal Health Office within DSVSA Constanta, which has a specific structure and responsibilities regarding the eradication of ASF at the local level based on the requirements of the Operational Manual for ASF (Terrestrial Code Online Access, n.d.) [18], develops the contingency plan and associated responsibilities. These steps are established by the Local Center for Disease

Control (CLCB), organized in three distinct divisions: the Local Decision Unit (ULD), the Local Operational Unit (UOL), which also supervises the Center for Field Investigations (CIT) and the Local Support Unit (ULS).

UDL is located within the institution of the county prefect, the permanent members being made up of the executive director of DSVSA Constanta, representatives of the Inspectorate for Emergency Situations and additional members from the decentralized structures in the region. The objectives of the UDL are to formulate and approve the strategic action plan for disease control, in accordance with the statutory requirements, to supervise the implementation of the action plan, to delimit the responsibilities of the UDL members by sectoral activities and territorial jurisdictions, to periodically evaluate the progress and trends of diseases, as well as the effectiveness of the measures implemented, and to take the necessary actions to strengthen these measures.

UOL is constituted at the county level of the DSVSA. The management of the UOL is entrusted to the executive director of DSVSA Constanta, the deputy director of DSVSA Constanta occupying the position of deputy head of the UOL; under their supervision, five departments are established: the Organization, Supply and Resources Department. (Human and Material), Department of Monitoring, Evaluation and Planning, Department of Epidemiology, Department of External Communication, Department: ICF this includes evaluation teams, monitoring team for the implementation of control measures, team responsible for road disinfectants, team for clinical examination of personnel in restricted areas.

The responsibilities of the ICF include executing the action plan in cases of disease outbreaks, documenting activities pertinent to the advancement and management of the disease to the CLCB, providing guidelines on notification of the outbreak by installing warning signage, and obstructing access routes to the affected farm using barriers such as strips, cables, grids, and breakwaters. In addition, it is imperative to ensure adequate regulation of the movement of animals in yards and holdings located in both protection and surveillance areas, as well as to raise awareness among animal owners and other stakeholders of the decisions and directives associated with control measures. Overseeing sanitation and disinfection processes for shelters and means of transport, along with the disposal of carcasses destined for destruction at a sewerage facility, is also a critical duty. In addition, it is mandated to organise investigations (examinations) and sampling in the designated areas, including the investigations necessary to lift the restrictions. Disposal of contaminated materials and post-cleaning and disinfection tools is also a necessary requirement.

The ULS is established at the level of the territorial administrative units, based on an order issued by the mayor. It is chaired by the mayor of the territorial

administrative unit and includes the deputy mayor of the locality, the representative of the local educational institutions, the representative of the local police station and the head of the Voluntary Service for Emergency Situations, the representatives of the local human health services, the official veterinarian and other actors involved in the fight against diseases (such as the County Directorate for Agriculture and Rural Development, the County Public Health Authority, The County Agency for Environmental Protection, the Environmental Guard and Romsilva, among others) are actively involved at local level. In response to the confirmation of an outbreak of African Swine Fever (ASF), the Veterinary Health Unit (ULS) provides assistance to the county inter-institutional working group (CIT) by providing staff, vehicles, logistical support and facilities, at the request of the County Local Control Council (CLCB), while ensuring that the veterinary staff in the field is promptly informed of any case of suspected epizootic, thus facilitating the activation of the CLCB. Moreover, the ULS has the task of executing the measures presented in the epizootic prevention and control program, disseminated by the National Veterinary Office (UOL), at the local level.

In fulfilling its responsibilities, CLCB applies both European Union regulations and national legislation by implementing surveillance programs aimed at monitoring the ASF situation, performing laboratory analyzes through diagnostic tests and confirming the appearance of the disease. Epidemiological methods are used to establish the origins of ASF disease, the mechanisms of its transmission and the necessary prevention and control strategies. The initial phase involves the collection of data through the surveillance program, including information on symptom onset, disease incidence and mortality among the domestic pig population on a specific farm or the wild boar population near the outbreak, along with the clinical status of the affected pigs. Based on an in-depth evaluation of the data collected, the epidemiology team classifies and synthesizes this information, subsequently identifying discrepancies and drawing conclusions on potential causal factors for disease transmission or associated risk elements. Finally, it proposes and executes strategies through intervention measures aimed at reducing the further spread of the disease and enlightening farmers, hunters and the wider public about the significance of their behaviour in relation to the spread of the disease. Eradication protocols are instituted by the CIT team upon official confirmation of ASF in a site, requiring immediate euthanasia of all pigs.

The CLCB has a mandate to establish a 3 km protection perimeter around the ASF outbreak and will continue with the slaughter of all pigs in the affected enclosure, which will be placed under official veterinary supervision to mitigate any potential for viral transmission during both the transport process and euthanasia. Sampling procedures are performed on all pigs at the time of euthanasia, in accordance with the Terrestrial Code Online Access to determine how the virus is introduced into the facility and to determine the duration of its presence prior to notification of the disease. The disposal of the carcasses and all hazardous materials resulting from the outbreak is carried out by an alternative method of neutralization, by burial in a location selected by the Environmental Protection Agency of Constanta (APM), the Administration of the Dobrogea Coastal Water Basin (ABADL) and the Autonomous County Water Administration (RAJA). Complete measures of mechanical cleaning and disinfection are instituted, along with the incineration of all sources of contamination. Appropriate disinfection measures shall be established at the entrances to the farm and in the stables. A comprehensive mapping of all areas in the protection area shall be carried out. The entry or exit of animals of any species in and/or in the protection area is strictly prohibited. Samples are taken from both sick and deceased animals and sent to the laboratory for confirmation. These samples are to be collected, recorded, statistically processed and used in ASF control and eradication efforts. Gatherings of animals of all species, including fairs, exhibitions, circuses, etc., are expressly prohibited. The application of measures in the protection zone will persist until the completion of the cleaning and disinfection protocols in the infected premises. All pigs located in all farms in the protection zone will be subject to clinical and laboratory evaluations for a period of 45 days. After completing these procedures and confirming the absence of the disease, the restrictions will be lifted.

The ASF surveillance area shall be demarcated within a radius of 10 km around the ASF outbreak and a mapping of all holdings in the surveillance area shall be carried out. The provision of information and education to breeders must be ensured.

Animal owners are mandated to carry out a passive clinical assessment of their pigs and are obliged to report any change in the health status of these animals to the officially appointed veterinarian. The movement of domestic animals outside the surveillance area is strictly prohibited, except for pigs designated for slaughter and those that have received authorization from local veterinary authorities. The gathering of animals of all species in the context of animal fairs and exhibitions is expressly prohibited. Any deceased or sick pig on a given holding must be promptly reported to the competent authority, which will initiate the necessary investigations in accordance with the protocols outlined in the Terrestrial Code Online Access [18]. Communication with the county forestry directorates and branches of the County Association of Sport Hunters and Fishermen (AJVPS) regarding the provisions of the CNCB is essential. The quantification and delimitation of areas inhabited by wild boars shall be evaluated to avoid any interaction with the surveillance area.

The compensation scheme was set up as a national support mechanism to compensate pig producers whose animals have been slaughtered because of the implementation of measures to reduce and eliminate the spread of ASF. The allocation of state aid functions as a compensatory instrument for pig farmers whose animals have been slaughtered, while promoting their maintenance on the free market and guaranteeing a basic income that allows them to resume their activity in the following year according to art. 5 of Annex no. 5 to Law no. 122/2023. In view of the implementation of the veterinary sanitary regulations, the financial damage incurred by pig producers because of the slaughter of pigs, together with the additional losses - in particular destroyed feed, costs related to subsequent disinfection, etc. - as well as the prohibition of pig breeding during the quarantine period, represent considerable obstacles in ensuring the food supply for the rural population. The reintegration of holdings affected in accordance with Article 5 of Council Directive 2002/60/EC of 27 June 2002 of 27 June 2002, as subsequently amended and supplemented, shall not take place until at least 40 days have elapsed since the completion of the cleaning and disinfection protocols (Council Directive 2002/60/EC of 27 June 2002 Laying down Specific Provisions for the Control of African Swine Fever and Amending Directive 92/119/EEC as Regards Teschen Disease and African Swine Fever (Text with EEA Relevance), 2002) [19].

In the context of small-scale operations, such as private households, the restocking process is prefaced by the introduction of sentinel pigs that have tested negative for ASF antibodies or come from farms that are not affected by ASF restrictions (Council Directive 2002/60/EC of 27 June 2002 Laying down Specific Provisions for the Control of African Swine Fever and Amending Directive 92/119/EEC as Regards Teschen Disease and African Swine Fever (Text with EEA Relevance), 2002) [19]. Sentinel pigs shall be strategically distributed throughout the holding in accordance with the provisions laid down by the veterinary authority. After a duration of 45 days, these pigs will be tested to establish the presence of antibodies against the ASF virus, according to the Operational Manual for ASF [18].

If the test results give a negative result, the complete restocking process can begin. In the case of commercial holdings, the restocking of pigs is carried out in accordance with the established legislative directives and is conditional on the total restocking of all pigs from holdings that are not subject to ASF restrictions. Pigs from the newly populated herd are subjected to serological evaluation in accordance with the Operational Manual for ASF (Terrestrial Manual Online Access, n.d.). Sampling for this investigation shall be carried out no earlier than 45 days after the arrival of the final group of pigs. The research team, composed of veterinarians, uses a variety of sources and methodologies to collect data in the field, with the aim of investigating and evaluating the epidemiological background of the disease. A standardized questionnaire is used to gather accurate information regarding the type of production, owner details, the number of additional farms owned by the same owner, the identification of animals and the total number of each animal species owned, as well as geographical coordinates and environmental characteristics such as neighboring farms or agricultural areas, as well as primary and secondary routes and arteries according to the Operational Manual for Intervention in ASF outbreaks (Operational Manual for Intervention in African Swine Fever Outbreaks – 4th Edition – 2019 - A.N.S.V.S.A., 2019) [20].

Data on available biosecurity facilities are also investigated, such as the existence of stables around the farm to prevent contact with wild boars or other pigs. If the farm has spaces for changing clothes, washing hands and disinfecting, in this case it must be specified which active substances are used. The investigation focuses on 30 days before the first signs of illness or suspicion of illness. Data is also collected when the owner uses artificial insemination, the date on which the inoculation took place, the name and address of the semen supplier and the traceability of the donor.

Data on the entry of contaminated meat products and by-products into the farm, if confirmed, the owner must provide details of origin and thus the epidemiological investigation must be extended and thus all participants and the main source of supply of contaminated meat and meat products are recorded. The checks of the activities carried out on the farm are carried out on all employees or family members, such as stable maintenance activities, hunting activities, wood cutting or mushroom picking, agricultural activities that provide animal feed, interaction with other farms.

If the farm is located near a hunting ground confirmed positive for ASF, it is recorded when the last positive case was confirmed by the Real Time PCR or ELISA method and when the last negative test was using the same analysis methods. As regards feed, the owner must specify whether he uses cereals from his own production in this case and whether he has noticed wild boar tracks on his agricultural land. In the event that the owner purchases the feed, the date of purchase, the source, the license plate of the vehicle used for transport and all deliveries to other pig farms are recorded at least 30 days before the first clinical signs from which the onset of ASF appears. It is also recorded if the pigs have been fed with kitchen scraps and if they have not been heat treated. As for the bedding used (straw), it is recorded if it was stored at least 90 days before the outbreak of the epidemic.

Sample analyses methods

Real time PCR analysis

The Real-Time PCR method was used to identify the specific genome targets of the ASFV using specific primers and probes following the WOAH Terrestrial Manual Chapter 3.9.1. [18]. Organ specimens and blood samples on anticoagulant (EDTA) were are DNA extracted and purified using IndiSpin Pathogen Kit (Indical Bioscience).

The preparation of Master Mix and DNA mixing. The amplification kit allows the realization of a fast, specific, and sensitive PCR test by using Tag Man enzyme. Reagents used were Mater Mix SSO Advance Universal Probes SuperMix 500 Bio Rad USA and specific ASFV Primer and Probe (Genentech) were regenerated in Nuclease Feree Water (Qiagen) to obtain 100 μ M stock solution and 10 μ M working solution which both were stored in the freezer at -20°C. All reagents were removed from the freezer and stored on colling block until use. The Real Time PCR assay used 5 μ l of DNA with Mater Mix SSO Advance Universal Probes SuperMix 500 Bio Rad USA in a final volume of 20 μ l following the manufacturers protocol.

In each PCR experiment, four controls were included: two positive controls (positive extraction control and positive mix control) and two negatives (negative extraction control and no templet control). The positive extraction control wa represented by the internal reference material (viral strain) strain characterized by the NRL IDSA Bucharest, and the positive mix control was represented by a previously extracted positive DNA. The negative extraction control was represented by water and no templet controls is a sample that does not contain biological material.

It is considered validated the test in which the positive test/samples and the positive controls are positive, and the negative test/samples and the negative controls are negative.

Real-time PCR reactions w performed on Applied Biosystems 7900HT Fast Real-Time PCR system uses fluorescent-based PCR chemistries to provide quantitative detection of nucleic acid sequences using real-time analysis. Software version SDSv2.4 and the following thermal profile: 3 min at 95°C (1 cycle), (15 s at 95°C, 60 s at 60°C) (45 cycle), 30 s at 40°C (1 cycle) (Figure 1). The result was considered negative for CT values \geq 39. [21] (Figure 2).

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Figure 1. Thermal Profile used for data collection.



Figure 2. Amplification plot showing the ASFV positive controls and the positive sample.

ELISA analysis

ELISA method detects antivirus antibodies in ASFV by immunoassay technique. Plasma and serum samples were analysed using the ID Screen African Swine Fever Indirect Screening Test and ELISA reader (Ledetect 96 Led Based & Channel Microplate Reader Austria).

The microwells are coated with ASF p32, p62 and p72 recombinant proteins. Test samples and controls were added to microwells. Anti-ASFV antibodies, if present, form an antigen-antibody complex. After washing, an anti-

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multi-species conjugate with horseradish peroxidase (HRP) was added to the wells. It binds to antibodies, forming an antigen-antibody-conjugate-peroxidase complex. After removing excess conjugate by washing the substrate solution (TMB) was added. The resulting staining is proportional to the quantity of specific antibodies present in the sample. In the presence of antibodies, a blue solution appears that turns yellow after adding the stop solution (Figure 3). In the absence of antibodies, no staining occurs. The microplate is read at 450nm (Figure 4).



Figure 3. The presence of antibodies coloured in yellow.

| - | - | A 18 1 1 1 | 1 22 | | | -721 | | - | _ | - | - | _ |
|---|-------|------------|------|------|---|------|-----|-------|------|-----|------|----|
| | | | 100 | # | - | | × | 0.845 | | (*) | • | |
| | 1 | 1 | × | Ξ. | | Ē | 8 | - | •• | - | 5 | 3 |
| | 10 | - 24 | 1 | | | | 3 | 1944 | | 38 | 20 | 3 |
| | | (1971) | 500 | 10 | • | 10 | 100 | 1993 | | • | | 2 |
| | | T | 1 | ÷ | | 1 | 8 | - | 2 | | - | i. |
| - | - (4) | 144 | 200 | - 14 | - | × | 14 | 1943 | - 24 | 3 | - 20 | 3 |
| | | 1.17 | 200 | | | * | 1 | (19) | | 1 | - 50 | 2 |
| | | 16. | | 1 | 3 | 12 | 12 | - | | | 10 | 3 |

Figure 4. Microplate reading at 450nm.

The plates are read at an optical density (OD) of 450nm using ELISA reader (Ledetect 96 Led Based &; Channel Microplate Reader within 5 min after adding the stopping solution. The samples that reacted positively and doubtful were be sent to NRL IDSA Bucharest. Positive and doubtful sample is kept for 60 days in the freezer at -20°C as counter evidence.

The validity of the results was ensured by using 2 positive and two negative controls. The validated test is considered when the mean optical density positive control (ODPC) value is > 0,350. The ratio of averages of positive and negative control values (ODPC and ODNC) is > 3.

Interpretation of results include calculating for each sample the percentage S/P according to the formula S/P%= (OD sample-OD NC) / (OD PC-ODNC) X 100

Samples with $%S/P \le 30\%$ are negative.

Samples with %S/P > 30% < 40% are dubious (S/P% between 30% and 40%).

Samples with $S/P \ge 40\%$ are positive.

When working on duplicate samples, the average of the two optical density values of the samples.

Statistical analysis

For statistical data analysis, IBM SPSS Software Descriptive Statistics for Windows, Version 29.0 (30-day trial version) was used. Nominal data were presented as absolute frequency and percentage, and continuous variables were expressed as mean and standard deviation. A value of the coefficient of statistical significance p<0.05 was considered significant. The analysis of the epidemiological curve was done using the Kruskal Wallis and Mann-Whitney U tests and adjusting the materiality threshold according to the number of comparisons (15 in our case).

Results

ASF was first reported in Romania in July 2017, starting with two outbreaks notified in a backyard holding with four pigs in Satu-Mare County [22]. As of 05th July 2018, ASF had been confirmed for the first time in Constanta County in one backyard farm in the province of Istria. The index case described during the investigation was a pregnant sow found dead in the stable. Following the notification of the official veterinarian in the affected area, samples were collected and sent to a laboratory for Real-Time PCR analyses, which were positive for ASF. The affected area has been under official surveillance, according to the control measures established by the PPA Operational Manual [23].

Subsequently, on 11th July 2018, an ASF outbreak was confirmed when seventeen more pigs died on a smallholding in the province of Cheia, approximately 32 km from Istria. The action was taken in both outbreaks to comply with At.5 of EU 429/2016 and according to EU 1099/2009 [24] relative to the method of killing and animal protection. In ASF, animals, products, and waste can be destroyed by burial and burning methods in an approved location. During

the killing, strict biosecurity procedures were followed by spraying the carcasses, tissues, blood, shelter, and yard with disinfectant. Several attempts were made to prevent the ASF virus spread by restricting animals, vehicles, and equipment to and from the outbreak and setting the protection zone (3km) and the surveillance zone (10 km) (Figure 5).

Despite the efforts to isolate each outbreak, 164 ASF outbreaks have been notified by the end of 2023.



Figure 5. The protection and surveillance zones of the ASF index case Istria measure a minimum of 3 km and 10 km respectively.

Situation of ASF outbreaks

Confirmed ASF outbreaks

An epidemiologic curve was used to identify how the ASF virus was transmitted. The curve indicates 93 new ASF outbreaks declared in 2018 (254 pigs and 4 wild boars), followed by a decrease, reaching 34 ASF outbreaks in 2019 (19 pigs and 50 wild boars) and tampering down to 3 ASF outbreaks in 2020 (4 pigs and 2 wild boars). In 2021, 11 ASF outbreaks were confirmed (62 pigs and 2 wild boars); in 2022, just 2 ASF outbreaks were confirmed (4 pigs and 1 wild boar), followed by 21 ASF outbreaks confirmed in 2023 (76 pigs) (Figure 6). This chart shows a propagated epidemic trend, as there is no common source of infection. The progress of the ASF is represented by the high number of confirmed domestic pig cases in 2018. A high number of notifications in wild boar and domestic pigs was confirmed in 2019, 2020, and 2021. However, in 2023, no cases of ASF in wild boars were reported.

Table 1 and graphic 1 shows the outbreaks of ASF by year and by species in which the disease occurred.

| Year | | Swine | ١ | Wild boar | Total number |
|------|--------|----------------|--------|----------------|--------------|
| | Number | Percentage (%) | Number | Percentage (%) | - |
| 2018 | 89 | 95.7 | 4 | 4.3 | 93 |
| 2019 | 8 | 23.5 | 26 | 76.5 | 34 |
| 2020 | 1 | 33.3 | 2 | 66.7 | 3 |
| 2021 | 9 | 81.8 | 2 | 18.2 | 11 |
| 2022 | 1 | 50 | 1 | 50 | 2 |
| 2023 | 21 | 100 | 0 | 0 | 21 |

 Table 1. ASF outbreaks occurred between 2018-2023



Graph 1. The annual distribution of ASF confirmed outbreaks by species in which the disease occurred.

Statistical analysis confirmed that more ASF outbreaks were identified in August (54 outbreaks), July (26), and September (25) (Table 2).

| Date | Swi | ine outbreaks | Wild | boar outbreaks |
|-----------|--------|----------------|--------|----------------|
| | Number | Percentage (%) | Number | Percentage (%) |
| January | 3 | 2.32 | 3 | 8.57 |
| February | 2 | 1.55 | 7 | 20 |
| March | 4 | 3.1 | 5 | 14.2 |
| April | 3 | 2.32 | 2 | 5.7 |
| May | 0 | 0 | 4 | 11.4 |
| June | 1 | 0.77 | 0 | 0 |
| July | 26 | 20.1 | 0 | 0 |
| August | 54 | 41.8 | 0 | 0 |
| September | 22 | 17 | 3 | 8.57 |
| October | 7 | 5.42 | 2 | 5.7 |
| November | 6 | 4.6 | 4 | 11.4 |
| December | 1 | 0.77 | 5 | 14.2 |
| Total | 129 | 100 | 35 | 100 |

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| Table 2. AST outbreaks comminatio | Table 2 | ASF | outbreaks | confirma | ation |
|-----------------------------------|---------|-----|-----------|----------|-------|
|-----------------------------------|---------|-----|-----------|----------|-------|

Number of susceptible animals

The average number of animals likely to be sick per outbreak was 28.05. The number of susceptible animals per outbreak was a minimum of 1 and a maximum of 237. The total number of susceptible animals during the study period was 3085. The average number of susceptible animals per outbreak ranged from 13 in 2022 to 25 in 2020. The maximum number of susceptible animals per outbreak ranged from 19 in 2022 to 237 in 2019.

The total number of susceptible animals was as follows: 1672 in 2018, 849 in 2019, 75 in 2020, 187 in 2021, 26 in 2022 and 276 in 2023 (Table 3).

| Animal species in which the disease occurred | Year of confirmatio n of the disease | Oubreaks number | Mean | Std. Deviation | Minimu m | Maximu m | Total susceptibl e animals |
|--|---|--------------------|-------|-------------------|-------------|-------------|----------------------------------|
| Swine | 2018 | 89 | 17,57 | 25,344 | 1 | 130 | 1564 |
| | 2019 | 8 | 14,25 | 21,137 | 1 | 66 | 114 |
| | 2020 | 1 | 68,00 | | 68 | 68 | 68 |
| | 2021 | 9 | 18,11 | 15,536 | 1 | 45 | 163 |
| | 2022 | 1 | 19,00 | | 19 | 19 | 19 |
| | 2023 | 21 | 13,14 | 15,650 | 1 | 61 | 276 |

Table 3. Number of susceptible animals

| | Maria Virginia Tanasa (Acreței), Natalia Roșoiu | | | | | | | | | | | |
|-----------|--|----|-------|--------|----|-----|-----|--|--|--|--|--|
| Wild boar | 2018 | 4 | 27,00 | 30,078 | 10 | 72 | 108 | | | | | |
| | 2019 | 26 | 28,27 | 45,669 | 2 | 237 | 735 | | | | | |
| | 2020 | 2 | 3,50 | 2,121 | 2 | 5 | 7 | | | | | |
| | 2021 | 2 | 12,00 | 14,142 | 2 | 22 | 24 | | | | | |
| | 2022 | 1 | 7,00 | | 7 | 7 | 7 | | | | | |

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Number of animals with clinical signs at the time of declaration of disease

At the time of disease declaration, the average number of animals per outbreak was 2.68. The minimum of sick animals at the date of disease declaration was 1, and the maximum was 42. The total number of sick animals at the time of the disease declaration was 440. Most animals sick at the date of disease declaration were in 2018 (258) (Table 4).

Table 4. Number of animals with clinical signs at the time of ASF declaration

| Animal species in which the | | | | | | | |
|-----------------------------------|------|----------|------|-----------|---------|--------|------------|
| disease | | Oubreaks | | Std. | | Maximu | Total sick |
| occurred | Year | number | Mean | Deviation | Minimum | m | animals |
| Swine | 2018 | 89 | 2,85 | 5,318 | 1 | 42 | 254 |
| | 2019 | 8 | 2,38 | 1,506 | 1 | 5 | 19 |
| | 2020 | 1 | 4,00 | | 4 | 4 | 4 |
| | 2021 | 9 | 6,89 | 9,117 | 1 | 23 | 62 |
| | 2022 | 1 | 4,00 | | 4 | 4 | 4 |
| | 2023 | 21 | 1,81 | 1,167 | 1 | 4 | 38 |
| Wild boar | 2018 | 4 | 1,00 | ,000 | 1 | 1 | 4 |
| | 2019 | 26 | 1,92 | 1,230 | 1 | 5 | 50 |
| | 2020 | 2 | 1,00 | ,000 | 1 | 1 | 2 |
| | 2021 | 2 | 1,00 | ,000 | 1 | 1 | 2 |
| | 2022 | 1 | 1,00 | | 1 | 1 | 1 |

Total animals with clinical signs since the beginning of the epizootic disease

The total number of animals confirmed with ASF disease since the start of the outbreaks was 489, with an average of 2.98 animals per outbreak, with a maximum at the beginning of 2018 (259 animals) (Table 5).

Table 5. The number of the ill animals confirmed since the beginning of the epizootic disease. The number of the ill animals confirmed since the beginning of the epizootic disease

| Animal species in which the | | | | | | | |
|-----------------------------------|------|----------|------|-----------|--------|--------|------------|
| disease | | Oubreaks | Mea | Std. | Minimu | Maximu | Total sick |
| occurred | Year | number | n | Deviation | m | m | animals |
| Swine | 2018 | 89 | 2,85 | 5,318 | 1 | 42 | 254 |
| | 2019 | 8 | 2,38 | 1,506 | 1 | 5 | 19 |
| | 2020 | 1 | 4,00 | | 4 | 4 | 4 |
| | 2021 | 9 | 6,89 | 9,117 | 1 | 23 | 62 |
| | 2022 | 1 | 4,00 | | 4 | 4 | 4 |
| | 2023 | 21 | 3,62 | 2,334 | 2 | 8 | 76 |
| Wild boar | 2018 | 4 | 1,25 | ,500 | 1 | 2 | 5 |
| | 2019 | 26 | 2,31 | 1,644 | 1 | 8 | 60 |
| | 2020 | 2 | 1,00 | ,000 | 1 | 1 | 2 |
| | 2021 | 2 | 1,00 | ,000 | 1 | 1 | 2 |
| | 2022 | 1 | 1,00 | | 1 | 1 | 1 |

Animals killed and destroyed

A total of 1985 animals were killed and destroyed: 1406 in 2018, 134 in 2019, 70 in 2020, 107 in 2021, 16 in 2022 and 252 in 2023 (Tabel 6).

 Table 6. Animals killed and destroyed

| Animal species in which the disease occurred | Year | Oubrea ks number | Mean | Std. Deviation | Minimu m | Maximu m | Total killed animals |
|--|------|------------------------|-------|-------------------|-------------|-------------|----------------------------|
| Swine | 2018 | 89 | 15,76 | 24,088 | 0 | 123 | 1403 |
| | 2019 | 8 | 12,50 | 20,771 | 0 | 63 | 100 |
| | 2020 | 1 | 68,00 | | 68 | 68 | 68 |
| | 2021 | 9 | 11,67 | 13,029 | 0 | 37 | 105 |
| | 2022 | 1 | 15,00 | | 15 | 15 | 15 |
| | 2023 | 21 | 12,00 | 15,238 | 0 | 57 | 252 |
| Wild boar | 2018 | 4 | ,75 | ,957 | 0 | 2 | 3 |
| | 2019 | 26 | 1,31 | 1,408 | 0 | 5 | 34 |
| | 2020 | 2 | 1,00 | ,000 | 1 | 1 | 2 |
| | 2021 | 2 | 1,00 | ,000 | 1 | 1 | 2 |
| | 2022 | 1 | 1,00 | | 1 | 1 | 1 |

PCR testing in the context of surveillance of ASF

In the period 2018-2023, a number of 2606 samples were taken for the PCR test, of which 518 (19.9%) in 2018, 538 (20.6%) in 2019, 360 (13.8%) in 2021, 445 (17.1%) in 2022, respectively 384 (14.7%) in 2023 (Table 7; Graph 2).

| Year | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | Total |
|-------|------|------|------|------|------|------|-------|
| Total | 518 | 538 | 360 | 361 | 445 | 384 | 2606 |
| % | 19,9 | 20,6 | 13,8 | 13,9 | 17,1 | 14,7 | 100,0 |

Table 7. Number of samples taken each year

Graph 2. Graphical representation of the number of samples taken each year



Number of samples analysed by Real Time PCR

The number of samples analysed in the period 2018-2023 by the PCR method was 6820, of which 942 in 2018, 1551 in 2019, 1168 in 2020, 1127 in 2021, 1132 in 2022, respectively 900 in 2023 (Table 8; Graph 3).

Table 8. The number of samples analysed by the Real Time PCR method per year

| Year | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 | Total |
|----------------|------|------|------|------|------|------|-------|
| No. of samples | 518 | 538 | 360 | 361 | 445 | 384 | 2606 |
| No. of samples | 942 | 1551 | 1168 | 1127 | 1132 | 900 | 6820 |



Graph 3. Graphical representation of the number of samples analysed per year

Place sampling

Most of the samples were taken from commercial farms (1556). 844 samples were taken from the hunting complex, and 206 (7.9%) from non-professional farms (Table 9; Graph 4).

Table 9. Year and place of sampling

Year and Place of sampling

| | | |] | Place of sampling | 5 | |
|-------|------|-------------|---------|-------------------|-----------------|--------|
| | | | | Commercial | | |
| | | | Hunting | industrial | Traditional pig | |
| | | | Complex | farming | farming | Total |
| Year | 2018 | Frequencies | 270 | 155 | 93 | 518 |
| | | % | 52,1% | 29,9% | 18,0% | 100,0% |
| | 2019 | Frequencies | 153 | 364 | 21 | 538 |
| | | % | 28,4% | 67,7% | 3,9% | 100,0% |
| | 2020 | Frequencies | 90 | 262 | 8 | 360 |
| | | % | 25,0% | 72,8% | 2,2% | 100,0% |
| | 2021 | Frequencies | 81 | 266 | 14 | 361 |
| | | % | 22,4% | 73,7% | 3,9% | 100,0% |
| | 2022 | Frequencies | 147 | 262 | 36 | 445 |
| | | % | 33,0% | 58,9% | 8,1% | 100,0% |
| | 2023 | Frequencies | 103 | 247 | 34 | 384 |
| | | % | 26,8% | 64,3% | 8,9% | 100,0% |
| Total | | Frequencies | 844 | 1556 | 206 | 2606 |
| | | % | 32,4% | 59,7% | 7,9% | 100,0% |



Graphic 4. Graphic representation of the place where the samples were taken each year

Context of sampling

The overwhelming majority of samples (97%) were collected in the context of surveillance of the spread of ASF and only 3% were collected on request (Table 10; Graph 5).

| | | | Context of sampl | ing | Total |
|-------|------|-----------|------------------|------------|--------|
| | | | Supervision | On request | |
| Year | 2018 | Frequency | 476 | 42 | 518 |
| | | % | 91,9% | 8,1% | 100,0% |
| | 2019 | Frequency | 509 | 29 | 538 |
| | | % | 94,6% | 5,4% | 100,0% |
| | 2020 | Frequency | 356 | 4 | 360 |
| | | % | 98,9% | 1,1% | 100,0% |
| | 2021 | Frequency | 360 | 1 | 361 |
| | | % | 99,7% | 0,3% | 100,0% |
| | 2022 | Frequency | 442 | 3 | 445 |
| | | % | 99,3% | 0,7% | 100,0% |
| | 2023 | Frequency | 384 | 0 | 384 |
| | | % | 100,0% | 0,0% | 100,0% |
| Total | | Frequency | 2527 | 79 | 2606 |
| | | % | 97,0% | 3,0% | 100,0% |

Table 10. The context of sampling each year



Types of samples

The types of samples were multiple, but mainly organs (71.3%). Other types of samples collected were blood on EDTA (28%), bone tissue (0.5%) and animal carcasses (0.3%) (Table 11; Graph 5)

Table 11. Type of samples taken each year

| | | | | T | ypes of | f samples | Total |
|-------|------|-----------|--------|-------|---------|---------------|--------|
| _ | | | Corpse | Organ | Bone | Blood on EDTA | |
| Year | 2018 | Frequency | 3 | 449 | 3 | 63 | 518 |
| | | % | 0,6% | 86,7% | 0,6% | 12,2% | 100,0% |
| | 2019 | Frequency | 2 | 441 | 9 | 86 | 538 |
| | | % | 0,4% | 82,0% | 1,7% | 16,0% | 100,0% |
| | 2020 | Frequency | 1 | 234 | 0 | 125 | 360 |
| | | % | 0,3% | 65,0% | 0,0% | 34,7% | 100,0% |
| | 2021 | Frequency | 2 | 223 | 0 | 136 | 361 |
| | | % | 0,6% | 61,8% | 0,0% | 37,7% | 100,0% |
| | 2022 | Frequency | 0 | 285 | 0 | 160 | 445 |
| | | % | 0,0% | 64,0% | 0,0% | 36,0% | 100,0% |
| | 2023 | Frequency | 0 | 225 | 0 | 159 | 384 |
| | | % | 0,0% | 58,6% | 0,0% | 41,4% | 100,0% |
| Total | | Frequency | 8 | 1857 | 12 | 729 | 2606 |
| | | % | 0,3% | 71,3% | 0,5% | 28,0% | 100,0% |



Graph 6. Graphical representation of the type of samples taken each year

Animal condition

38.9% of the animals from which samples were taken were dead, 30.9% were shot, 28.9% were with clinical signs of disease. Only 1.2% of the samples were taken from emergency cuts and 0.1% from normal cuts (Table 12; Graphic 7)

Table 12. Clinical status of animals at the time of sample collection per year

| | | | Clinical status of animals at the time of harvest | | | | | | |
|-------|------|-----------|---|-------|-------|------------------|-----------------|--------|--|
| | | | With | | | | Emergen | L | |
| | | | clinical signs | Shot | Death | Normal slaughter | cy slaughter | Total | |
| Year | 2018 | Frequency | 65 | 262 | 191 | 0 | 0 | 518 | |
| | | % | 12,5% | 50,6% | 36,9% | 0,0% | 0,0% | 100,0% | |
| | 2019 | Frequency | 96 | 134 | 308 | 0 | 0 | 538 | |
| | | % | 17,8% | 24,9% | 57,2% | 0,0% | 0,0% | 100,0% | |
| | 2020 | Frequency | 126 | 87 | 146 | 0 | 1 | 360 | |
| | | % | 35,0% | 24,2% | 40,6% | 0,0% | 0,3% | 100,0% | |
| | 2021 | Frequency | 138 | 80 | 143 | 0 | 0 | 361 | |
| | | % | 38,2% | 22,2% | 39,6% | 0,0% | 0,0% | 100,0% | |
| | 2022 | Frequency | 168 | 143 | 106 | 2 | 26 | 445 | |
| | | % | 37,8% | 32,1% | 23,8% | 0,4% | 5,8% | 100,0% | |
| | 2023 | Frequency | 161 | 100 | 120 | 0 | 3 | 384 | |
| | | % | 41,9% | 26,0% | 31,3% | 0,0% | 0,8% | 100,0% | |
| Total | | Frequency | 754 | 806 | 1014 | 2 | 30 | 2606 | |
| | | % | 28,9% | 30,9% | 38,9% | 0,1% | 1,2% | 100,0% | |



Graphic 7. Graphical representation of the clinical status of the animals at the time of sample collection per year

No. Positive sample

A total of 218 positive samples were determined from 152 samples (Table 13).

 Table 13. The number of samples taken with a positive result and the total number of samples analysed in which we obtained positive PCR test results during the period 2018-2023

| | No. Positive sample | |
|---|---------------------|--|
| Number of samples with positive samples | 152 | |
| Number of positive samples | 218 | |

Most positive samples were determined in 2018 (112 samples) (Table 14; Graph 8).

 Table 14. The number of samples taken with a positive result and the total number of samples analysed in which we obtained positive PCR test results for each year

| | No. Positive sample | | | | | |
|---|---------------------|------|------|------|------|------|
| | Year | | | | | |
| | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 |
| Number of samples with positive samples | 82 | 26 | 2 | 11 | 3 | 28 |
| Number of positive samples | 112 | 44 | 5 | 20 | 3 | 34 |



Graphic 8. Graphical representation of the number of samples taken each year

Most of the positive samples (162) were determined from samples taken from non-professional farms (Table 15).

 Table 15. Number of samples taken with a positive PCR test result and place of sampling during the period 2018-2023

| Place of sampling | Number of samples with pos samples | itiveNumber of positive samples |
|---------------------------|------------------------------------|------------------------------------|
| Hunting Complex | 28 | 41 |
| Commercial holding (farm) | 3 | 15 |
| Non-professional holding | 121 | 162 |

Most positive samples (218) were determined from samples collected through the surveillance programme (Table 16).

 Table 16. Number of samples taken with a positive PCR test result and the context of sampling in the period 2018-2023

| Context of sampling | Number of samples positive samples | withNumber samples | of | positive |
|---------------------|------------------------------------|-----------------------|----|----------|
| Supervision | 152 | 218 | | |
| On request | 0 | 0 | | |

A total of 218 positive samples were determined over the period 2018-2023, of which the majority (103) were blood samples on EDTA (Table 17)

 Table 17. Number of samples taken with a positive PCR test result and sample matrix for the period 2018-2023

| | Number of samples with | | | | |
|---------------|------------------------|----------------------------|--|--|--|
| Matrix Probe | positive samples | Number of positive samples | | | |
| Corpse | 4 | 5 | | | |
| Organ | 72 | 85 | | | |
| The | 12 | 25 | | | |
| Blood on EDTA | 64 | 103 | | | |

A total of 218 positive samples were determined over the period 2018-2023, of which most (102) were taken from animals showing clinical signs of disease (Table 18).

 Table 18. Number of samples taken with a positive PCR test result and the condition of the animals at the time of collection during the period 2018-2023

| Animal condition | Number of samples w positive samples | vithNumber of positive samples |
|--------------------------------|---|--------------------------------|
| With clinical signs of illness | 63 | 102 |
| Shot | 14 | 14 |
| Death | 72 | 99 |
| Normal cutting | 0 | 0 |
| Emergency slaughter | 3 | 3 |

We have also considered the negative results obtained from the PCR test. The negative findings have an important role in the epidemiologist and in understanding the dynamics of ASF disease. Knowing these negative outcomes is essential for improving understanding ASF disease patterns and refining surveillance strategies, 6602 negative samples were determined out of 2459 samples taken during the period 2018-2023 (Table 19; Graph 9).

 Table 19. The number of samples taken with a negative result and the total number of samples analysed in which we obtained negative PCR test results during the period 2018-2023

| | | | | No. samples negative | | |
|---------------------------------------|--------|------|------|----------------------|------|------|
| | Year | | | | | |
| | 2018 | 2019 | 2020 | 2021 | 2022 | 2023 |
| Number of samples winnegative samples | ith440 | 512 | 358 | 350 | 443 | 356 |
| Number of negative samples | 830 | 1507 | 1163 | 1107 | 1129 | 866 |

Graphic 9. Graphical representation of samples with negative PCR test result for each year



Most of the samples were taken from commercial farms (1553). 817 samples were taken from the hunting complex, and 1553 from non-professional farms (Table 20).

 Table 20. Number of samples taken with a negative PCR test result and place of sampling for the period 2018-2023

| Place of sampling | Number o samples | of samples | with | negativeNumber samples | of | negative |
|---------------------------|---------------------|------------|------|---------------------------|----|----------|
| Hunting Complex | 817 | | | 1086 | | |
| Commercial holding (farm) | 1553 | | | 5312 | | |
| Non-professional holding | 89 | | | 204 | | |

Most of the 2380 samples were collected in the context of surveillance of the spread of ASF and only 79 were collected on request (Table 21).

 Table 21. Number of samples taken with a negative PCR test result and the context of sampling for the period 2018-2023

| Context of sampling | Number of samples with negative | Number samples samples | of | negative |
|---------------------|---------------------------------|---------------------------|----|----------|
| Supervision | 2380 | 6243 | | |
| On request | 79 | 359 | | |

The types of samples were multiple, but predominantly there were organs 1786. Other types of samples collected were blood on EDTA 669, bone tissue 0 and animal carcasses 4 (Table 22).

 Table 22. Number of samples taken with a negative PCR test result and sample matrix for the period 2018-2023

| Matrix Probe | Samples collected | Number of negative samples |
|---------------|-------------------|----------------------------|
| Corpse | 4 | 4 |
| Organ | 1786 | 4179 |
| The | 0 | 0 |
| Blood on EDTA | 669 | 2419 |

Of the animals from which samples were taken, 942 were found dead, 793 were shot, 695 were with clinical signs of disease. Only 27 of the samples were taken from emergency pruning and 2 from normal pruning (Table 23).

Table 23. Number of samples taken with a negative PCR test result and the condition of the animals at the time of collection during the period 2018-2023

| Animal condition | Number of sample negative samples | es withNumber samples | of neg | gative |
|--------------------------------|-----------------------------------|--------------------------|--------|--------|
| With clinical signs of illness | 695 | 2498 | | |
| Shot | 793 | 1057 | | |
| Death | 942 | 3009 | | |
| Normal cutting | 2 | 2 | | |
| Emergency slaughter | 27 | 36 | | |

ELISA testing in the context of surveillance of ASF

ELISA testing

ELISA testing in the context of ASF surveillance. Between 2019 and 2023, 4248 ELISA samples were analysed, of which 2045 were in 2019, 265 were in 2020, 1216 were in 2021, 415 were in 2022 and 317 were in 2023 (Table 23). Most positive samples were determined in 2019 (25) and 2021 (19) (Table 29).

Number of samples taken

In the period 2019-2023, a total of 693 samples were taken for the ELISA test, of which 214 (30.9%) in 2019, 91 (13.1%) in 2020, 115 (16.6%) in 2021, 153 (22.1%) in 2022, respectively 120 (17.3%) in 2023 (Table 24; Graph 10).

 Table 24. Number of samples collected and analysed using the ELISA method in the period 2019-2023

| | 2019 | 2020 | 2021 | 2022 | 2023 | Total |
|-----------|------|------|------|------|------|-------|
| Frequency | 214 | 91 | 115 | 153 | 120 | 693 |
| % | 30,9 | 13,1 | 16,6 | 22,1 | 17,3 | 100,0 |

Graph 10. Graphical representation of the number of samples collected and analysed using the ELISA method in the period 2019-2023



Number of samples analysed

The number of samples analysed in the period 2019-2023 by the ELISA method was 4248, of which 2045 in 2019, 265 in 2020, 1216 in 2021, 415 in 2022, respectively 317 in 2023 (Table 23; Graphic 11).

 Table 23. Number of samples taken and total number of samples analysed using the ELISA method over the period 2019-2023

| | |] | Number of | samples | |
|-----------------------------|------|------|-----------|---------|------|
| | Year | | | | |
| | 2019 | 2020 | 2021 | 2022 | 2023 |
| Number of samples collected | 214 | 91 | 115 | 153 | 120 |
| Number of samples analysed | 2045 | 265 | 1216 | 405 | 317 |

Graph 11. Graphical representation of the total number of samples analysed using the ELISA method over the period 2019-2023



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Place of sampling

Most of the samples were taken from hunting complexes (559). 66 samples were taken from commercial farms, and 68 (9.8%) from non-professional farms (Table 24; Graph 12)

 Table 24. Number of samples taken and place of sampling, to be analysed using the ELISA method for the period 2019-2023

| | | | Place of sam | pling | | |
|-------|------|-----------|--------------------|------------------------------------|--------------------------|--------------|
| | | | Hunting Complex | Commercial industrial (farm) | Traditional j farming | pig Total |
| Year | 2019 | Frequency | 140 | 35 | 39 | 214 |
| | | % | 65,4% | 16,4% | 18,2% | 100,0% |
| | 2020 | Frequency | 89 | 0 | 2 | 91 |
| | | % | 97,8% | 0,0% | 2,2% | 100,0% |
| | 2021 | Frequency | 81 | 24 | 10 | 115 |
| | | % | 70,4% | 20,9% | 8,7% | 100,0% |
| | 2022 | Frequency | 147 | 5 | 1 | 153 |
| | | % | 96,1% | 3,3% | 0,7% | 100,0% |
| | 2023 | Frequency | 102 | 2 | 16 | 120 |
| | | % | 85,0% | 1,7% | 13,3% | 100,0% |
| Total | | Frequency | 559 | 66 | 68 | 693 |
| | | % | 80,7% | 9,5% | 9,8% | 100,0% |

Graph 12. Graphical representation of the number of samples taken and the place of sampling, to be analysed using the ELISA method for the period 2019-2023



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Context of sampling

Most samples (89.8%) were collected in the context of ASF spread surveillance and only 10.2% were collected upon request (Table 25; Graph 13).

 Table 25. Number of samples taken and context of sampling, to be analysed using the ELISA method for the period 2019-2023

| | | | Context of samp | oling | |
|-------|------|-----------|-----------------|------------|--------|
| | | | Supervision | On request | Total |
| Year | 2019 | Frequency | 173 | 41 | 214 |
| | | % | 80,8% | 19,2% | 100,0% |
| | 2020 | Frequency | 91 | 0 | 91 |
| | | % | 100,0% | 0,0% | 100,0% |
| | 2021 | Frequency | 92 | 23 | 115 |
| | | % | 80,0% | 20,0% | 100,0% |
| | 2022 | Frequency | 148 | 5 | 153 |
| | | % | 96,7% | 3,3% | 100,0% |
| | 2023 | Frequency | 118 | 2 | 120 |
| | | % | 98,3% | 1,7% | 100,0% |
| Total | | Frequency | 622 | 71 | 693 |
| | | % | 89,8% | 10,2% | 100,0% |

Year and Context of sampling

Graph 13. Graphical representation of the number of samples taken and the context of the sampling, to be analysed using the ELISA method for the period 2019-2023



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Matrix probe

The tests of two types of blood sample were analysed, blood samples predominantly (96.4%), and 3.6% of the samples were blood serum (Table 26; Graph 14).

 Table 26. Number of samples taken and sample matrix, to be analysed using the ELISA method for the period 2019-2023

| | | | Matrix Probe | | Total |
|-------|------|-----------|--------------|-------------|--------|
| | | | Blood | Blood serum | |
| Year | 2019 | Frequency | 193 | 21 | 214 |
| | | % | 90,2% | 9,8% | 100,0% |
| | 2020 | Frequency | 90 | 1 | 91 |
| | | % | 98,9% | 1,1% | 100,0% |
| | 2021 | Frequency | 112 | 3 | 115 |
| | | % | 97,4% | 2,6% | 100,0% |
| | 2022 | Frequency | 153 | 0 | 153 |
| | | % | 100,0% | 0,0% | 100,0% |
| | 2023 | Frequency | 120 | 0 | 120 |
| | | % | 100,0% | 0,0% | 100,0% |
| Total | | Frequency | 668 | 25 | 693 |
| | | % | 96,4% | 3,6% | 100,0% |

Year and Sample Matrix

Graph 14. Graphical representation of the number of samples taken and the sample matrix, to be analysed using the ELISA method for the period 2019-2023



Condition of the animals

81.5% of the animals from which samples were taken were shot, 15.7% were with clinical signs of disease and 2.5% of the samples were taken from animals found dead. Only 0.3% of the samples were taken from normal cuts (Table 27; Graph 15).

Table 27. Number of samples taken and the clinical status of the animals at the time of collection, to be analysed using the ELISA method for the period 2019-2023

| Year a | and Clir | nical Conditio | n of Animals | | | | |
|--------|----------|----------------|------------------|------------|-------|---------|--------|
| | | | Clinical status | of animals | 1 | | Total |
| | | | With clinica | ıl | | Normal | |
| | | | signs of illness | Shot | Death | cutting | |
| Year | 2019 | Frequency | 71 | 136 | 6 | 1 | 214 |
| | | % | 33,2% | 63,6% | 2,8% | 0,5% | 100,0% |
| | 2020 | Frequency | 1 | 88 | 2 | 0 | 91 |
| | | % | 1,1% | 96,7% | 2,2% | 0,0% | 100,0% |
| | 2021 | Frequency | 27 | 85 | 2 | 1 | 115 |
| | | % | 23,5% | 73,9% | 1,7% | 0,9% | 100,0% |
| | 2022 | Frequency | 6 | 143 | 4 | 0 | 153 |
| | | % | 3,9% | 93,5% | 2,6% | 0,0% | 100,0% |
| | 2023 | Frequency | 4 | 113 | 3 | 0 | 120 |
| | | % | 3,3% | 94,2% | 2,5% | 0,0% | 100,0% |
| Total | | Frequency | 109 | 565 | 17 | 2 | 693 |
| | | % | 15,7% | 81,5% | 2,5% | 0,3% | 100,0% |

Graphic 15. Graphical representation of the number of samples taken and the condition of the animals at the time of collection, to be analysed using the ELISA method for the period 2019-2023



No. Positive sample

A total of 51 positive samples were determined from 35 samples (Table 28).

 Table 28. Number of samples taken with a positive result and the total number of samples analysed in which we obtained positive ELISA test results for the period 2019-2023

| | No. Positive sample |
|---|---------------------|
| Number of samples with positive samples | 35 |
| Number of positive samples | 51 |

Most positive samples were determined in 2019 (25 samples) and 2021 (19 samples) (Table 29; Graph 16).

 Table 29. The number of samples taken with a positive result and the total number of samples analysed in which we obtained positive ELISA test results per year

| | | | No. P | ositive s | ample |
|---|------|------|-------|-----------|-------|
| | Year | | | | |
| | 2019 | 2020 | 2021 | 2022 | 2023 |
| Number of samples with positive samples | 22 | 4 | 6 | 3 | 0 |
| Number of positive samples | 25 | 4 | 19 | 3 | 0 |

Graph 16. Graphical representation of the number of samples taken with a positive result and the total number of samples analysed in which we obtained positive ELISA test results per year



Most positive ELISA samples (51) were determined from samples collected during the period 2019-2023 through the surveillance programme (35) (Table 30).

 Table 30. Number of samples taken with a positive ELISA result and context of sampling for the period 2019-2023

No. Positive sample

| Context of sampling | Number of samples with positive samples | Number of positive samples |
|---------------------|---|----------------------------|
| Supervision | 35 | 51 |
| On request | 0 | 0 |

A total of 51 positive samples were determined over the period 2019-2023, of which the majority (46) were blood samples and 6 were blood serum samples (Table 31).

 Table 31. Number of samples taken with a positive ELISA test result and sample matrix for the period 2019-2023

| | Number of samples with positive | | |
|--------------|---------------------------------|----------------------------|--|
| Matrix Probe | samples | Number of positive samples | |
| Blood | 31 | 45 | |
| Blood serum | 4 | 6 | |

A total of 51 positive samples were determined over the period 2019-2023, of which the majority (44) were taken from wild boars shot (Table 32).

Table 32. Number of samples taken with a positive ELISA test result and the condition of the animals at the time of collection during the period 2019-2023

| | No. Positive sample | | | |
|--------------------------------|------------------------------------|--------------------------------|--|--|
| Animal condition | Number of samples positive samples | withNumber of positive samples | | |
| With clinical signs of illness | 4 | 6 | | |
| Shot | 30 | 44 | | |
| Death | 1 | 1 | | |
| Normal slaughtering | 0 | 0 | | |

No. samples negative

The number of negative samples was 4198 out of 669 samples (Table 33).

 Table 33. The number of samples taken with a negative result and the total number of samples analysed in which we obtained negative ELISA test results for the period 2019-2023

| | No. samples negative |
|---|----------------------|
| Number of samples with negative samples | 669 |
| Number of negative samples | 4198 |

A total number of 4198 negative samples were determined from 669 samples taken during the period 2019-2023 (Table 34; Graph 17)

 Table 34. The number of samples taken with a negative result and the total number of samples analysed in which we obtained negative ELISA test results per year

| | | | No. samples negative | | |
|---|------|------|----------------------|------|------|
| | Year | | | | |
| | 2019 | 2020 | 2021 | 2022 | 2023 |
| Number of samples with negative samples | 198 | 88 | 112 | 151 | 120 |
| Number of negative samples | 2020 | 261 | 1197 | 402 | 318 |

Graph 17. Graphical representation of the number of samples taken with a negative result and the total number of samples analysed in which we obtained negative ELISA test results per year



Most of the samples were taken from hunting complexes (535). 68 from non-professional farms and 66 from commercial farms (Table 35).

 Table 35. Number of samples taken with negative ELISA test result and place of collection for the period 2019-2023

| | No. of samples | |
|-------------------------------|------------------|------------------|
| | collected with | Number of |
| Place of sampling | negative results | negative samples |
| Hunting Complex | 535 | 680 |
| Commercial industrial farming | 66 | 1934 |
| Traditional pig farming | 68 | 1584 |

Most of the 598 samples were collected in the context of surveillance of the spread of ASF and only 71 were collected on request (Table 36).

Table 36. Number of samples taken with a negative ELISA test result and the context of sampling in the period 2019-2023

| Background to sampling | No. of samples collected with negative results | Number of negative samples |
|------------------------|--|----------------------------|
| Surveillance programs | 598 | 2261 |
| On request | 71 | 1937 |

The types of samples were multiple, but mainly there were 644 blood samples and 25 blood serum samples (Table 37).

 Table 37. Number of samples taken with negative ELISA test result and sample matrix for the period 2019-2023

| MatrixProbe | Number of samples negative samples | withNumber samples | of | negative |
|--------------------|------------------------------------|-----------------------|----|----------|
| Blood | 644 | 3248 | | |
| To be bloodthirsty | 25 | 950 | | |

Of the animals from which samples were taken were shot (542), 109 were with clinical signs of the disease. Only 16 samples were taken from dead animals, and 2 samples were taken from normal slaughters (Table 38).

Table 38. Number of samples taken with a negative ELISA result and the condition of the animals at the time of collection for the period 2019-2023

| Animal to privilege | Number of samples with negativ samples | e Number of negative samples |
|--------------------------------|---|---------------------------------|
| With clinical signs of illness | 109 | 3209 |
| Shot | 542 | 942 |
| Death | 16 | 16 |
| Normal slaughtering | 2 | 31 |

Discussions

In this original article, we statistically analysed the data from 2018 to 2023 to understand the trend of the ASF virus in Constanta County and the results indicates 93 new ASF outbreaks declared in 2018 (254 pigs and 4 wild boars), followed by a decrease, reaching 34 ASF outbreaks in 2019 (19 pigs and 50 wild boars) and tampering down to 3 ASF outbreaks in 2020 (4 pigs and 2 wild boars). In 2021, 11 ASF outbreaks were confirmed (62 pigs and 2 wild boars); in 2022, just 2 ASF outbreaks were confirmed (4 pigs and 1 wild boar), followed by 21 ASF outbreaks confirmed in 2023 (76 pigs) (Tabel 4). A maintenance of the epidemiological curve is observed, the difference between years in the number of outbreaks, in the number of susceptible animals and in the number of sick animals at the date of declaration is not statistically significant, as shown by the results of the Kruskal-Wallis test below, both for pigs and wild boars.

In the case of pigs, however, there is a significant difference in the number of confirmed sick animals since the beginning of the epizootic in the period under analysis 2018-2023.

Since the Kruskal Wallis test does not tell us which years the differences are statistically significant, we compared the years two by two applying the Mann-Whitney U test and adjusting the materiality threshold according to the number of comparisons (15 in our case), so that p = 0.05/15 = 0.003.

| Animal species in which the disease occurred | Analysis Method | Outbreaks number | Susceptible animals | Sick animals at the time of declaration of disease | Ill animals confirmed since the beginning of the epizootic disease |
|--|----------------------|---------------------|------------------------|---|--|
| Swine | Kruskal- Wallis H | ,000 | 4,154 | 4,952 | 14,982 |
| | df | 5 | 5 | 5 | 5 |
| | Asymp. Sig. | 1,000 | ,527 | ,422 | ,010 |
| Wild boar | Kruskal- Wallis H | ,000 | 4,673 | 7,430 | 7,790 |
| | df | 4 | 4 | 4 | 4 |
| | Asymp. Sig. | 1,000 | ,323 | ,115 | ,100 |

Statistical Aspects of Data Collected from African Swine Fever Virus Outbreak's in Constanta County

There were significant differences in the number of confirmed sick animals since the beginning of the epidemic between 2018 and other years (2019, 2020, 2021, 2022, 2023).

However, during 2019-2023, no statistically significant differences are observed in the number of confirmed sick animals since the beginning of the epizootic.

Conclusions

 Table 38. Statistical analysis

The study involved surveillance actions carried out by official veterinarians and hunters who collected a total of 6820 samples for PCR typing from 2018-2013 and a total number of 4248 samples analysed ELISA over the period 2019-2023 the data obtained from the test were statistically analysed using IBM SPSS Statistics for Windows, version 29.0 emphasizing the advantage of using reliable and advanced statistical tools that can lead to a better understanding and management of ASF disease.

Following the statistical analysis, we concluded that the study does indeed present nominal data as absolute frequencies and percentages, which helps to understand the distribution of categorical variables. Continuous variables are expressed by mean values and standard deviations, providing a clear picture of the central trend and variability of the data. This approach improves the interpretability of the results. By establishing a significance level of p<0.05, the paper establishes a standard for determining the statistical significance of findings. This criterion is crucial for validating the results and ensuring that they are not due to random chance, thus contributing to the reliability of the research results.

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