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Professional paper

AFRICAN SWINE FEVER IN THE REPUBLIC OF NORTH MACEDONIA

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Abstract

African swine fever is a highly contagious viral disease of domestic and wild pigs, which is characterized by rapid spread, high morbidity and mortality. It is transmitted directly and indirectly and can occur in hyperacute and chronic forms. In the acute form, the disease is characterized by high fever, skin rash and massive hemorrhage in the lymphatic organs. The nature of the disease can change, when it acquires endemic status in domestic pigs, accompanied by a significant reduction in mortality. The African swine fever virus is unique, with double-stranded DNA, the only member of the Asfarviridae family. It has the specificity of being an DNA virus transmitted by arthropods, is resistant to temperatures from 4C0 to 20 and pH fluctuations. It survives in serum at room temperature for 18 months, while in blood at 4 degrees Celsius for up to six years, at 37 degrees for up to a month, and at 55 degrees for 30 minutes. Putrification does not immediately destroy the virus, persisting in feces for 11 days. Infected pigs at different stages of the disease excrete the agent 24 to 48 hours before the onset of fever, but titers are low. The virus is present in all physiological secretions and excretions, including nasal, oral, pharyngeal, conjunctival, genital, urinary, and fecal. Surviving pigs excrete the virus intermittently for up to a month. In these pigs, viremia persists for up to 8 weeks and the virus can be found in lymphoid tissues for up to 6 months. The incubation period for contact infection in pigs varies from 5 to 15 days according to the virus strain. The diagnosis must be confirmed in the laboratory based on methods for detecting the virus and specific antibodies. The materials needed for the laboratory are blood with EDTA anticoagulant and without anticoagulant, tonsils, spleen and lymph nodes. The most useful and appropriate test is PCR. From the survey during the period 2022-2024, a total of 33337 samples sent to the laboratory of the Veterinary Research Institute were analyzed. These results were obtained for the years 2022, 11143 samples were taken for analysis, of which 107 cases were positive for the African plague virus, 2023 out of 7562 samples analyzed, 208 cases were positive, while during the period 2024 out of 4632 samples analyzed, 321 cases were positive.

Keywords: Virus, African swine fever, clinical signs, incubation.

1. Introduction

1.1. History of African Swine Fever: In the early 1900s, African swine fever (ASF) was reported in East Africa as an acute hemorrhagic fever that killed almost all infected domestic pigs. The source of infection was identified as a virus that spread from an ancient sylvatic cycle (Montgomery R., Plowright W). Since then, the African swine fever (ASF) virus has spread to most countries in sub-Saharan Africa (Mulumba-Mfumu LK). The transcontinental spread of ASF first occurred in Portugal in 1957 and 1960 and from there to other countries in Europe, the Caribbean, and Brazil (Wilkinson PJ.). Eradication was achieved by the mid-1990s, except in Sardinia, where the disease remains endemic. In 2007, the disease entered a new era of transmission in Georgia in the Caucasus, as ASF spread to the Russian Federation, Ukraine, and Belarus, and in 2014 to the EU Baltic States and Poland. By 2018, the infection had also spread to Belgium, Hungary, the Czech Republic, Romania, Bulgaria, Slovakia, and Serbia (Abrahantes JC.,). In 2018, the situation worsened significantly when African swine fever virus was detected in China, which contains half of the world's pig population. The widespread outbreak in China has been followed by a spread to Mongolia, Vietnam, Cambodia, North

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Korea, Myanmar, Laos, and the Philippines (Figure 1). Further spread to Asia and other territories is likely (. World Organ. Anim. Health. 2012, Food Agric Organ. 2019, Linda K. Dixon). The high socio-economic impact of African swine fever virus comes from lost business in the pig production chain, disease control costs, and loss of trade. Large outbreaks can result in dramatic reductions in the size of national pig farms and inflation of prices for pork and pork products. It can also have a devastating psychological impact on farmers and cause mortality in wild boar populations.

1.2. Etiology of the Virus: ASFV is a large double-stranded DNA virus and is the only member of the Asfarviridae family (Alonso C
). The genome varies in length between approximately 170 and 193 kbp, mainly due to the gain or loss of multigene family members. Core genes include those required for replication and cytoplasmic transcription of the virus genome (Alejo A, Matamoros T)and most of the 68 virus proteins detected in virus particles (Alejo A, Matamoros T., Hernáez B, Guerra M, Dixon LK). Many ASFV genes inhibit.

Image 1. Pig sampling

Type i interferon antiviral responses

Type I interferon activates the major early innate antiviral response of the host. This response is induced when host pattern recognition receptors recognize specific molecular patterns associated with the pathogen and activate downstream signaling pathways, which result in the activation of transcription factors such as IRF3 to increase the transcription of type I interferon genes. After translation, these are secreted from infected cells and bind to receptors on the infected cell or neighboring cells. This results in the activation of JAK/STAT signaling pathways and leads to the transcription of several hundred interferon-stimulated genes. These include genes for proteins that induce an antiviral state in cells to limit virus replication and others, including cytokines and chemokines, that activate the host's innate and adaptive immune responses. Host defenses, including type I interferon, the host's major antiviral pathway, and programmed cell death, or apoptosis (see sidebar titled Type I Interferon Antiviral Responses). Deletion of these genes from the viral genome offers an attractive route to produce rationally attenuated vaccine candidates. Half or more of the genes are of unknown function (Dixon LK, Chapman DAG). Precise editing of the DNA polymerase and the DNA repair system encoded by the virus results in a low mutation rate in the viral DNA. The lack of related viruses means that recombination with other viruses is highly unlikely. Thus, the risk of the viral genome crossing the species barrier is considered negligible.

Pathogenesis in domestic and wild pigs: The host range of ASFV is restricted to pigs and soft-bodied ticks of the genus Ornithodoros. In wild boar hosts in Africa, ASFV infection causes mild clinical signs and can result in long-term persistent infections (Jori F, Jori F, Vial L, Penrith ML). In contrast, most ASFV isolates cause acute hemorrhagic fever, with a mortality rate approaching 100%, in domestic pigs and wild boar (Pietschmann J,Sanchez-Cordon PJ, Montoya M). Diseases observed in domestic pigs and wild boar include acute and peracute forms (Sánchez-Vizcaíno JM, de Carvalho Ferreira HC), which are caused by highly virulent isolates and result in death within 4 to 15 days after infection. Moderately virulent isolates cause lower mortality (30-70%). Isolates with low virulence result in low or no mortality and lack of vascular lesions. However, signs of chronic disease may be observed. Clinical signs of acute ASF virus include high fever, loss of appetite and lethargy, and increasing morbidity. Bloody diarrhea, vomiting, and abortion may also be observed. Infection is associated with very high levels of virus in the blood (up to 109 TCID50/mL) and tissues. Wild boar (Sus scrofa) show the same acute signs of disease as domestic pigs (Blome S, Gabriel C,Sanchez-Cordon PJ, . Sánchez-Vizcaíno JM, Mur L). Most isolates circulating in Europe, the Russian Federation, and Asia cause the acute form of the disease, although some isolates with reduced virulence have been obtained from infected wild boar in the Baltic States (Sánchez-Vizcaíno JM, Mur L, Gomez-Villamandos JC, Carrasco L.,Gallardo C, Nurmoja) Animals that recover from infection may remain infected for several months (de Carvalho Ferreira HC).

3. Methodology

The identification of African Swine Fever (ASF) is an important process for the management and control of this viral disease that affects wild and domestic pigs. The methodology for the identification of ASF involves several important steps, including monitoring, testing, and data analysis. The main steps of this methodology are described below. Monitoring and surveillance where observation of clinical symptoms is carried out where farmers and veterinary specialists should be aware of the clinical symptoms of African Swine Fever, which include: high temperature, signs of depression or reduced appetite, skin changes, such as redness or hematoma, vomiting, diarrhea, high mortality especially in acute cases. Reporting of cases by farmers and veterinarians where they should report any suspicious case to veterinary authorities to enable a rapid response then diagnostic testing for African Swine Fever is carried out which includes several laboratory methods: serological tests where these tests identify antibodies to the ASF virus in the blood of pigs. The most common methods include the ELISA (Enzyme-Linked Immunosorbent Assy) method and immunofluorescence. Molecular tests such as the PCR (Polymerase Chain Reaction) method are used to identify genetic material of the virus in samples taken from pigs, such as blood, tissue or body fluids. In some cases, virus isolation from the samples taken may be required to confirm its presence and blood samples should be taken from suspicious pigs to perform serological and molecular tests. While tissue samples in cases of death of pigs, tissue samples (such as liver, kidney and lymph) should be taken for further analysis. Samples of body fluids, such as abdominal fluid, can also be taken for analysis. Then, data analysis is performed, epidemiological analysis from the data collected from suspected and confirmed cases and analyzed to identify trends and geographical distribution of the disease in our case in all regions included within the territory of North Macedonia from the period 2022-2024. All identified cases, as well as test results, should be documented and reported to the relevant authorities to enable a Effective management of the situation. In isolation and destruction in case of confirmation of the disease, it is necessary to isolate the affected population and destroy the infected pigs to prevent further spread, disinfection of the farm after the destruction of infected pigs is done to prevent future outbreaks. Education of farmers is also important on the symptoms, preventive measures and management of the disease

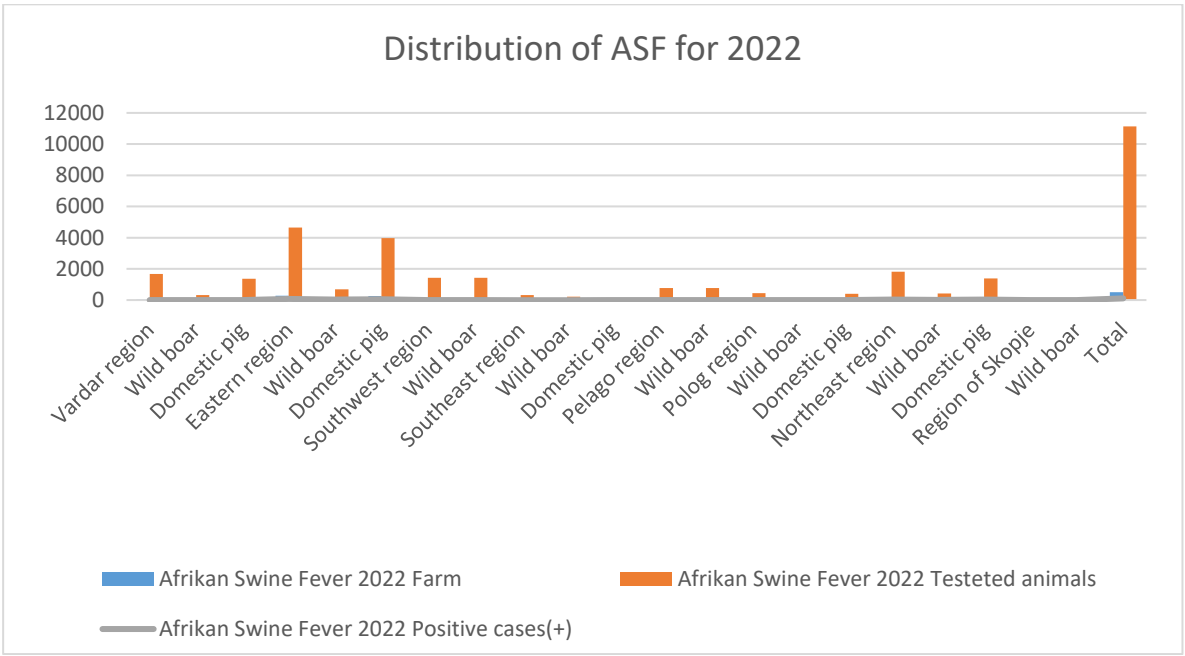
is essential to prevent future outbreaks, with this achieved in improving control strategies although there is currently no vaccine for ASF, research on vaccine development is in progress. It is important that the authorities also develop clear policies for the management of African Swine Fever, including support for farmers and strategies to prevent the spread. ASF requires a multifaceted approach that includes continuous monitoring, accurate testing, analysis of data and rapid response. This methodology is essential to protect the pig population and minimize the economic and social impact of this disease. The ELISA test method is a common method for identifying antibodies to the African Swine Fever virus in pig blood samples. This test is important for diagnosing the disease and monitoring outbreaks. The purpose of the ELISA test is to identify antibodies produced by the pig immune system in response to infection with the ASF virus, and this helps in identifying infected pigs and carefully managing the disease. In our case, we have obtained pig blood samples (serum) that are necessary for testing. The reagents for the test include: ASF virus antigen, enzyme-linked antibodies, enzyme substrate. The blood samples are processed to obtain serum, antigen I ASF virus is placed on the test plate and incubated to allow binding to the antibodies in the serum, the sera of the tested pigs are added to the plate, allowing the antibodies to bind to the antigen. Enzyme-linked antibodies are added to bind the antibodies present, then washing is done to remove any unbound antibodies, the enzyme substrate is added and if there is binding, a reaction occurs that produces a color. The reaction is stopped with a stopping solution. In measuring the color intensity, the measurement is done with a spectrophotometer at a certain valence (usually 450nm). The results are then interpreted in the case of a positive result, the color intensity indicates the presence of antibodies to the ASF virus, suggesting a possible infection.

4. Discussion

Table 2 shows data on the distribution of African Swine Fever (ASF) for 2023 in several different regions In the table, we have the total number of pigs tested in each region, including wild and domestic pigs, where the number of positive cases of infection with African Swine Fever is noted to show how many of the pigs tested with positive results. The Vardari region has the highest number of pigs tested 1108 and a considerable number of positive rates 34. The Southern region shows a high distribution of infection, especially wild boar farms while the Northern region has a low number of positive cases, but there are still some cases reported. The percentage of infection by calculating the percentage of positive cases in relation to the number of pigs tested, can be estimated as the rate of infection in each region and this helps in identifying the regions with the highest number of positive cases. at risk. Trends in some regions, such as the South and Vardar regions, show a higher trend of infection, which may require stronger control and management measures. In conclusion, during the 2023 period, the distribution of African Swine Fever shows that some regions are more affected than others. This requires special attention from the authorities to prevent the further spread of the disease. Control measures, as well as the isolation of positive cases and continuous monitoring of the pig population, are necessary to manage the situation. To analyze the African Swine Fever (ASF) table for 2024 and to compare it with 2023, we have examined several main aspects: the 2024

Table. 1 Data on the distribution of African Swine Fever (ASF) for 2022

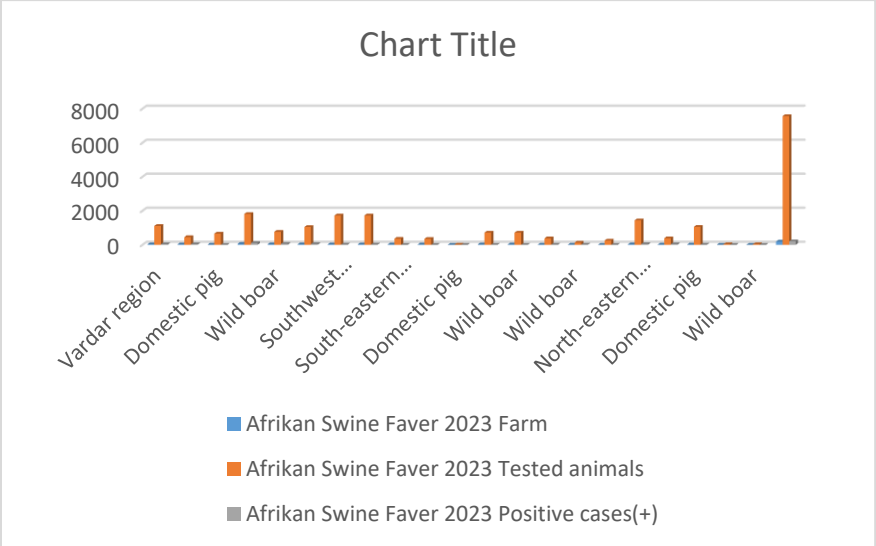
Afrikan Swine Fever 2022			
Region	Farm	Testeted animals	Positive cases(+)
Vardar region	38	1672	0
Wild boar	16	312	0
Domestic pig	22	1360	0
Eastern region	285	4649	76
Wild boar	24	685	27
Domestic pig	261	3964	49
Southwest region	14	1433	0
Wild boar	14	1433	0
Southeast region	15	328	1
Wild boar	11	220	1
Domestic pig	4	108	0
Pelago region	14	777	0
Wild boar	14	777	0
Polog region	8	447	0
Wild boar	5	43	0
Domestic pig	3	404	0
Northeast region	131	1824	30
Wild boar	11	429	8
Domestic pig	120	1395	22
Region of Skopje	3	13	0
Wild boar	3	13	0
Total	508	11143	107



Graf.1.Distribution of ASF for 2022

Table.2 Data on the distribution of African Swine Fever (ASF) for 2023

Afrikan Swine Faver 2023			
Region	Farm	Tested animals	Positive cases(+)
Vardar region	31	1108	34
Wild boar	23	451	34
Domestic pig	8	657	0
Eastern region	55	1811	105
Wild boar	27	762	58
Domestic pig	28	1049	47
Southwest region	25	1723	14
Wild boar	25	1723	14
South-eastern region	21	356	0
Wild boar	20	349	0
Domestic pig	1	7	0
Pelago region	20	715	2
Wild boar	20	715	2
Polog region	9	379	10
Wild boar	8	129	10
Domestic pig	1	250	0
North-eastern region	27	1437	43
Wild boar	13	382	37
Domestic pig	14	1055	6
Region of Skopje	3	33	0
Wild boar	3	33	0
Total	191	7562	208

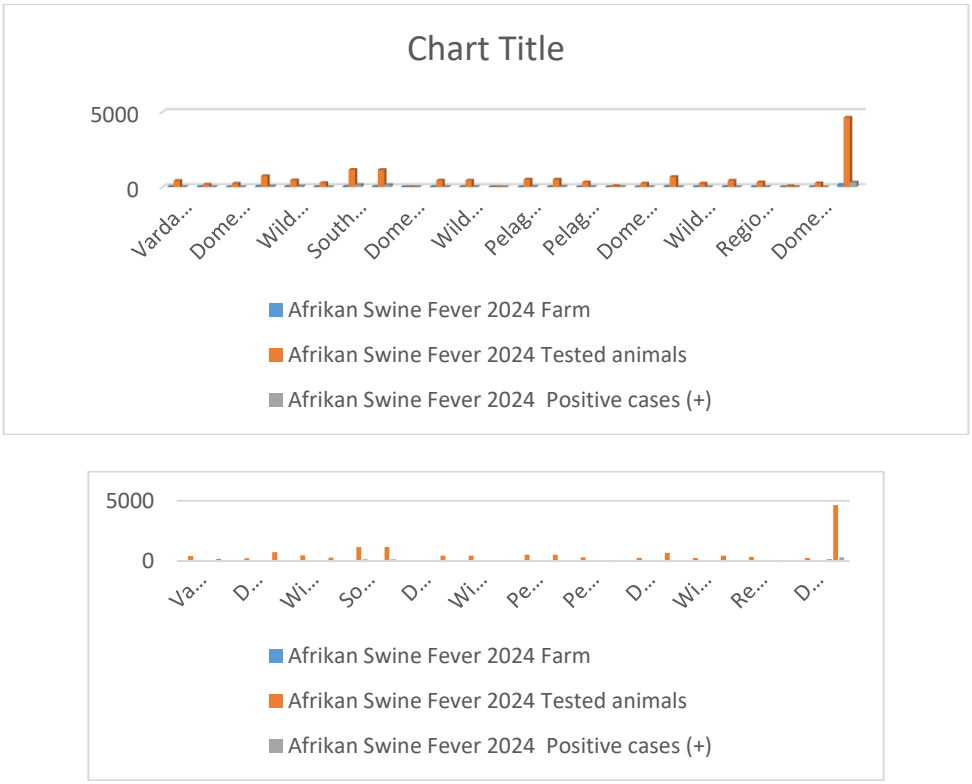


Graf 2 ASF 2023 distribution by regions

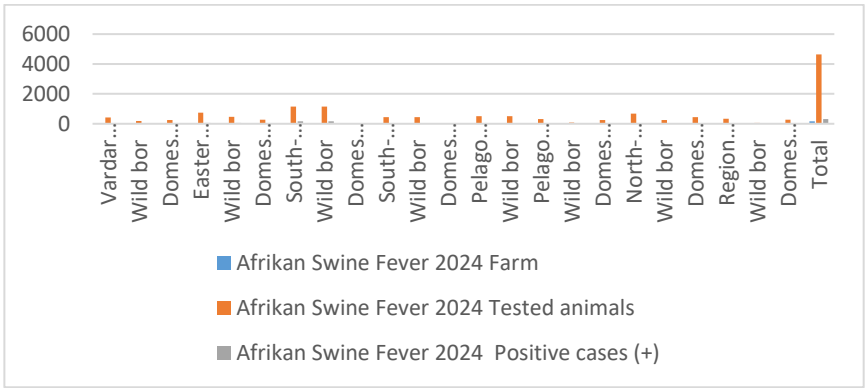
table analysis, the number of pigs tested in all regions included, where in the Vardar region we have 18 positive cases, the region Eastern 87 positive cases, Southwestern region with 161 positive cases, Southern region 19 positive cases, Skopje region with 7 positive cases and Northern region with 55 positive cases, where in total there are 4632 pigs tested and 3211 cases are positive. Number of pigs tested compared to 2023, 2024 figures show an increase in the number of pigs tested in some regions, especially in the Southwestern region. The number of positive cases has increased in some regions, such as the Southwestern and Eastern regions, indicating a deterioration of the situation. Some regions, such as the Vardar and Skopje Region, have lower numbers of positive cases compared to 2023. The percentage of infection by calculating the percentage of positive cases in relation to the number of pigs tested, it can be estimated that some regions have a higher rate of infection, which requires strong measures control. In conclusion, the data for 2024 show an increase in the number of pigs tested and an increase in positive cases in some regions, suggesting that the African Swine Fever (ASF) situation seems to be worsening in some areas. This requires special attention and immediate measures to prevent the further spread of the disease. To analyze the African Swine Fever table for 2022 with the years 2023 and 2024, we have examined several key aspects such as the analysis of the 2022 table, the comparison with 2023 and 2024 where in 2023 the number of pigs tested has increased, indicating an effort to better monitor the situation while during 2024 the number of pigs tested has increased further especially in the South-West region, where the positive cases in 2023 have increased in some regions, indicating a deterioration of the situation, while in 2024 positive cases have increased in some regions, suggesting that the situation continues to be problematic. The conclusion for the period 2022-2024 is that the number of pigs tested has increased, but there has also been an increase in positive cases in some regions, indicating that African Swine Fever continues to pose a threat to the pig industry. Where the most affected regions such as the Southwest and the East have shown a significant increase in positive cases, requiring strong control and management measures,. The situation requires special attention from the authorities to prevent the further spread of the disease to protect the pig population and in these situations clear strategies are developed for monitoring and managing the situation, including vaccination, educating farmers on preventive measures. The period 2022-2024 shows a complicated situation for African Swine Fever, with the need for continued commitment to manage and control its spread.

Table.3 Data on the distribution of African Swine Fever (ASF) for 2024

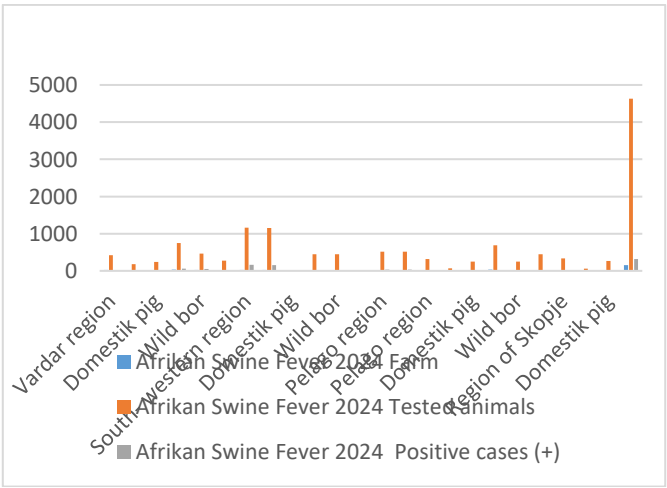
Afrikan Swine Fever 2024			
Region	Farm	Tested animals	Positive cases (+)
Vardar region	22	423	18
Wild boar	17	179	18
Domestic pig	5	244	0
Eastern region	33	746	56
Wild boar	23	468	55
Domestic pig	10	278	1
South-western region	25	1159	161
Wild boar	23	1153	155
Domestic pig	2	6	6
South-eastern region	20	448	19
Wild boar	18	443	19
Domestic pig	2	5	0
Pelago region	12	516	37
Wild boar	12	516	37
Pelago region	5	321	18
Wild boar	4	71	18
Domestic pig	1	250	0
North-eastern region	31	688	5
Wild boar	13	245	5
Domestic pig	18	443	0
Region of Skopje	10	331	7
Wild boar	5	63	7
Domestic pig	5	268	0
Total	158	4632	321



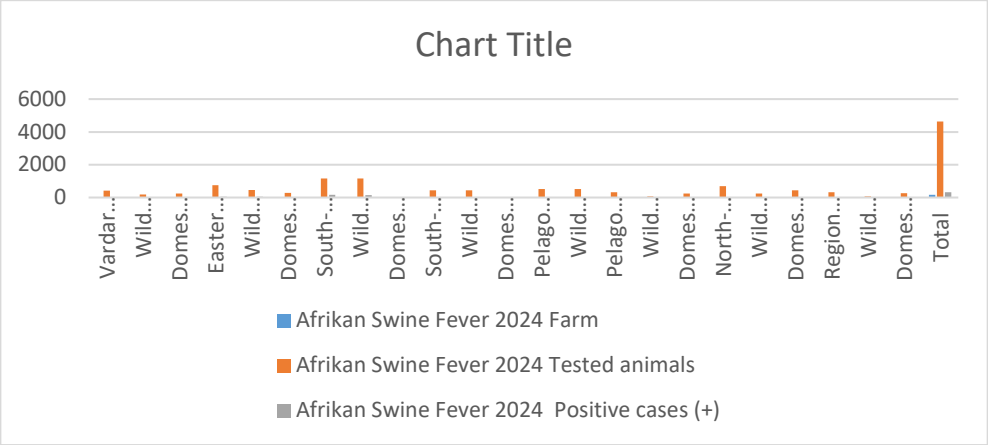
Graf.*4 Data on the distribution of African Swine Fever (ASF) Region Vardar*



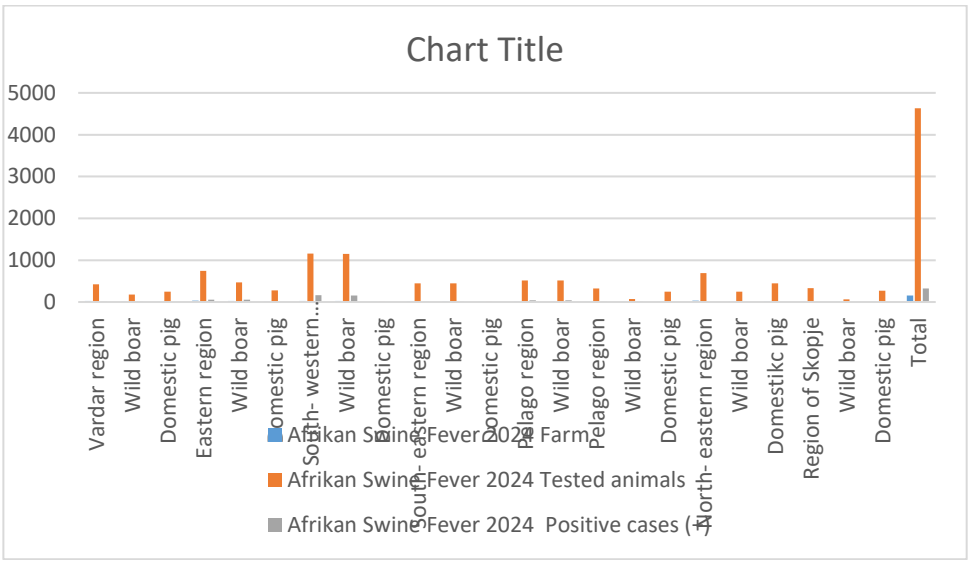
Graf.5 Data on the distribution of African Swine Fever (ASF) Eastern Region



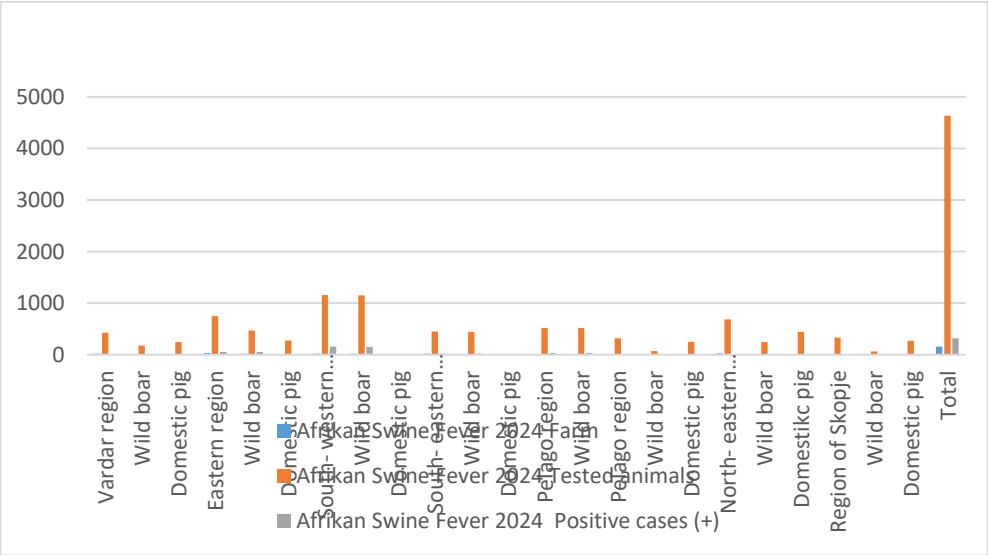
Graf.6 Data on the distribution of African Swine Fever (ASF) South-western Region



Graf.7 Data on the distribution of African Swine Fever (ASF) South-eastern Region



Graf.8 Data on the distribution of African Swine Fever (ASF) Pelago Region



Graf.9 Data on the distribution of African Swine Fever (ASF) North-eastern Region

5. Conclusion

African Swine Fever (ASF) is a viral disease that affects wild and domestic pigs, causing major economic losses for farmers and the meat industry. During the period 2022-2024, North Macedonia has experienced a complicated situation regarding this disease, which has required special attention from the relevant authorities and institutions. In 2022, North Macedonia reported a limited number of positive cases of ASF, with the affected regions being the southern and eastern ones, but the number of cases was relatively low, suggesting that the disease was under control. During 2023, the situation began to deteriorate, with an increase in the number of positive cases in some regions. The number of pigs tested increased, reflecting an effort to better monitor the situation, with positive cases reaching 321, indicating a significant increase and a wider spread of the disease. In 2024, the increase continued with regions such as the South-West and the East being particularly affected, requiring immediate measures to prevent the further spread of ASF. Factors contributing to the spread of African swine fever and as risk factors are the transport of pigs between farms and markets, where infected pigs can be transported without being identified, increasing the risk of spreading the disease. Wild pigs also constitute a possible source of infection. In some cases, contacts between wild and domestic pigs can lead to the spread of the virus. Lack of education is also a preventive and management factor, many farmers are not aware of the symptoms of the disease and the modes of transmission. When positive cases are identified, it is important that they are immediately isolated to prevent the spread of the disease and this includes the destruction of pigs and disinfection of farms. With this research we conclude that North Macedonia during this period 2022-2024 has developed policies and strategies to manage the situation including financial support for affected farmers and the creation of a general management plan, veterinary authorities have increased efforts to monitor and test the pig population and identify positive cases in time. The spread of the disease has increased concerns about food security, affecting the supply of meat and other products of pig origin. The impact on rural communities, which depend on livestock for their livelihoods, are particularly affected by the economic consequences of African Swine Fever. All this is done due to the further prevention of the disease and to protect the pig industry in the country.

The complex structure of ASF, its large genome and the diversity of viral genomes, add to the difficulty of developing a vaccine. The structure and function of the main ASF proteins, infection and immune mechanisms need to be fully understood and the main immune gens need to be identified. The mechanisms of ASF replication and the process of virus entry and endosomal transport, many details about the specific process of virus replication still remain unclear. Until a vaccine is available, biosecurity may be the most important measure to prevent the spread of the ASF virus. Pathological diagnosis of ASF and research into laboratory diagnostic technology occupy an important position in the prevention and control of ASF also in North Macedonia.

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