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AFRICAN SWINE FEVER: A REVIEW OF THIS EXOTIC DISEASE IN BRAZIL

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Abstract: African Swine Fever is a highly contagious viral disease that affects domestic and wild swine, and causes mortality rates of up to 100% in the most acute forms. It can be transmitted by direct or indirect contact with infected pigs and contaminated materials, and also by tick vectors. It is considered endemic in Africa but has been causing new outbreaks in Europe, Asia and even Central America in recent years. It is a global threat, because there is no vaccine or treatment for this disease. The recommendation of the World Organization for Animal Health is the sanitary slaughter of all exposed animals, causing great socioeconomic damages. Brazil has already registered the virus in the past and is now considered a country free of the disease. Implements restrictive import and biosecurity policies to prevent reintroduction of the virus. Early detection and diagnosis are the keys to preventing the spread of African Swine Fever, which can derail swine farming in a region for years. This study reviews the literature on the disease, with approaches on etiology, pathogenesis, epidemiology, clinical manifestations, necropsy findings, diagnostic methods, therapies, and control and prevention strategies.
Keywords: African Swine Fever, Asfivirus, swine, tick, epidemiology.

INTRODUCTION

African Swine Fever (ASF) is a highly contagious hemorrhagic disease caused by a virus of the Asfarviridae family, which affects domestic and wild swine and crosses. As the name suggests, the first cases were recorded in Africa at the beginning of the last century, where the disease is also known as African Swine Fever. But outbreaks have been recorded periodically on other continents. Mortality can reach 100% of infected animals, and the sanitary slaughter of the entire exposed herd is recommended as an immediate measure to contain the rapid spread of the disease, which causes great economic losses and can even make swine farming in the affected region unfeasible (Figure 1). Although it is not a zoonosis, ASF is on the list of diseases of mandatory notification to national authorities and the World Organization for Animal Health (OIE), as the impact of infection can cross borders and give rise to a pandemic. To date, there is no vaccine available, nor treatment (GALINDO & ALONSO, 2017).

Figure 1 - Animals slaughtered on farms in Russia to contain the spread of ASF.

Source: Korniyenko (2017).

The virus is highly resistant to environmental challenges. It can survive freezing, the putrefaction process and remain stable for months in tissues, secretions and excretions. Transmission occurs either by direct contact with infected pigs (live or dead) or by ingestion of material from them (meat, blood, feces, feed containing swine derivatives, leftovers of undercooked human food, cured or smoked sausages), or even by contact with fomites, injection needles, equipment, shoes, clothing, vehicles, etc. An important peculiarity is that the virus also spreads through the bite of soft ticks of the genus Ornithodoros, which act as biological reservoirs and vectors (PROBST et al., 2017).

The disease is characterized by high fever, leukopenia, erythema, and bloody diarrhea.

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It can present in severe acute (which leads to death in a few hours), subacute or chronic forms. The clinical signs are similar to those of Classical Swine Fever (CSF), caused by a virus of the Flaviviridae family, and for a long time it was thought that ASF outbreaks in Africa were caused by the same disease, but in an aggravated form. But with viral isolation, it was seen that PSA has a different etiological agent, and that it is possible to make a differential laboratory diagnosis (MCVEY et al., 2016).

Today ASF is considered an endemic disease in most sub-Saharan African countries. And it is also present in Europe and Asia. In the last decade, new outbreaks have been recorded in countries that, until then, were free of the disease, such as Moldova, the Czech Republic, Romania, Hungary, Bulgaria, Slovakia, Serbia and Greece. The same happened in Asia, including China – a country that is the biggest producer and biggest consumer of pork in the world. Between 2016 and 2020, a total of 832,698 cases were reported to the OIE globally, with 8,202,702 animals killed and euthanized (Figure 2) (OIE, 2020).

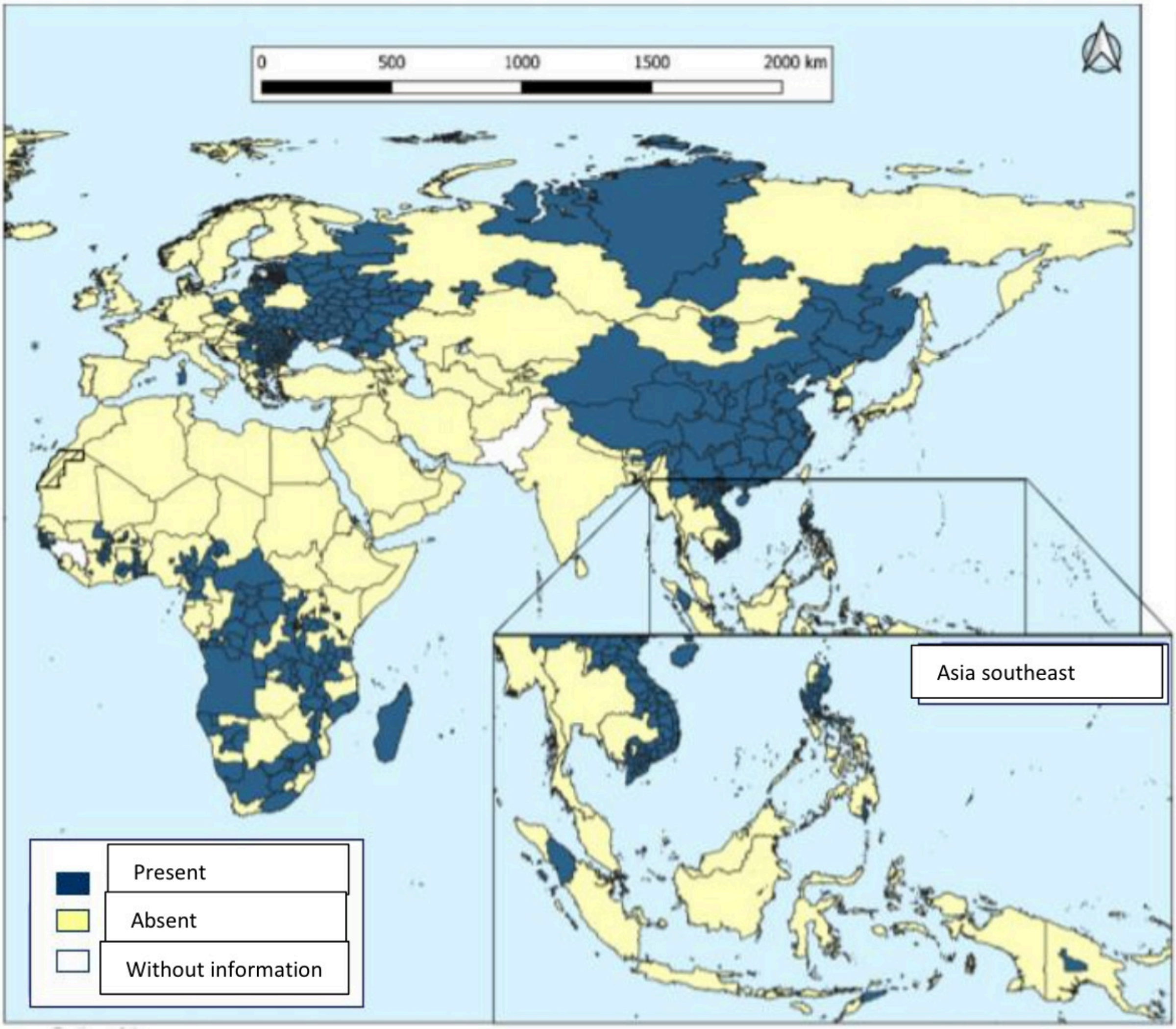

Figure 2 - Global situation of PES (2016-2020).

Source: OIE (2020).

In July 2021, an outbreak was recorded in the Americas for the first time since the 1980s. The virus was detected in domestic swine farms in the Dominican Republic and later in Haiti. The origin is not clear, but illegal animal trade and food transit are suspected. Thanks to intervention measures and sanitary slaughter, the disease did not spread across the continent. The Inter-American Institute for Cooperation on Agriculture (IICA) is now coordinating efforts to eradicate the disease in the Americas (IICA, 2022).

Latin American countries, including Brazil, had ASF outbreaks in the 1970s, but they were soon brought under control. The disease was considered eradicated in Brazilian soil in 1984 and, since then, it has been classified as exotic in the country. Prevention here, as in other disease-free countries, is based on the implementation of strict import policies for pork products, and on strict biosecurity measures. The Brazilian Agricultural Research Corporation (Embrapa), linked to the Ministry of Agriculture, Livestock and Supply (MAPA), calculates that, if the virus is reintroduced in Brazil, losses to swine farming could exceed US$ 5.5 billion in the first outbreak year. The consequences would be very serious, as Brazil is now the 4th largest producer and 4th largest exporter of pork in the world. Hence the great importance of disseminating information about the disease and how to avoid it (BRASIL, 2018).

LITERATURE REVIEW

ETIOLOGIC AGENT AND PATHOGEN

The etiologic agent of African swine fever is a DNA virus of the genus Asfivirus, the only member of the family Asfarviridae. It is 175 to 215 nm in diameter and is formed by a nucleoprotein center within an icosahedral capsid, surrounded by an outer envelope containing lipids (Figure 3). 23 different genotypes have been identified by genetic sequencing, most of them highly virulent. The infection of swine in general is acquired mainly through the oronasal route. The virus initially replicates in the pharyngeal mucosa, tonsils and regional lymph nodes. The infection then spreads through the bloodstream to other lymph nodes, bone marrow, spleen, lung, liver, and kidneys. The virus can also infect megakaryocytes, endothelial cells, renal epithelium and hepatocytes (QUINN et al., 2005).

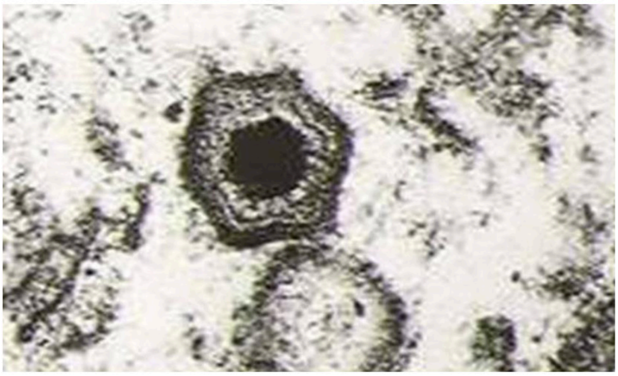

Figure 3 - Electromicrograph of an ASF-causing virus. Source: CISA-INIA (2015).

In the case of transmission via the tick, the target cells are monocytes and macrophages, where the virus replicates before being released into the circulation by budding in the plasma membrane or disintegration of the host cell. Virions contain more than 50 different proteins that promote viral replication and also appear to modulate the protective antiviral response of the infected animal (MCVEY et al., 2016).

When released into nature with the carcass, viscera, secretions or excretions of the host, the ASF virus is quite stable, being able to resist important physical and chemical variations:

- TEMPERATURE: highly resistant to low temperatures and freezing (can survive years in frozen carcasses), but is inactivated if exposed to 56°C for 70 minutes or to 60°C for 20 minutes;

• pH: can withstand a wide range between 4 and 13;

• DISINFECTANTS: susceptible to ether and chloroform; inactivated by sodium hydroxide 8/1,000 (30 minutes), hypochlorites – solutions between 0.03% and 0.5% (30 minutes), formalin 3/1,000 (30 minutes), orthophenylphenol 3% (30 minutes) and compounds iodinated;

• SURVIVAL: remains viable for long periods in blood (up to 1.5 years at 4°C), faeces and tissues, especially in raw or undercooked infected pork (150 days in deboned meat at 4°C) (OIE, 2019).

EPIDEMIOLOGY OF THE DISEASE

ASF was originally identified in East and South Africa in the early 20th century. The disease apparently established itself in a sylvatic cycle involving transmission between feral swine species Phacochoerus africanus (warrior, figure 4A) and Potamochoerus larvatus (African hog, Figure 4B) respectively) and biological vectors - ticks Ornithodoros genus. As theferal swine act as reservoirs and do not develop the clinical disease, they eventually spread the virus to domestic swine farms in Kenya in the 1920s, and later throughout sub-Saharan Africa, devastating entire herds. Until today, the disease is considered endemic in Africa and, it is believed, impossible to be eradicated, due to the large number of asymptomatic wild pigs and ticks in the environment, which perpetuate the sylvatic cycle (OKOTH et al., 2012).

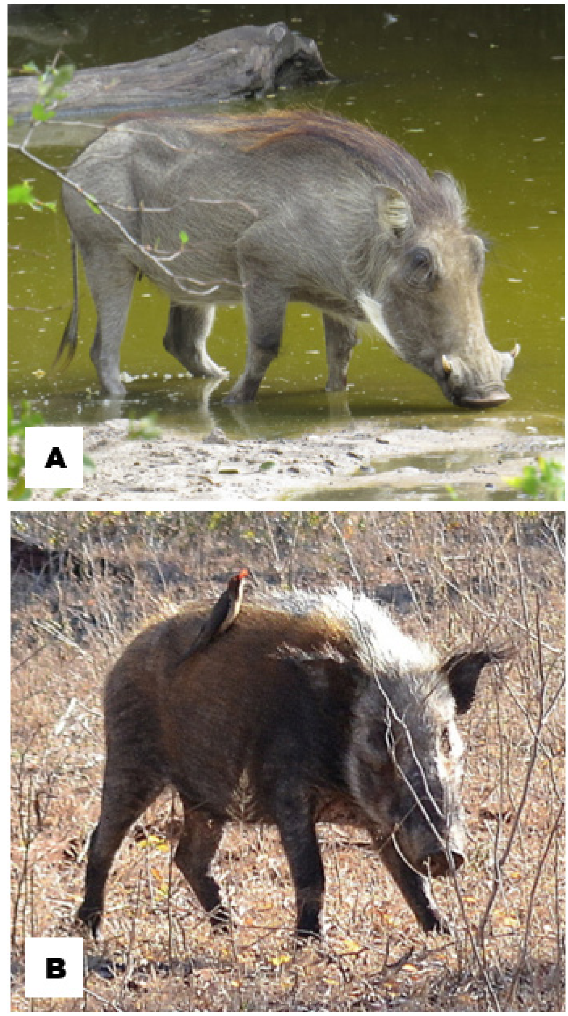

Figure 4 - ASF reservoirs in Africa: A) Phacochoerus africanus; B) Potamochoerus larvatus. Sources: LOUIS (2015) and COOPER (2015), respectively.

In Europe, ASF was introduced in the 1950s because of leftover meals from a plane from Angola, which contained contaminated pork derivatives. The disease spread to other European countries, and was considered endemic on the continent until the end of the century. Contaminated food from aircraft is also identified as the cause of the introduction of the virus in Latin America in the 70s, including Brazil (DANZETTA et al., 2020).

In the Brazilian case, food scraps from a flight departing Portugal were diverted by an airport police officer in 1978 to feed pigs on a farm he kept in Paracambi, Rio de Janeiro (Figure 5A). More than 200 animals died in 13 days (Figure 5B), and the breeder ended up selling the carcasses, as well as live pigs, fearing further damage. So he spread the PSA to 10 other states. At the time, Brazilian pig farming was still very rudimentary, with a low level of technology and widespread clandestine slaughter, which created favorable conditions for the spread of the disease (MOURA et al., 2010).

Figure 5 - ASF outbreak in Brazil, 1978: A) leftover diverted flight food; B) dead animals in Paracambi, RJ. Source: MOURA et al. (2010).

When the virus was identified, the Brazilian government declared an emergency and implemented strict sanitary measures, with mass slaughter, to contain the disease. The last positive case was registered in 1981. In 1984, Brazil was declared free from ASF – a status that remains until today (MOURA et al., 2010).

ASF affects domestic or wild pigs of any age. It is transmitted mainly by direct contact with infected animals, through oral and nasal secretions, aerosols, injuries in fights, ingestion of carcasses; by indirect contact with excreta, blood, products derived from contaminated swine; via fomites, vehicles, equipment, etc.; by the bite of the vector tick; or, still, the infection can happen mechanically through the action of blood-sucking insects, such as mosquitoes and flies. Studies have revealed that the so-called stable fly (Stomoxys calcitrans), for example, can retain and transmit infective amounts of the virus for up to 2 days after feeding on a sick pig (ROVID, 2019).

The epidemiology varies in different parts of the world, according to habitat, vectors and types of breeding. Currently, four cycles with different routes of viral transmission are recognized:

- WILD CYCLE: occurs in Sub-Saharan Africa, as already mentioned, and involves African wild swine (Phacochoerus africanus and Potamochoerus larvatus) and tick vectors Ornithodoros spp.;
- TICK CYCLE: ticks Ornithodoros spp. infected transmit the virus to domestic pigs (Sus scrofa domesticus);
- DOMESTIC CYCLE: domestic pigs become infected by consuming products derived from infected pigs (such as meat, blood, fat, bones, bone marrow, skins; and human food waste, such as salami and sausages); after a few days they begin to eliminate the virus through secretions and excretions;
- BOAR-HABITAT CYCLE: has been detected in recent outbreaks in Europe; There is direct transmission of the virus from infected wild boar (Sus scrofa scrofa) to other

susceptible ones, and indirectly by intraspecies necrophagy (BRASIL, 2018).

Each epidemiological cycle is independent, but inter-cycle transmissions can also occur. In vectors, transmission can be sexual, transovarian and transstadial (Figure 6).

CLINICAL MANIFESTATIONS

The incubation period for the ASF virus is 4 to 19 days. The clinical manifestations observed depend on the virulence of the strain and the transmission route, and the disease can present in hyperacute, acute, subacute and chronic forms (Chart 1). In general, the first sign of infection in a herd is the sudden death of several animals, even before they present lesions. This is the hyperacute form of the disease, caused by the more virulent genotypes of PSA, as well as the acute form. Both the hyperacute and acute forms usually appear at the beginning of the outbreak, and are characterized by a mortality that can reach 100% between 4 and 9 days after infection, and by a rapid spread of the virus. Once the disease is established in the region, a wide range of clinical manifestations is expected, with the emergence of subacute and chronic forms of the disease, and a decline in the mortality rate (GALLARDO et al., 2015).

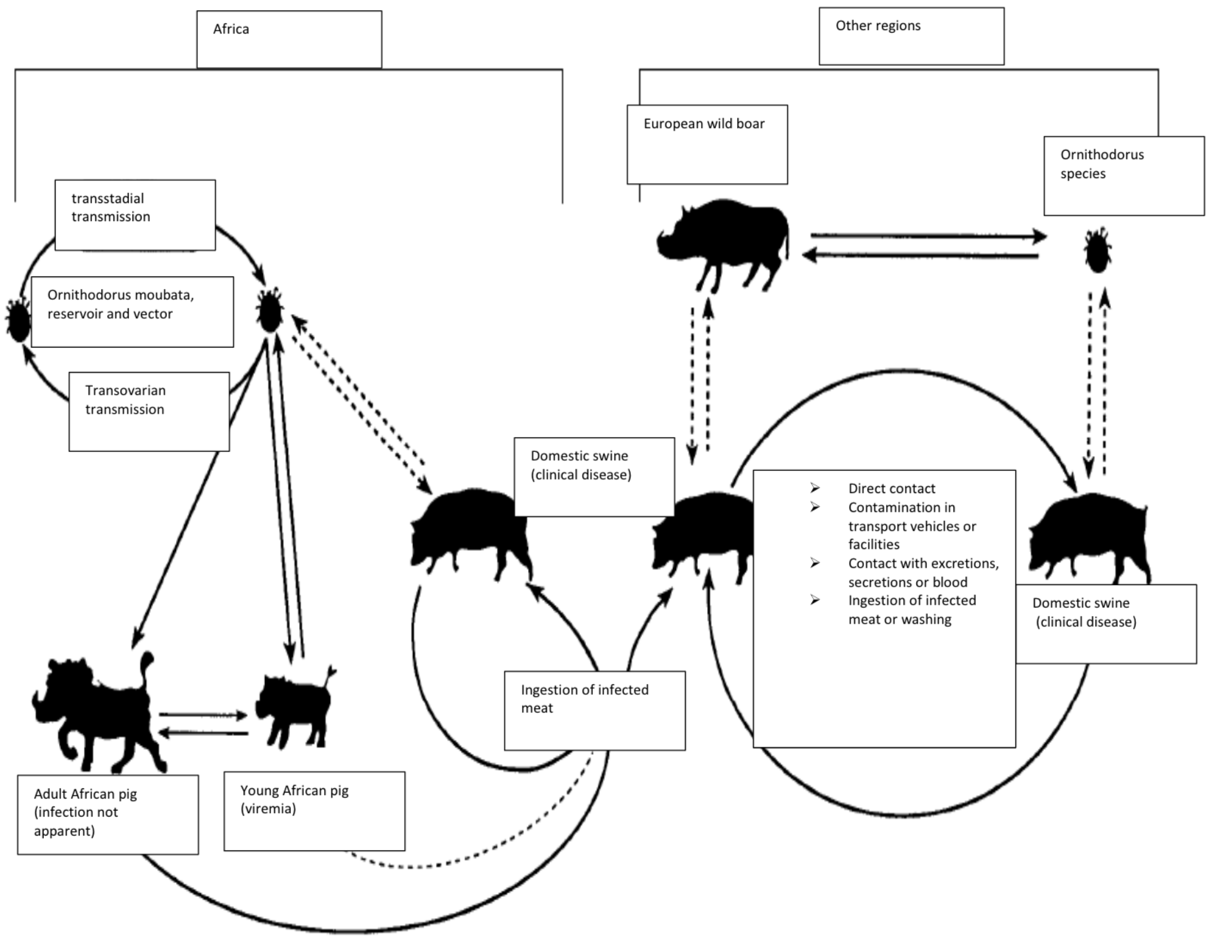

Figure 6 - Transmission of the ASF virus in domestic and wild swine populations, and in tick vectors.

Source: Quinn et al. (2005).

PSA forms	Clinical manifestations
hyperacute (high virulence)	• Sudden death with few signs
acute (high virulence)	• Fever (40.5°C to 42°C) • Leukopenia and thrombocytopenia (48-72 hours) • Skin erythema and hemorrhages – tips of ears, tail, distal extremities, ventral part of the chest and abdomen • Anorexia, apathy, cyanosis, and incoordination (24-48 hours before death) • Increased pulse and respiratory rate • Vomiting, diarrhea (sometimes bloody) and eye secretions • Abortions in pregnant females • Death between 6-13 days, or up to 20 days In domestic pigs, the mortality rate usually approaches 100%.
Subacute (moderate virulence)	• Clinical signs similar to the acute form, but less intense • Hyporexia and depression • Duration of illness of 5-30 days • Abortion in pregnant females • Lower mortality rate (wide range, 30% to 70%)
chronicle (moderate or low virulence)	• Weight loss, irregular temperature spikes, respiratory signs, chronic ulcers and skin necrosis, arthritis • Pericarditis, lung adhesion, joint swelling • Low mortality

Table 1: Forms and clinical manifestations of African Swine Fever.

Source: Adapted from OIE (2019).

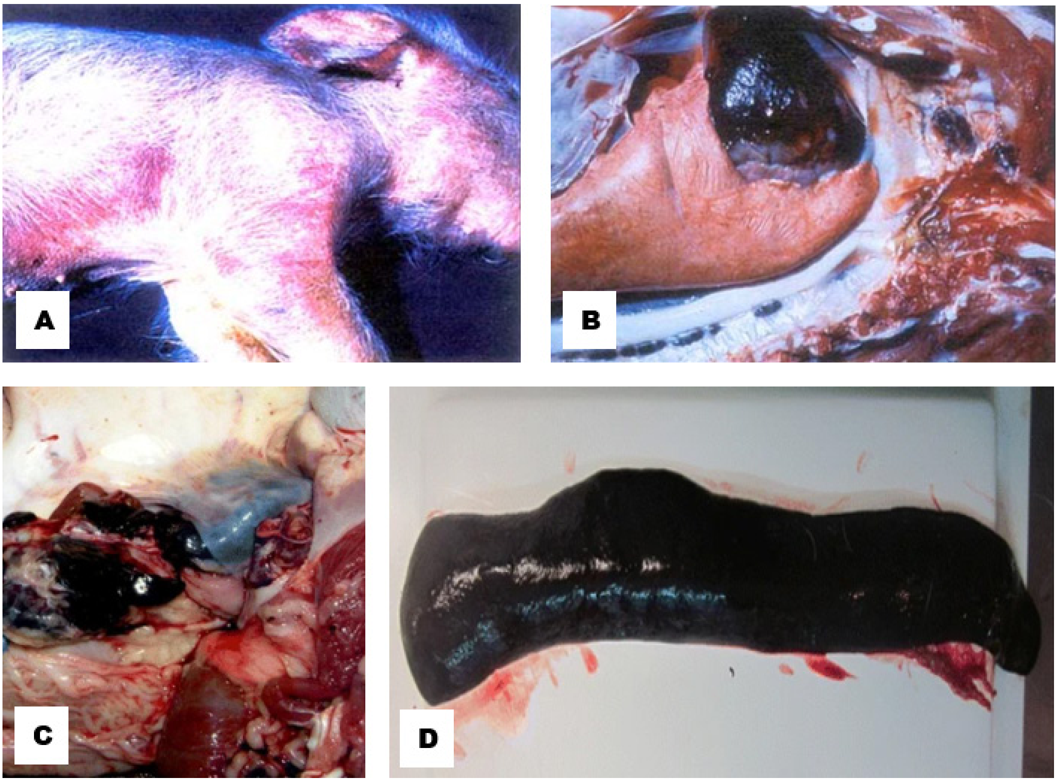

Figure 7 - Necropsy findings for the acute form of PSA: A) extensive hemorrhages in the skin; B) hemorrhages in multiple organs and in the serous lines of the cavities; C) enlarged and hemorrhagic lymph node, with the appearance of a blood clot; D) hyperemic splenomegaly. Sources: CISA-INIA (2015) and FAO (2000).

Pigs infected with milder forms of the disease can survive for several weeks. Some even recover completely, but remain infected subclinically – totally asymptomatic – for the rest of their lives, acting as hosts for the virus. The mechanisms responsible for the recovery of sick animals are still poorly understood. Studies have concluded that they develop an intense humoral response, but neutralizing antibodies are not demonstrable in the serum of recovered pigs. Cellular immunity is suspected to be the main protective factor. In endemic areas, ASF often progresses to subacute and chronic forms, due to the establishment of median and low virulence strains. (QUINN et al., 2005). They cause mild and nonspecific clinical signs (mild fever, hyporexia, depression, miscarriage, etc.), which can be confused with other illnesses.

NECROPSY FINDINGS

The lesions observed at necropsy vary depending on the clinical presentation of PSA. In general, animals that die in the hyperacute form have the carcass in good condition. In the acute form, it is common to find congestion and extensive hemorrhages in the carcass, skin (Figure 7A) and in internal organs (mainly lymph nodes, kidneys and intestines - Figures 7B and 7C), bloody fluid in the thoracic and abdominal cavities, spleen with increased volume (Figure 7D), pulmonary edema and trachea filled with respiratory secretions (sometimes with blood), among other findings (FAO, 2000).

In the subacute form, the macroscopic lesions are similar to those found in the acute form, but less intense. There may be fluid in cavities if there is heart failure; enlarged and often hemorrhagic lymph nodes, etc. In the chronic form, the lesions are not specific but can persist for months. Animals may present with fibrinous pleuritis and pericarditis, consolidation of the pulmonary lobe, joint swelling and areas of skin necrosis. Microscopic lesions are more evident in lymphoid tissues and include extensive necrosis of both mononuclear phagocytic cells and lymphocytes. In fulminant cases of hyperacute PSA, it is common to note endothelial cell necrosis and pulmonary vessel thrombosis (MCVEY et al., 2016).

DIAGNOSIS

Early diagnosis of ASF is essential to implement sanitary and biosecurity measures, and prevent the spread of the disease. But this is not an easy task due to the different forms of presentation of the disease and its complex epidemiology. Confirmation has to be done in the laboratory, and for this it is possible to make use of two types of methods: serological/immunological (for antibody detection) or parasitological/viral isolation (antigen detection). Each has its advantages and limitations. The sample for viral isolation can be blood collected from live animals or tissues recovered at necropsy – especially spleen, kidneys, tonsils, lymph nodes, lungs and bone marrow. From them, a viral culture is made in a specific medium and one of the identification techniques is applied: hemadsorption test, direct immunofluorescence or PCR (Figure 8). Viral isolation is used especially in areas of outbreak of the hyperacute and acute form of the disease, where animals die before they can seroconvert. The limitation of the method is that it has low sensitivity for situations where viremia is low (OIE, 2019).

Figure 8 - PCR kit for PSA amplification and molecular detection. Source: Ingenetix (2019).

The serological/immunological method uses laboratory kits to detect antibodies in the blood, serum or plasma of the animal with suspected disease or viremia. This is a very useful method for ASF control and eradication programs, and in endemic areas, to identify pigs that survived the disease and became asymptomatic carriers of the virus. The main limitations are that the sick animal needs to be at a specific stage of infection for antibodies to be detected, and chronic patients do not always have high titers because they may have intermittent viremia. The most used serological tests are the ELISA (Immunoenzyme Assay - quantitative test), the RIFI (Indirect Immunofluorescence Reaction - quantitative test, also known by the acronym in English IFA), and the immunoperoxidase and Immunoblotting tests (immunoblotting). ELISA is the test prescribed for international trade, and is usually confirmed by IFA or immunoperoxidase and immunoblot tests (GALLARDO et al., 2015).

In Brazil, ASF is part of the PNSS (National Swine Health Program), and is one of the diseases that must be notified to MAPA in case of suspicion. The diagnosis is made exclusively in an official laboratory of the Federal Government, Lanagro, in Minas Gerais. It is also worth remembering that the differential diagnosis of ASF must include, primarily, Classical Swine Fever and, secondarily, other diseases such as salmonellosis, erysipellosis, pasteurellosis, circovirus (PDNS), PRRSV, anthrax and intoxication by anticoagulants (BRASIL, 2018).

TREATMENT

There is no treatment for PSA, only supportive treatment (FAO, 2000).

PREVENTION AND CONTROL

Just as there is no treatment, there is still no vaccine against the ASF virus. Largely because the animals that survived the disease lack neutralizing antibodies. The control and prevention measures recommended by the World Organization for Animal Health, for outbreak regions, is the immediate sanitary slaughter of all animals exposed to the virus, with adequate disposal of carcasses and livestock residues (incineration or digging of deep ditches), followed by the implementation of strict biosecurity measures such as: thorough cleaning and disinfection with effective products (already mentioned in item 2.1); sanitary vacuum (at least 1 month, but each country has its specific legislation); designation of the infected zone, with restriction of movement of swine in it; conducting a detailed epidemiological investigation to track possible sources of infection and sites of spread; monitoring of the infected zone and surroundings etc. For already infected countries and endemic regions, the OIE recommends restricting the movement of swine (Figure 9), to avoid contact between domestic animals and wild animals, and with tick vectors and their habitat (OIE, 2019).

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Figure 9 - German rangers install a fence on the Polish border to keep wild boars out. Source: JEON (2020).

The Food and Agriculture Organization of the United Nations (FAO) also recommends preventive measures such as: instituting immediate quarantine for farms with suspected or confirmed cases of ASF; do not feed swine with washing that contains pork meat and derivatives, or, in the best case scenario, cook this food for 30 minutes and cool it before serving the creation; disinfection of vehicles, equipment, clothes and shoes at the entrance and exit of properties, etc. (Figure 10). (FAO, 2000).

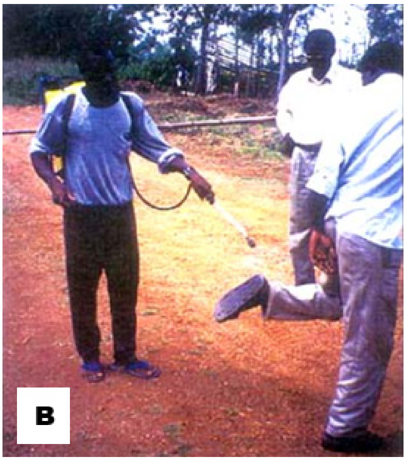

Figure: 10 - Biosecurity measures against African Swine Fever: A) tire disinfection; B) disinfection of shoes. Source: FAO (2000).

In Brazil, as in other disease-free countries, prevention aims to prevent the introduction and spread of the virus. Strict import policies are in place to ensure that neither live infected pigs nor pork products from ASF-affected regions enter the country. Proper disposal (heat treatment) of food waste from aircraft, ships or vehicles from countries with the disease is also mandatory. Embrapa carries out educational campaigns, training and qualifying veterinarians and producers to recognize the disease, and subsidizing the competent bodies in decision-making (BRASIL, 2018).

FINAL CONSIDERATIONS

ASF has become a re-emerging disease in recent years, with the virus being reported in once-free countries, including China - the world's biggest producer of pork, which saw the price of the product hit a record high in March 2021. the outbreak registered in Central America in 2021, there is a real threat that ASF will be reintroduced throughout the continent and even in Brazil, which could have very serious implications for a country whose economy is substantially dependent on agribusiness as a growth vector.

With the globalized world and intense international trade, the authorities are committed to inspecting imports at customs at ports and airports. But it is almost impossible to control what travelers carry in their suitcases, even across land borders. Almost 2 years ago, UK authorities announced that they had confiscated almost 300 kilos of illegal meat and dairy products from the luggage of passengers who disembarked at Northern Ireland's international airport. The seizure was made in a single month, and one detail drew attention: laboratory tests detected traces of the ASF virus in the material examined.

A study published in 2018 concluded that other ingredients used in animal and human food, such as soybean meal and pie, wet cat and dog food, and pork casings for sausages can maintain an infectious viral load during international travel from Asia to the United States, even after 30 days of temperature and humidity fluctuations.

For now, ASF remains a disease without treatment and commercial vaccine. Some live attenuated vaccines developed decades ago even managed to induce some protection against homologous strains of the virus in some pigs, but the animals in the experiment became carriers and developed chronic lesions. In 2020, a novelty appeared in England. The Pirbright Institute announced that it has achieved 100% success with a new recombinant vaccine developed by them, which uses a harmless vector virus and 8 selected genes from the ASF virus. According to the researchers, 100% of the immunized pigs survived a lethal dose of African Swine Fever virus, although they manifested clinical signs. This vaccine is still under development, as are others in China, the United States and Vietnam.

In June 2022, Vietnam even announced that an attenuated vaccine developed by the country's Ministry of Agriculture, in partnership with US scientists, had achieved a 100% rate of efficacy and safety in clinical trials analyzed by the US Department of Agriculture. In July, the Vietnamese government issued a commercial license to produce and administer 600,000 doses of the immunizer on national soil. But use was suspended a month later, due to the death of dozens of vaccinated animals.

For countries that are free of the disease, such as Brazil, the only alternative is still prevention and surveillance. There is no control over the movement of wild animals between South American countries, for example. A single outbreak on the continent could be enough for the virus to spread to all neighboring countries if not detected quickly. Hence the importance of educational campaigns so that veterinarians, producers and even hunters become aware of the disease and play a role in the disease prevention chain.

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