

**PROTOCOL OF STEP-BY-STEP INSTRUCTIONS FOR
IDENTIFYING THE SOURCE OF AFRICAN SWINE FEVER AND
PREVENTING ITS SPREAD FOR JOINT PREVENTIVE AND
LIQUIDATION MEASURES
(REFERENCE DOCUMENT)**

Developed under Activity 1.5. “Increasing awareness on emerging
diseases and the prevention of cross-border epidemics”
of the international project

“Development of screening and monitoring systems for emerging
diseases and prevention of cross-border epidemics in the current context”

ROUA00136,

co-financed by the EU under the Interreg NEXTRomania – Ukraine
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PREAMBLE

This Protocol of step-by-step instructions for identifying the source of the African swine fever pathogen and preventing its spread (hereinafter - the Protocol) was developed by PI "Educational and research institute of biotechnology and biosafety" Agreement No. 151 dated September 22, 2026 (hereinafter — the Agreement), concluded with Odesa State Agrarian University (OSAU) as Partner 3 (PP3) of the international project "SMS-RO-UA: Development of screening and monitoring systems for emerging diseases and prevention of cross-border epidemics under current conditions," ROUA00136, co-financed by the EU under the Interreg NEXT Romania – Ukraine Programme 2021–2027 (hereinafter — the Project).

The document was prepared as part of the implementation of Activity 1.5, "Raising awareness of emerging diseases and preventing cross-border epidemics" (Deliverable D.1.5.1) of the international project.

The purpose of this Protocol is to provide veterinary medicine specialists, state veterinary and sanitary control bodies, hunting enterprises, businesses operating in the pig-farming sector, research institutions, and other interested parties with step-by-step practical instructions for:

- identifying sources and factors driving the spread of the African swine fever (ASF) virus in the chain "wild boar – environmental objects/contaminated fomites – domestic pig – pig-farming products";

- assessing pathogen transmission routes (direct contact, alimentary route, mechanical transmission via fomites, movement of infected carcasses and pig-farming products) and risk factors in a cross-border context, in particular with regard to wild boar population migration;

- conducting joint epizootiological investigations, monitoring of wild boar populations, and laboratory studies in line with the "One Health" principles and WOA recommendations;

- harmonizing approaches to surveillance, early detection, notification, and control of ASF between Ukraine and Romania in the Lower Danube area, where

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viral circulation among wild boar populations constitutes a shared cross-border risk.

Preparation of this Protocol draws on:

- results of joint field studies and laboratory analyses carried out under the Project, in particular monitoring of wild boar populations and environmental objects as factors in viral circulation and spread;
- the Joint Technical Specification (TOR) agreed by the partners, which defines unified methods for collecting pathological material from domestic pigs and wild boars, its delivery, preparation, and testing;
- the use of real-time polymerase chain reaction (real-time PCR) as the reference (“gold standard”) method for laboratory diagnosis of ASF, combined with serological methods (ELISA, IFA, Immunoblot, IPT) for detecting animals that have recovered from infection;
- experience and findings from previous international projects and scientific activity;
- guidance documents of WOA, FAO, and the current regulatory framework of Ukraine and the EU.

This Protocol was prepared with due regard to, and is consistent with, the following documents:

- Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases (“Animal Health Law”) — African swine fever is classified as a category A disease subject to immediate notification and mandatory eradication measures;
- Commission Delegated Regulation (EU) 2020/687 of 17 December 2019 on rules for the prevention and control of certain listed diseases;
- Commission Implementing Regulation (EU) 2023/594 of 16 March 2023 laying down special disease control measures for African swine fever (repealing Regulation (EU) 2021/605), as subsequently amended by Regulations (EU) 2024/2139 and (EU) 2025/1924 — the profile and most current EU regulatory act, directly governing zoning, restrictive measures, and control of animal and product movement in connection with ASF;

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- Law of Ukraine No. 1206-IX of 04.02.2021 “On Veterinary Medicine and Animal Welfare”;
- Law of Ukraine No. 4718-IX of 16.12.2025 “On Amendments to Certain Laws of Ukraine Regarding the Alignment of Regulation in the Fields of Veterinary Medicine, Animal Welfare, and Feed with the Acts of European Union Law”;
- Resolution of the Cabinet of Ministers of Ukraine No. 1009 of 28.09.2021 “On Approval of the Procedure for Carrying Out Anti-Epizootic Measures”;
- Order of the Ministry of Agrarian Policy and Food of Ukraine No. 111 of 07.03.2017 “On Approval of the Instruction on the Prevention and Control of African Swine Fever”;
- The Plan of Measures for the Prevention, Monitoring, Diagnosis, and Eradication of African Swine Fever in Ukraine, approved by the State Service of Ukraine on Food Safety and Consumer Protection (2024);
- European Veterinary Big Data Strategy 2022–2027;
- WOAHA Terrestrial Animal Health Code, section “Infection with African swine fever virus.”

Implementation of the SMS-RO-UA project supports the delivery of the EU Strategy for the Danube Region (EUSDR) as it relates to environmental protection and the economic stability of the agricultural sector, and is consistent with the goals of the EU Biodiversity Strategy for 2030 and the European Green Deal. The Danube Delta and the adjacent forest-steppe and floodplain areas are habitat to substantial wild boar populations, and their migration between Ukraine and Romania creates a persistent cross-border risk of introducing and sustaining the circulation of the ASF virus, which calls for a joint, coordinated approach to wild boar monitoring, farm biosecurity, and outbreak response..

RELEVANCE

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African swine fever (ASF) remains one of the most dangerous transboundary animal diseases, posing a serious threat to pig farming, food security, and the economic stability of many countries around the world [1, 2].

The disease is characterized by high lethality among domestic pigs and wild boar, the absence of effective means of specific prevention and treatment, and the pathogen's substantial resistance in the environment and in animal-origin products [3, 4]. Accordingly, timely identification of the source of infection and the implementation of effective biosecurity measures are key elements of disease control [5].

The spread of ASF over recent decades has become global in scale. Since 2007, following the introduction of the virus into the Caucasus region, the disease has spread rapidly across Eastern and Central Europe and subsequently reached large parts of Asia and several Caribbean countries [2, 6, 7].

According to the World Organisation for Animal Health, ASF continues to be recorded in many countries around the world, causing substantial economic losses due to animal deaths, forced culling of livestock, restrictions on international trade, and the cost of anti-epizootic measures [6, 7].

For Ukraine, the ASF problem remains particularly relevant because of the substantial wild boar population, active animal migration processes, intensive movement of pig-farming products, and difficult conditions for controlling certain territories [8, 9]. Additional risks arise from the ongoing military conflict, which can adversely affect the functioning of the veterinary surveillance system, farm biosecurity, and monitoring of the epizootic situation [10].

An effective response to ASF cases requires a comprehensive approach to epizootiological investigation. Establishing the source of virus introduction, determining its transmission routes, and identifying factors in its spread are necessary conditions for containing an outbreak and preventing the further spread of the disease [5, 8]. Standardizing investigation procedures, sample collection, risk analysis, and the implementation of control measures improves the effectiveness of anti-epizootic measures and ensures compliance with international recommendations [5, 7].

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Accordingly, the development and implementation of a step-by-step instruction for identifying the source of the African swine fever pathogen, preventing its spread, and eliminating it is an important tool for veterinary medicine specialists, state control bodies, and business operators. Such a document will support prompt decision-making, improve the effectiveness of epizootiological investigations, and strengthen the biosecurity system in pig farming [5, 7].

GENERAL CHARACTERISTICS OF THE AFRICAN SWINE FEVER PATHOGEN

The causative agent of African swine fever (ASF) is the African swine fever virus (African swine fever virus, ASFV), which belongs to the genus *Asfivirus*, family *Asfarviridae*, and is the only known member of this family capable of infecting vertebrate animals [1].

ASFV is a large, enveloped, double-stranded DNA virus with a complex multilayered structure and a genome ranging from 170 to 193 thousand base pairs, depending on the isolate [3].

The virus is characterized by high genetic variability. More than 24 genotypes of ASFV have so far been described on the basis of p72 gene analysis, although most disease cases in Europe and Asia are associated with genotype II, which was introduced into Georgia in 2007 and subsequently spread across large parts of Eurasia [2, 6].

ASF is characterized by exceptional environmental resistance. The virus can retain its infectious properties for extended periods in blood, tissue, animal carcasses, manure, feed, and pork products, particularly at low temperatures. In frozen meat, the virus can survive for years, and in smoked or salted meat products, for several months [5, 6, 7]. This is why contaminated animal-origin products are considered an important factor in the interregional and international spread of ASF.

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The virus is transmitted both directly and indirectly. Infection can occur through contact with sick or infected animals, through infected feed, water, vehicles, equipment, staff clothing and footwear, and through biological waste and slaughter products from infected pigs [5, 8]. The high concentration of virus in the blood, secretions, and excretions of sick animals accounts for its rapid spread within holdings.

The pathogenicity of ASF depends on the properties of the particular strain. Highly virulent isolates can cause an acute course of disease with lethality of up to 90–100%, whereas strains of moderate or low virulence can cause subacute or chronic forms of the disease [2]. Clinical signs include high body temperature, depression, anorexia, skin cyanosis, hemorrhagic lesions of internal organs, respiratory and neurological disorders, often ending in the animal's death

IMPACT OF THE AFRICAN SWINE FEVER VIRUS ON HUMAN HEALTH

Despite the disease's substantial impact on livestock farming and the economy, there is currently no scientific evidence that ASFV is able to infect humans or cause illness in people [5, 7].

According to international animal health and food safety organizations, ASF is characterized by narrow species specificity and, under natural conditions, affects only members of the family Suidae, including domestic pigs and wild boar [2]. No cases of human infection have been recorded, even among pig farm workers, veterinarians, hunters, meat-processing industry workers, and laboratory personnel who have prolonged contact with sick animals or infected material [5, 7].

Consuming meat or meat products obtained from pigs infected with the ASF virus does not pose a risk to human health. At the same time, such products can serve as a source of viral spread among susceptible animals if used to feed pigs or improperly disposed of as food waste [8]. This is why veterinary and

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sanitary measures controlling the movement of pork are important for preventing the spread of the disease.

Although ASF is not a zoonotic infection, its indirect impact on people is substantial. Mass outbreaks of the disease lead to significant economic losses due to animal deaths, culling of livestock, trade restrictions, and reduced pork production [2]. This can negatively affect food security, farm household incomes, and employment in the livestock sector [4].

The ASF problem takes on particular significance under conditions of military conflict, economic instability, and disruption of veterinary control systems. Under such conditions, the risk of uncontrolled disease spread among pig populations increases, which can cause additional socioeconomic consequences for the population and the state as a whole [8].

In summary, African swine fever does not pose a direct threat to human health and is not a zoonosis. However, the disease has a substantial indirect impact through economic losses, disruption of food security, and the need for large-scale anti-epizootic measures. Effective ASF control is therefore an important element of ensuring the sustainable development of livestock farming and protecting society's economic interests [5, 6].

EPIZOOTOLOGY OF AFRICAN SWINE FEVER

The epizootiological features of ASF, as an emerging and transboundary disease, are shaped by the complex interaction between the virus, susceptible animals, environmental factors, and anthropogenic factors that facilitate the spread of infection.

The natural hosts of the African swine fever virus are members of the family Suidae, in particular domestic pigs (*Sus scrofa domesticus*), wild boar (*Sus scrofa*), and several species of African wild pigs [4]. In endemic regions of Africa, an important role in sustaining viral circulation is played by the natural sylvatic cycle “wild pigs – soft ticks of the genus *Ornithodoros*,” in which ticks act as

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biological vectors and a reservoir of infection [14]. In European and Asian countries, the main significance lies with populations of domestic pigs and wild boar (the so-called “contact (domestic) transmission cycle”), while the role of ticks (the “sylvatic transmission cycle”) in sustaining the epizootic process remains limited or absent.

The source of the pathogen is sick and recovered pigs, which shed the virus in blood, saliva, nasal discharge, feces, urine, and other biological material [5]. The high concentration of virus in the blood accounts for the disease’s substantial contagiousness and facilitates transmission through direct contact between animals.

Transmission of the virus can occur both horizontally and mechanically. The main mechanisms of spread are direct contact between infected and susceptible animals, and contact with infected carcasses, slaughter products, feed, water, vehicles, equipment, and staff clothing and footwear [6, 7, 8]. Particular danger is posed by the use of food waste or feed contaminated with pork products containing viable virus.

Wild boar play an important role in sustaining and spreading ASF in the natural environment. Infected animals can travel substantial distances, facilitating the introduction of the virus into new territories. In addition, the carcasses of dead wild boar can remain a source of infection for a long time, owing to the high resistance of ASFV in tissue and the environment [8, 12].

One of the key features of ASF epizootology is the virus’s high resistance in the external environment. ASFV can persist for a long time in blood, tissue, animal carcasses, manure, and pork products, particularly at low temperatures [3]. This property considerably complicates disease control and facilitates its cross-border spread.

Following the introduction of the virus into Georgia in 2007, ASF spread rapidly across Eastern Europe, including Ukraine, and subsequently reached large parts of the European Union and Asia [2]. The spread of the infection is largely linked to human activity, in particular the illegal movement of live animals, meat products, and inadequate farm biosecurity [14].

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In Ukraine, ASF continues to be a serious epizootic problem because of viral circulation among wild boar populations, intensive movement of animals and animal-origin products, and the difficulty of controlling certain territories [10]. Under martial law, additional risk factors include disruption of the veterinary surveillance system, restrictions on monitoring activities, and complications in controlling animal movement..

ROUTES OF TRANSMISSION OF THE AFRICAN SWINE FEVER PATHOGEN

Transmission of the African swine fever virus occurs through various routes, which accounts for the disease's high capacity to spread among both domestic pigs and wild boar populations. Features of the virus's biology, its resistance in the environment, and its substantial concentration in the blood and tissues of infected animals create numerous mechanisms of infection transmission [1, 14].

Direct contact route. The most effective transmission route is direct contact between healthy animals and sick or infected pigs. The virus is present in high concentrations in the blood, saliva, nasal discharge, feces, urine, and other biological fluids of infected animals. Transmission can occur during shared housing of pigs, transportation, or contact through damaged skin or mucous membranes [5, 6, 7].

Transmission via infected carcasses and biological material. The carcasses of dead pigs and wild boar are an important source of infection. The virus remains viable for a long time in the tissue, blood, and bone marrow of dead animals, particularly at low temperatures. Contact between susceptible animals and carcasses or their remains can result in infection even a long time after the death of the infected animal [12].

Alimentary route. Infection is possible through consumption of feed or food waste contaminated with the ASF virus. Particular danger is posed by waste

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containing insufficiently heat-treated pork or products from infected animals. This mechanism is considered one of the main causes of the interregional and international spread of the disease [5, 13].

Mechanical transmission via environmental objects. Contaminated objects and environmental items (fomites) play an important role in spreading the virus. These include vehicles, equipment, tools, protective clothing, staff footwear, feed containers, hunting equipment, and other items that have come into contact with infected animals or their secretions [6, 7]. Inadequate disinfection of such objects considerably increases the risk of introducing the virus into disease-free holdings.

Transmission via pig-farming products. The virus persists for a long time in pork, meat products, sausages, smoked products, and frozen meat. Illegal movement or sale of infected products is one of the most important factors in the cross-border spread of ASF [2, 5]. This is why controlling the movement of animal-origin products is one of the priority prevention measures.

Transmission via wild boar. Wild boar populations play a key role in sustaining viral circulation under natural conditions. Transmission of the infection occurs both through direct contact between animals and through contact with infected carcasses. Movement of infected wild boar facilitates the spread of the virus over substantial distances and the formation of new disease foci [12, 8].

Vector-borne transmission. In regions of Africa endemic for ASF, the vector-borne transmission route involving soft ticks of the genus *Ornithodoros* is important. Ticks can become infected while feeding on infected animals, retain the virus for a long time, and pass it to subsequent generations and other animals [4]. For most European countries, including Ukraine, this mechanism has no substantial epizootiological significance.

The role of humans in spreading ASF. Humans are not susceptible to ASF and do not become ill with African swine fever, but human activity is nonetheless considered one of the main factors in the disease's spread. Failure to comply with biosecurity requirements, illegal transport of animals and pig-farming products, use of contaminated feed, and improper disposal of carcasses and food waste considerably increase the risk of new outbreaks [6, 7, 8].

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In summary, the spread of ASF occurs through a set of interconnected transmission mechanisms, among which the most significant are direct contact between animals, infected pig-farming products, contaminated environmental objects, wild boar populations, and the human factor. Establishing the specific transmission route during an epizootiological investigation is one of the key tasks for identifying the source of infection and implementing effective control measures..

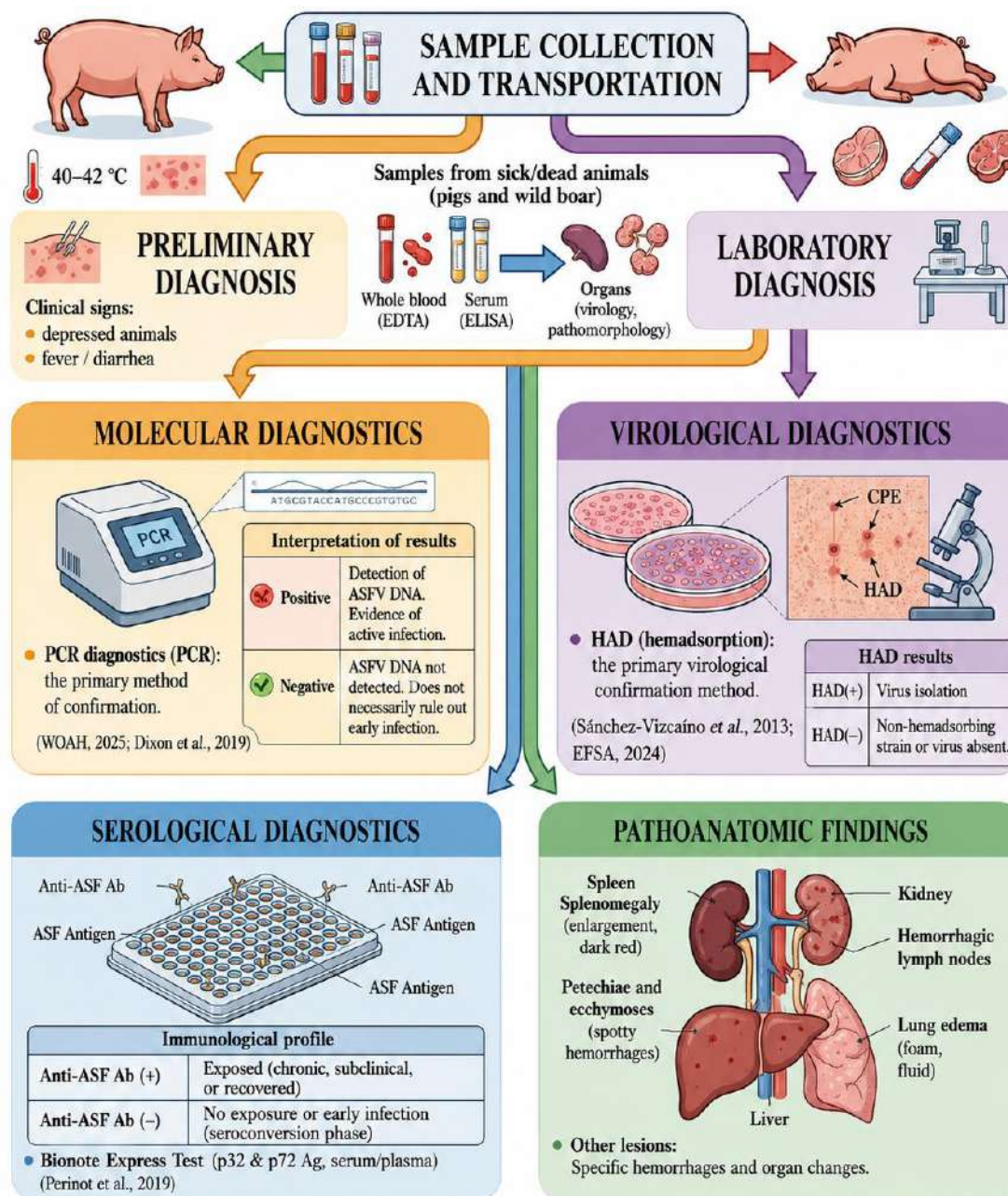
DIAGNOSIS OF AFRICAN SWINE FEVER

Diagnosis of African swine fever (ASF) (Figure 1) is based on a comprehensive assessment of epizootiological data, clinical signs, pathological changes, and laboratory test results. Because the clinical manifestations of ASF can resemble other hemorrhagic diseases of pigs, a definitive diagnosis is established exclusively on the basis of laboratory confirmation [5, 6].

Epizootiological diagnosis. Suspicion of ASF arises with a sudden increase in mortality among pigs, the appearance of fever of undetermined origin, recorded deaths of wild boar, and the detection of risk factors for virus introduction into a holding [14]. Information on animal movement, contact with wild boar, use of food waste for feed, purchase of animals without veterinary control, and violations of biosecurity requirements is of particular importance [8].

Clinical diagnosis. Clinical signs depend on the virulence of the strain, the age of the animals, and the form of the disease. The acute form is typically characterized by: an increase in body temperature to 40.5–42.0 °C, depression and anorexia, reddening or cyanosis of the skin in the ear, abdominal, and limb areas, difficulty breathing, vomiting and diarrhea (sometimes with blood), loss of motor coordination, abortion in pregnant sows, and high mortality within 5–10 days of the onset of clinical signs [1, 8].

However, these symptoms are not pathognomonic and can also be observed in classical swine fever, salmonellosis, pasteurellosis, swine erysipelas, and other diseases [5].



.Figure 1 — Diagnostic scheme for African swine fever

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Pathological diagnosis. Necropsy of animals that have died most often reveals enlargement and hemorrhagic lesions of the lymph nodes, an enlarged, dark-red spleen, multiple hemorrhages in internal organs, hemorrhagic changes in the kidneys, pulmonary edema, accumulation of serohemorrhagic exudate in the thoracic and abdominal cavities, and hemorrhages on serous and mucous membranes [2].

At the same time, pathological changes also do not allow reliable differentiation of ASF from a number of other infectious pig diseases, and therefore require laboratory confirmation.

Laboratory diagnosis. Laboratory methods are the basis for confirming a diagnosis of ASF. Blood, blood serum, spleen, lymph nodes, lungs, kidneys, tonsils, and bone marrow from dead or emergency-slaughtered animals are used for testing [6].

Molecular-genetic methods. Real-time polymerase chain reaction (real-time PCR) is the main method for detecting ASFV genetic material. The method is characterized by high sensitivity, specificity, and speed of results, and is recommended by the World Organisation for Animal Health as the “gold standard” for laboratory diagnosis of ASF [6].

Virological methods. Isolating the virus in cell culture or primary macrophage cultures confirms the presence of viable virus. The method is highly specific but requires specialized high-biosafety-level laboratories and considerable time [1].

Serological methods. Enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay (IFA), immunoblotting (Immunoblot), and the immunoperoxidase test (IPT) are used to detect antibodies to ASF.

Serological methods are particularly useful for monitoring and identifying animals that have survived infection or been infected with strains of reduced virulence [11].

Differential diagnosis. ASF must be differentiated from:
classical swine fever;
swine erysipelas;

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salmonellosis;
pasteurellosis;
actinobacillary pleuropneumonia;
porcine reproductive and respiratory syndrome (PRRS);
septicemic forms of bacterial infections;
poisoning and other conditions accompanied by hemorrhagic syndrome
[5].

The importance of laboratory confirmation. In line with international standards, a definitive diagnosis of ASF can be established only after laboratory confirmation of the presence of the virus, its genetic material, or specific antibodies [6]. Timely and reliable diagnosis is the foundation of an effective epizootiological investigation, the prompt implementation of quarantine measures, and the prevention of further spread of the infection.

CLINICAL COURSE OF AFRICAN SWINE FEVER

The clinical manifestations of African swine fever depend on the virulence of the viral strain, infectious dose, age and physiological condition of the animals, as well as housing conditions and the presence of concurrent diseases [1, 6, 7]. The course of the disease can be peracute, acute, subacute, or chronic.

Peracute form. The peracute, or fulminant, form is observed with infection by highly virulent strains of the pathogen and is characterized by sudden death of animals without pronounced clinical signs, or after a brief rise in body temperature. Mortality can reach 100% [6, 7].

Acute form. The acute form is the most common with infection by highly virulent ASFV isolates. The incubation period usually lasts from 4 to 19 days, and clinical signs include an increase in body temperature to 40.5–42.0 °C, depression, lethargy, and refusal to eat, animals crowding near sources of warmth, reddening or cyanosis of the skin in the ear, tail, abdominal, and limb areas, conjunctivitis and lacrimation, serous or mucous nasal discharge, shortness of

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breath and difficulty breathing, vomiting and diarrhea (sometimes with blood), loss of motor coordination, an unsteady gait, and abortion in pregnant sows [2, 5].

Death usually occurs within 6–13 days of the appearance of the first clinical signs, and lethality can reach 90–100% [1].

Subacute form. The subacute course is more often recorded with infection by strains of moderate virulence. In such cases the clinical signs are less pronounced and can include:

- intermittent rises in body temperature;
- reduced appetite;
- depression;
- difficulty breathing;
- emaciation;
- intermittent diarrhea;
- growth retardation in young animals [4].

The duration of the disease can range from several weeks to a month, and the mortality rate ranges from 30 to 70%.

Chronic form. The chronic form is found mainly with infection by low-virulence strains and is characterized by a prolonged course of disease. Affected animals may show:

- emaciation;
- growth retardation;
- intermittent fever;
- arthritis;
- chronic respiratory disorders;
- necrotic skin lesions;
- enlarged lymph nodes [11].

Animals may remain alive for several months, but are often a source of viral spread among susceptible livestock.

Clinical manifestations in wild boar. In wild boar, the disease predominantly runs an acute course and is accompanied by high mortality.

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Sudden death of animals, or a brief period of depression, fever, and loss of motor coordination before death, is most often recorded [12].

Features of clinical diagnosis. The clinical signs of ASF are not specific and can resemble the manifestations of classical swine fever, swine erysipelas, salmonellosis, pasteurellosis, porcine reproductive and respiratory syndrome, and other infectious diseases [5]. A definitive diagnosis is therefore possible only after laboratory testing.

A sudden increase in mortality, fever of unknown origin, hemorrhagic lesions, and wild boar deaths should be regarded as grounds for immediate notification of the competent authorities and for carrying out laboratory diagnosis for African swine fever [7].

PATHOLOGICAL CHANGES IN AFRICAN SWINE FEVER

Pathological changes in African swine fever depend on the virulence of the viral strain, the duration of the disease, and its clinical form. The most characteristic lesions are observed in the acute and peracute course of the disease (Figures 2, 3) and are associated with the development of generalized hemorrhagic syndrome, impaired microcirculation, damage to the vascular system, and pronounced immunopathological processes [1, 7].

General pathological changes. Carcasses of dead pigs often show signs of emaciation or, conversely, satisfactory body condition, depending on the duration of the disease. Multiple hemorrhages, congestive hyperemia, and areas of cyanosis may be found on the skin of the ears, neck, chest, abdomen, perineum, and limbs [2].

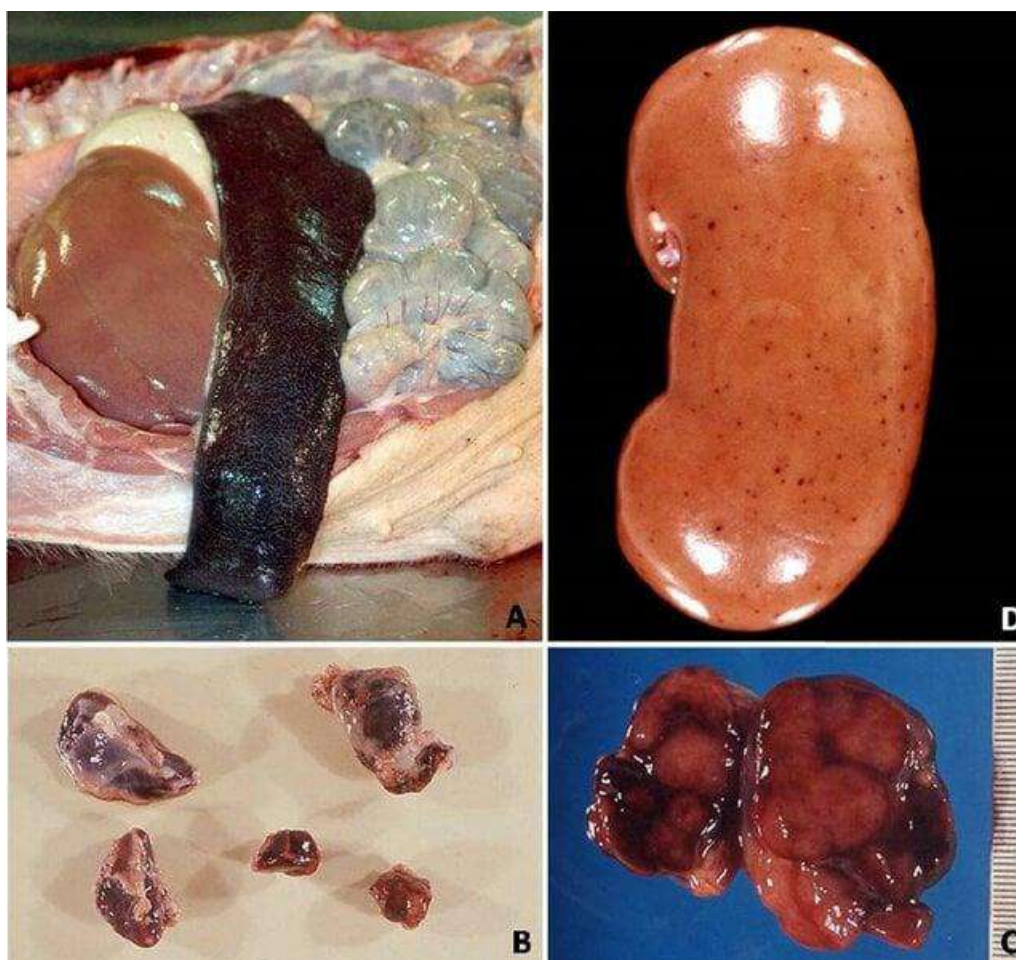


Figure 2 — Acute form of ASF. (A) An enlarged, purplish-black spleen occupies the entire abdominal cavity (congestive splenomegaly). (B) Several lymph nodes show hemorrhages of varying severity, most pronounced in the medulla. (C) Cross-sections of lymph nodes showing a marbled pattern. (D) Petechial hemorrhages in the renal cortex [2].

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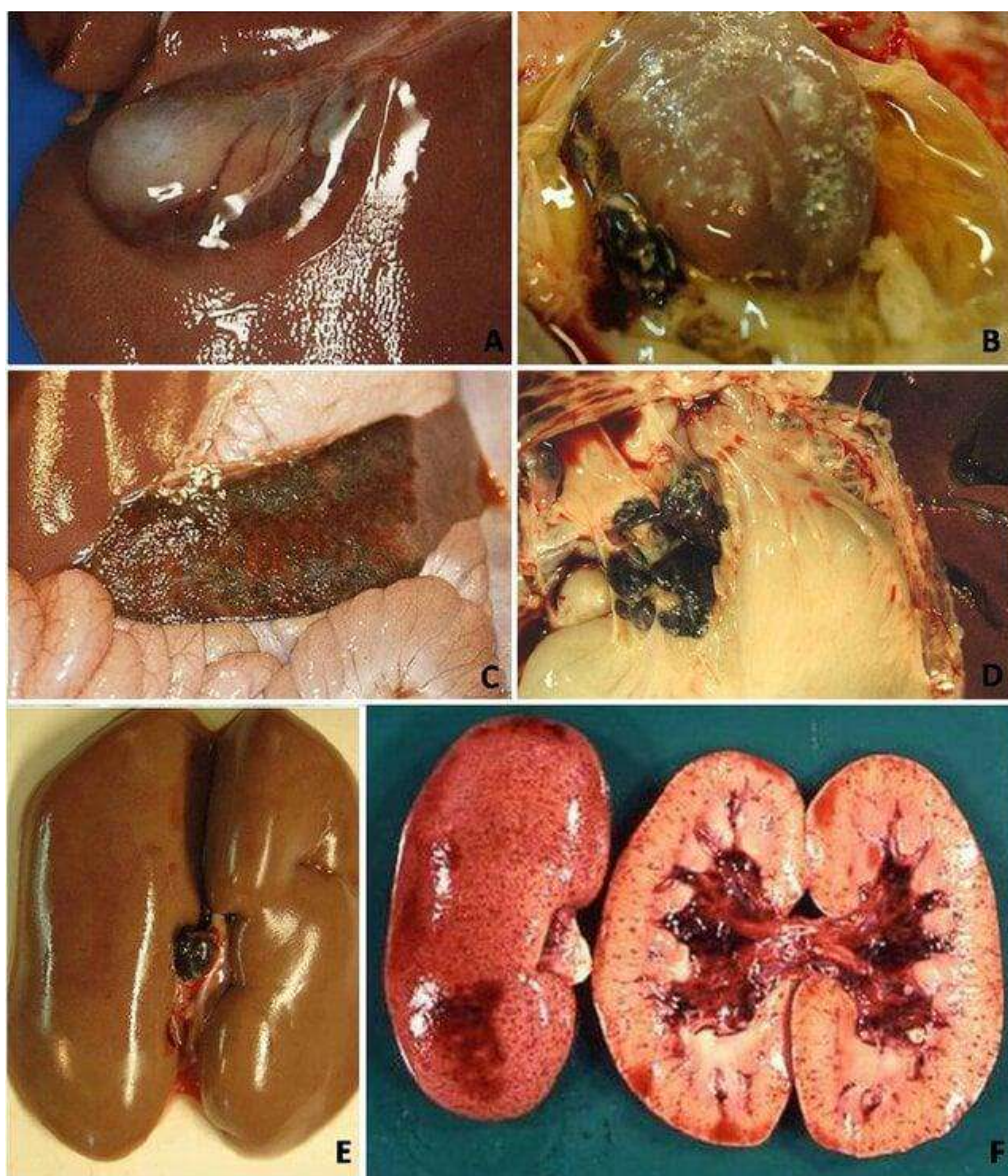


Figure 3 — Subacute form of ASF Marked edema of the gallbladder wall (A) and perirenal tissue (B). (C) Spleen with partial congestive splenomegaly. Hemorrhagic and edematous gastrohepatic (D) and renal (E) lymph nodes. (F) Kidneys with pronounced hemorrhage in the cortex, medulla, and pelvis [2].

ASF is characterized by:

- multiple petechial and ecchymotic hemorrhages;

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- serohemorrhagic infiltrates in the subcutaneous tissue;
- edema of varying severity;
- congestion in internal organs;
- hemorrhagic diathesis [15].

Changes in the lymph nodes. One of the most characteristic signs of ASF is a marked enlargement of the lymph nodes. The gastrohepatic, renal, mesenteric, submandibular, and mediastinal lymph nodes are most commonly affected. They become dark red or almost black in color, swollen, and moist on section as a result of massive hemorrhage [6, 13].

Changes in the spleen. The spleen is one of the most severely affected organs in the acute form of ASF. It is considerably enlarged, dark red or almost black in color, with a tense capsule and softened pulp. In many cases the mass of the spleen can increase two- to fourfold compared with normal [1].

Changes in the kidneys. The kidneys frequently show numerous punctate and patchy hemorrhages in the cortex, pelvis, and beneath the capsule. The organs may be enlarged, congested, and edematous. Renal hemorrhages are considered one of the characteristic signs of the hemorrhagic course of ASF [5].

Changes in the lungs. The lungs in ASF are usually enlarged, edematous, and congested. Serohemorrhagic pulmonary edema, blood stasis, and focal hemorrhages are often observed. Serous or serohemorrhagic exudate may accumulate in the thoracic cavity [7].

Changes in the heart. Multiple hemorrhages of varying size are often found on the epicardium and endocardium. Petechiae and ecchymoses on the serous membranes, in the atrial area, and near the base of the great vessels are particularly characteristic [2].

Changes in the liver. The liver is usually enlarged, congested, and shows signs of congestive hyperemia. In some cases small hemorrhages and degenerative changes in the parenchyma may be observed [4].

Changes in the gastrointestinal tract. Hemorrhages, edema, and catarrhal-hemorrhagic inflammation may be found in the mucosa of the stomach and

intestine. The most pronounced changes are often localized in the large intestine and cecum [5].

Changes in the serous membranes and body cavities. Serohemorrhagic exudate frequently accumulates in the thoracic, abdominal, and pericardial cavities. Multiple hemorrhages of varying intensity may be observed on the pleura, peritoneum, and pericardium [6, 7].

Features of pathological changes in different forms of the disease. In the peracute course, characteristic changes may be poorly developed because of the rapid death of animals. In the acute form, the classical picture of generalized hemorrhagic syndrome is observed, with pronounced lesions of the spleen and lymph nodes. The subacute and chronic forms are characterized by less pronounced hemorrhagic changes, while emaciation, foci of necrosis, fibrinous inflammation of serous membranes, arthritis, and pneumonia are more often found [11].

Pathological changes in ASF have important diagnostic value and allow a well-founded suspicion of the disease to be formed. However, none of the signs described is absolutely specific, so a definitive diagnosis must be confirmed by laboratory testing in accordance with WOAHP requirements and national regulatory documents [5, 6, 7].

CONTROL AND PREVENTION MEASURES FOR AFRICAN SWINE FEVER

African swine fever (ASF) is a particularly dangerous transboundary animal disease for which no widely available and broadly implemented means of specific treatment currently exist. Disease control therefore relies on the timely detection of infection cases, strict compliance with biosecurity requirements, prompt quarantine measures, and effective epizootiological investigation of the sources and routes of pathogen spread [5, 7].

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Farm biosecurity. Biosecurity is the most effective means of preventing the introduction of the ASF virus into holdings. The main measures are:

- keeping pigs in enclosed conditions without contact with wild boar;
- controlling access by outsiders to the holding's territory;
- installing sanitary checkpoints and disinfection barriers;
- regular disinfection of premises, vehicles, and equipment;
- using dedicated clothing and footwear for personnel;
- prohibiting the use of untreated food waste to feed pigs;
- controlling the origin of feed and water;
- restricting contact between different production groups of animals [5, 8].

Particular attention should be given to backyard-type holdings, where the risk of virus introduction is often higher because of a lower level of biosecurity.

Epizootic monitoring and early detection. Timely detection of the disease is a key condition for preventing large-scale spread of the infection. The monitoring system should include:

- continuous clinical surveillance of pig populations;
- laboratory testing of suspect disease cases;
- examination of pathological material from dead pigs;
- passive and active monitoring of wild boar populations;
- prompt notification of the competent authorities of cases of elevated mortality or suspected ASF [7, 8].

Measures in the event of suspected ASF. If ASF is suspected, the following must be done:

- immediately notify the local branch of the State Service on Food Safety and Consumer Protection;
- halt the movement of pigs, feed, and pig-farming products;
- isolate suspect animals;
- arrange the collection of pathological material for laboratory testing;
- keep records of all contact animals and possible transmission factors;
- conduct a preliminary epizootiological investigation [10].

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Measures upon confirmation of ASF. After laboratory confirmation of the disease, quarantine restrictions are introduced in accordance with the current legislation of Ukraine and WOAHA recommendations. The main measures include:

- establishing quarantine;
- delineating the epizootic focus and the protection and surveillance zones;
- prohibiting the movement of pigs and pig-farming products;
- culling (depopulation) of sick, suspect, and contact animals;
- safe disposal of carcasses, feed, and waste;
- cleaning and disinfecting premises, vehicles, and equipment;
- conducting an epizootiological investigation to establish the source of infection and possible routes of its spread [5, 7].

Control of wild boar populations. Wild boar play an important role in sustaining viral circulation under natural conditions. To reduce the risk of ASF spread, the following are carried out:

- monitoring population numbers;
- examining animals found dead;
- controlled management of wild boar numbers;
- searching for and safely disposing of the carcasses of dead animals;
- preventing the feeding of wild boar in affected regions [8, 12].

Disinfection and decontamination. The ASF pathogen is relatively resistant in the external environment, so effective disinfection is important for eliminating disease foci. Decontamination relies on preparations based on:

- sodium hydroxide;
- sodium hypochlorite;
- formaldehyde;
- glutaraldehyde;
- other agents recommended by veterinary authorities [5].

Thorough mechanical cleaning of surfaces from organic contamination is mandatory before disinfection.

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Public information and staff training. The effectiveness of prevention depends substantially on the level of awareness among animal owners, hunters, pig farm workers, and veterinary specialists. Information and outreach work should focus on:

- raising awareness of the clinical signs of ASF;
- promoting biosecurity principles;
- explaining the rules for handling the carcasses of dead animals;
- preventing illegal movement of pigs and pig-farming products;
- timely reporting of suspect disease cases [5, 6].

The importance of epizootiological investigation. One of the most important elements of ASF control is establishing the source of virus introduction and the factors in its spread. A detailed epizootiological investigation makes it possible to identify transmission mechanisms, assess risks to neighboring holdings, and determine the additional control measures needed to eliminate an outbreak and prevent the disease from recurring [6, 7, 8].

The comprehensive application of biosecurity, monitoring, quarantine, disinfection, and epizootiological investigation measures is the basis for effective prevention and control of African swine fever, both in Ukraine and in other countries around the world.

Consequences of failing to comply with ASF control measures.

Failure to comply with prevention, biosecurity, and control requirements for African swine fever (ASF) (Figure 4) creates the preconditions for rapid spread of the virus among domestic pigs and wild boar, the emergence of new epizootic foci, and substantial economic losses. Given the disease's high contagiousness, the absence of effective treatment, and the pathogen's considerable environmental resistance, even minor violations of veterinary-sanitary requirements can lead to large-scale outbreaks of infection [1, 6]. Spread of the disease among pig populations. The most obvious consequence of non-compliance with control measures is the introduction and rapid spread of the virus within a holding. Under intensive pig-farming conditions, the disease can affect the entire livestock population within a short time. For highly virulent ASFV

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strains, the lethality rate can reach 90–100%, leading to mass animal deaths or the need for culling as part of anti-epizootic measures [2].

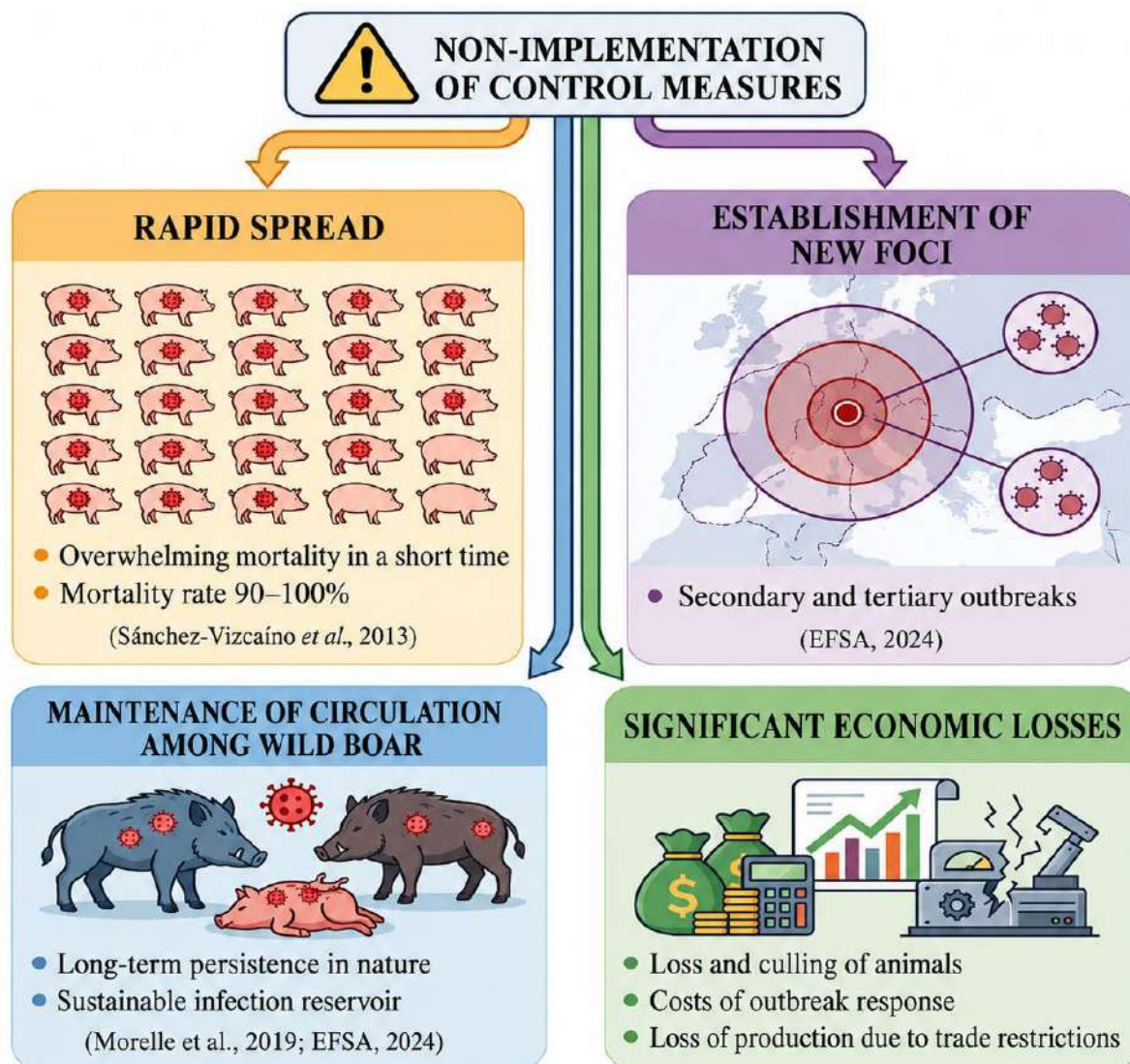


Figure 4 – Consequences of failing to comply with ASF control measures

Formation of new epizootic foci. Violations of animal movement rules, transportation of pig-farming products, non-compliance with quarantine restrictions, and inadequate disinfection can facilitate the spread of the virus beyond the initial focus. In such cases, secondary and tertiary outbreaks occur,

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which considerably complicates disease eradication and lengthens the duration of quarantine measures [8].

Sustained circulation of the virus among wild boar. Delayed detection and disposal of dead animal carcasses, lack of monitoring of wild boar populations, and inadequate control of hunting activity contribute to the long-term persistence of the virus in the natural environment. As a result, a stable natural reservoir of infection forms, which can remain a source of new disease cases among domestic pigs for years [12].

Substantial economic losses. ASF outbreaks are accompanied by significant economic losses for animal owners and the state. The main losses include:

- death and forced culling of pig populations;
- costs of eliminating disease foci;
- disinfection measures;
- disposal of carcasses and contaminated products;
- compensation payments to animal owners;
- reduced productivity of the pig-farming sector;
- job losses in the livestock sector [4, 5].

Trade restrictions. Detection of ASF outbreaks leads to the introduction of domestic and international restrictions on the movement of live pigs, meat, and pig-farming products. Such restrictions negatively affect the country's export potential, producer competitiveness, and the development of the agricultural sector as a whole [7].

Threat to food security. In regions with developed pig farming, substantial losses of livestock can lead to reduced pork production, market shortages, and rising prices. This can negatively affect the population's food security and the availability of protein-rich foods [5].

Complications in conducting epizootiological investigations. Delayed reporting of disease, concealment of animal deaths, or violations of the rules for recording pig movements considerably complicate the establishment of the

source of infection and its transmission routes. As a result, the risk of further viral circulation and repeated disease outbreaks increases [8].

Social consequences. ASF outbreaks can have substantial social consequences for the population, particularly in rural areas where pig farming is an important source of income. Mass culling of animals, restrictions on economic activity, and economic losses negatively affect the wellbeing of farmers and smallholder households [4].

In summary, failure to comply with African swine fever control measures poses a serious threat to animal health, the economic stability of holdings, food security, and the country's epizootic wellbeing. Strict compliance with biosecurity requirements, timely detection of the disease, and prompt anti-epizootic measures are necessary conditions for effectively combating ASF and preventing its further spread.

ADVANTAGES OF COMPLYING WITH AFRICAN SWINE FEVER PREVENTION AND CONTROL MEASURES

Compliance with a comprehensive set of veterinary-sanitary, preventive, and anti-epizootic measures is the foundation of effective control of African swine fever (ASF). Timely implementation of biosecurity, monitoring, and epizootiological surveillance principles makes it possible to minimize the risk of virus introduction into holdings, reduce the likelihood of its further spread, and ensure the stable functioning of the pig-farming sector [5, 7].

Preventing virus introduction into holdings. One of the main advantages of complying with biosecurity measures is a substantial reduction in the risk of the ASF pathogen entering a holding. Controlling access to the farm's territory, properly disinfecting vehicles and equipment, using safe feed, and preventing contact between pigs and wild boar considerably reduce the likelihood of an outbreak [5, 8].

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Preserving pig populations. Timely implementation of preventive measures makes it possible to avoid mass animal deaths or their forced culling in the event of an outbreak. This helps maintain farm productivity and preserve the genetic potential of pig populations [1].

Reducing potential economic losses. Compliance with control measures is economically advantageous, since it helps avoid costs associated with eliminating infection foci, culling animals, disinfecting premises, disposing of carcasses, and making compensation payments. In addition, stable epizootic wellbeing supports the sustainable development of pig-farming enterprises and the preservation of jobs [4].

Preventing further spread of ASF. Strict compliance with quarantine requirements, veterinary control, and animal movement rules makes it possible to contain an outbreak at an early stage and prevent the virus from spreading to other holdings, regions, or countries [7].

Preserving the epizootic wellbeing of territories. Regular monitoring, early detection of disease cases, and epizootiological investigations help maintain a stable epizootic situation and reduce the risk of forming permanent foci of viral circulation among domestic pigs and wild boar [8].

Preserving trade opportunities. Epizootic wellbeing with respect to ASF is an important condition for international trade in live animals and pig-farming products. Compliance with international standards makes it possible to maintain access to domestic and foreign markets, avoid trade restrictions, and support the development of the agricultural sector [6, 7].

Improving the effectiveness of epizootiological investigation. Proper record-keeping of livestock numbers, animal movements, veterinary measures, and timely reporting of suspected disease considerably facilitates the identification of the source of infection and its transmission routes. This enables rapid, well-founded decisions on containing and eliminating a disease focus [5].

Guaranteeing food security. Maintaining stable pork production helps ensure the population's supply of quality animal-origin products and supports the

state's food security. Preventing large-scale ASF outbreaks helps avoid product shortages and sharp market price fluctuations [4].

Improving preparedness for epizootic emergencies. Systematic compliance with prevention measures, staff training, and regular updates to response plans improve the readiness of veterinary services and animal owners to act promptly in the event of suspected ASF. This helps shorten response times and improve the effectiveness of anti-epizootic measures [5]. In summary, compliance with African swine fever prevention and control measures protects pig populations from infection, helps preserve the economic stability of holdings, supports the epizootic wellbeing of territories, and creates the preconditions for the sustainable development of the pig-farming sector. A comprehensive approach to biosecurity and monitoring remains the most effective tool for combating ASF in the absence of widely available means of specific prevention.

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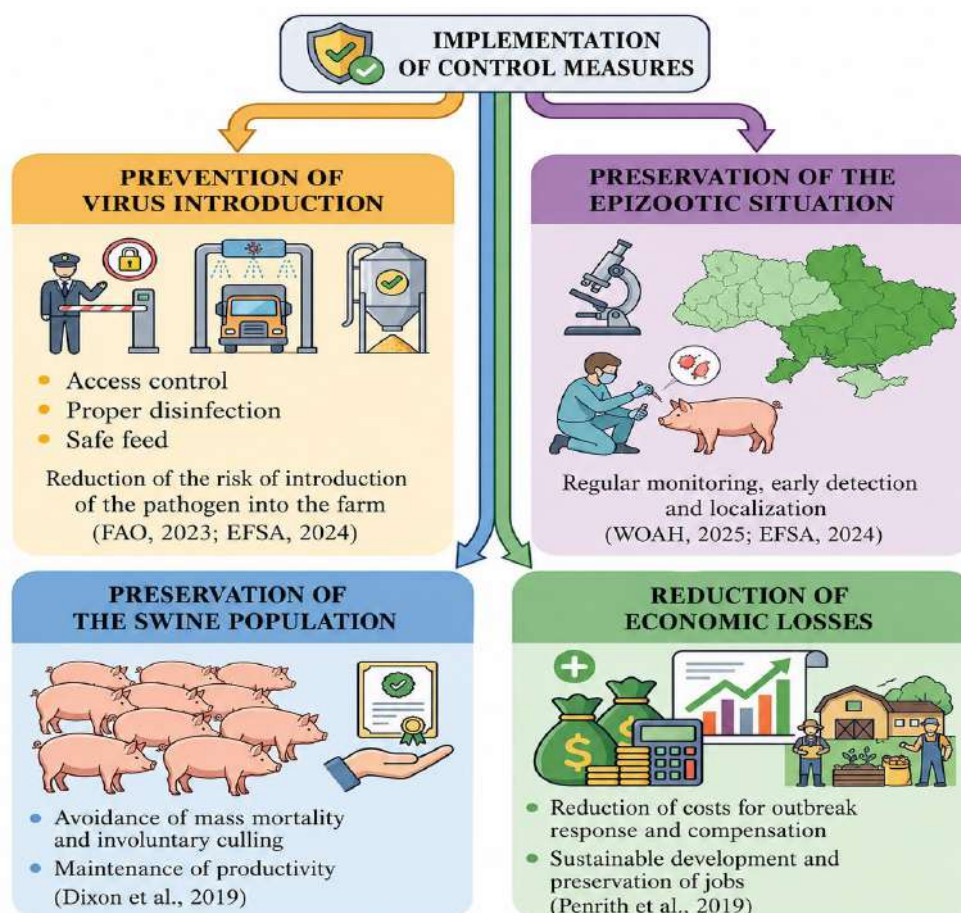


Figure 5 - Advantages of complying with ASF control measures

CONCLUSIONS AND OUTLOOK

- The transmission chain “wild boar – environmental objects (carcasses, soil, fomites) – domestic pig” is confirmed as the leading mechanism of ASF spread in the region studied. The pathogen’s exceptional resistance in the environment, in tissue, and in pig-farming products means that the infection is capable of persisting and spreading considerably longer and more widely than is captured by clinical cases recorded among domestic livestock.
- Molecular-genetic diagnosis by real-time PCR is the priority and reference approach at every stage of investigation. Combined with serological

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methods (ELISA, IFA, Immunoblot) for detecting animals that have recovered from infection, this combination provides the highest diagnostic reliability and forms the basis of the Protocol as the recommended approach for unified cross-border monitoring.

- The lack of a harmonized approach between Ukraine and Romania remains a critical gap, particularly given the shared wild boar populations in the border forest-steppe and floodplain areas of the Danube Delta. Before the SMS-RO-UA project, monitoring of wild boar numbers, recording of animal deaths, and the exchange of outbreak data by the two countries took place without adequate coordination. The Joint TOR and the Protocol developed are a first step toward closing this gap.

- Wild boar populations remain the key, and practically uncontrollable, natural reservoir of the virus. Unlike diseases with a clearly defined biological vector, ASF control shifts its main focus from treatment (which is unavailable) to passive surveillance of dead animals, population management, and the safe disposal of carcasses — these measures are the most effective tool for containing the pathogen in the natural environment.

- The absence of means of specific prevention (a vaccine) substantially increases the importance of biosecurity and epizootiological investigation. At present, no ASF vaccine has broad international recognition or use in the EU and Ukraine, so the basis of control remains farm biosecurity, early detection, quarantine restrictions, and identification of the source of virus introduction.

Implementation of the “One Health” principle in its cross-border dimension remains the central strategic benchmark for the further development of the ASF monitoring system. Specific perspectives, taking EU priorities into account, are set out below.

- Developing a GIS platform for predictive monitoring of wild boar populations. The GIS platform developed under the project has potential for integrating data on wild boar movement and numbers, locations where dead animals have been found, and the results of field studies. Predictive modeling of

ASF spread risk using artificial intelligence algorithms is consistent with the European Veterinary Big Data Strategy 2022–2027.

- Harmonizing Ukraine’s national legislation with EU standards. In the context of Ukraine’s status as an EU candidate country, the approach to joint standardized data collection established in this Protocol is a practical tool for aligning Order of the Ministry of Agrarian Policy No. 111/2017 and Resolution of the Cabinet of Ministers No. 1009/2021 with the requirements of Implementing Regulation (EU) 2023/594 and its subsequent updates.

- Strengthening passive surveillance of wild boar as a priority tool. Experience from EU countries shows that detecting and examining the carcasses of dead wild boar is a more effective method of early ASF detection than active culling. It is recommended to expand the network of collection points and laboratory testing facilities for pathological material from dead wild boar in the border zone.

- Incorporating the results into educational programs. The materials in this Protocol, together with the Practical Guide to Assessing Emerging Diseases, are recommended for integration into the training programs of veterinary medicine faculties at OSAU and partner institutions, in line with the goals of the Erasmus+ Programme and the EU Strategy for the Danube Region (EUSDR).

- Engaging hunting enterprises and raising public awareness. The project’s findings underscore the need for systematic public information campaigns, particularly among hunters, smallholder households, and pig farm owners in the border zone, with an emphasis on rules for handling wild boar carcasses, preventing supplementary feeding, and prohibiting the use of untreated food waste to feed pigs.

- Building a cross-border network for laboratory and population monitoring of wild boar. The SMS-RO-UA project lays the groundwork for a sustainable cross-border network among laboratories and potential new participants from neighboring countries with experience in controlling ASF among wild boar populations. Such a network is consistent with the priorities of

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EU macro-regional strategies and could be supported under future Interreg or Horizon Europe programmes.

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