

African swine fever: An overview

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Abstract

African swine fever (ASF) is a highly contagious viral disease of pigs, which causes about 100% mortality in domestic pigs. It creates huge economic loss through out the world although it has no zoonotic importance. For a long period, ASF remained endemic in Africa and few European countries but recently it has been reported in China and North Eastern states of India. The disease is transmitted to domestic pigs through contact with sick animals, infected wild boars consumption of contaminated feed and also by biological vectors (soft ticks of the genus *Ornithodoros*). The most common and suitable techniques to detect and identify ASFV is the polymerase chain reaction. The serological techniques play an important role in the surveillance program. Till date there is no effective vaccine developed so far, that is why most of the countries adopted the quarantine procedure and slaughter as effective control measures. This review throws light into the nature of the virus, epidemiology, transmission pattern, prevention and control of spread of the disease.

Key words: African swine fever, Outbreaks

Introduction

According to the release of the 2019 International Committee on Taxonomy of Viruses (EC 51, Berlin, Germany, July 2019), the family *Asfarviridae* has been included in the order of *Asfuvirales* and the class of *Pokkesviricetes*. Apart from this official nomenclature, it has been controversially discussed to include African swine fever virus (ASFV) into the tentative order of *Megavirales* containing the monophyletic but heterogeneous clade of nucleo-cytoplasmic large DNA viruses (NCLDV).

The disease is characterized by disseminated hemorrhages on spleen, lymph nodes, kidney, gastrointestinal and respiratory tracts of swine causing a high mortality rate. ASFV affects both domestic and wild pigs of all breeds and ages. The wild suids and arthropod of the *Ornithodoros* genus are the natural hosts of this virus.

African swine fever virus replicates predominantly in the cytoplasm of infected cells (Iyer *et al.*, 2006). The ASFV particles have a multilayered structure with an overall

icosahedral morphology and a diameter of about 200 nm. It is made up of a genome-containing nucleoid, which is successively covered by a broad protein core-shell, an inner lipid membrane, an icosahedral protein capsid, and an outer lipid membrane. The virus enters into the cell by cell clathrin-mediated endocytosis and cell drinking mechanism (Salas and Andres, 2013). Neither proper treatment protocols nor vaccines are available for the prevention and control of the disease (Bastos *et al.*, 2014).

An effective vaccine is not developed yet mainly due to the immuno-modulation developed by the virus which interferes with the cellular signaling pathway (Revilla *et al.*, 2018). ASFV infection induces the modulation of Type-III interferon (IFN γ) production by T lymphocytes (Revilla *et al.*, 1992). The most relevant source of IFN production *in vivo* after ASFV virulent infection is most likely dendritic cells (DCs) (Golding *et al.*, 2016). ASFV-encoded A238L, a multifunctional protein, has been shown to deregulate the level of pro-inflammatory molecules and cytokines by

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inhibiting the activation of nuclear factor-kappa B (NF- κ B) ((Neilan *et al.*, 1997). CD2v another ASFV-encoded virus protein causes the adsorption of extracellular virus particles and red blood cells around virus-infected cells. CD2v protein expression helps virus dissemination in pigs and it also has a role in impairing bystander lymphocyte function. This may be mediated either by direct interaction of CD2v extracellular domain with ligands on lymphocytes or by an indirect mechanism involving interaction of the CD2v cytoplasmic tail with host proteins involved in signalling or trafficking pathways (Dixon *et al.*, 2004). The unavailability of an applicable ASF vaccine is partly due to the complex nature of the virus, which encodes various proteins associated with immune evasion (Teklue *et al.*, 2020).

History

ASF was first identified in Kenya in 1910 (Montgomery, 1921). After that disease has circulated in several African countries. In 1957 ASFV was introduced into Portugal through contaminated food waste from African airline flights and ships, which was fed to pigs and they controlled the outbreak by early detection of disease in the field followed by a rapid laboratory diagnosis, as well as enforcement of strict sanitary measures (Sánchez-Vizcaino *et al.*, 2012). But it re-established in 1960 in Portugal probably because of improper management of pig carcass which died of ASFV and spread to Spain (Bech-Nielsen *et al.*, 1995). Portuguese veterinary services conducted a national campaign to the farmers by providing information of vector which was involved in the transmission of ASFV, detection of anti-tick antibodies in domestic and wild boars, as well as the destruction or isolation of the pig pens where ticks were present (Danzetta *et al.*, 2020). Before it was finally eradicated in 1995, ASF remained endemic in the Iberian Peninsula (Sánchez-Vizcaino *et al.*, 2012).

In 1978 ASF was spread and remained endemic in several parts of Sardinia (Mur *et al.*,

2016). Then the disease was reported from Europe and different countries of South and Central America. However, by utilizing rigorous disease control programs (biosecurity measures, awareness of disease, quarantine) it was successfully eradicated (Cwynar *et al.*, 2019). For many years, ASF remained endemic only in African states and Sardinia (Costard *et al.*, 2009). Though, in 2007 ASF was detected in Europe, namely Georgia, from where it was spread to neighbouring states Armenia, Azerbaijan and the Russian Federation (Sánchez-Vizcaino *et al.*, 2013). During 2007-2017, more than 1,000 outbreaks have been reported and resulted in deaths of approximately 800,000 pigs in 46 regions across Russia (Sánchez-Vizcaino *et al.*, 2013). There was a huge loss in pork production from 1,119 tons in 2007 to 608 tons in 2017 (Kolbasov *et al.*, 2018). In 2012 and 2013, Ukraine and Belarus additionally revealed an ASF episode (Sánchez-Vizcaino *et al.*, 2015). In 2014, ASF surfaced at the European Union flare-ups in Lithuania, Latvia, Estonia and Poland (Gavier-Widen *et al.*, 2015; Wozniakowski *et al.*, 2016).

In August 2018, ASF was reported for the first time, in Shenyang, a north eastern city of China (Wang *et al.*, 2018). As China is the world's largest pig producer, the outbreaks of ASF had a huge impact on both local and the world pig industry. The disease spread swiftly, reaching almost all the territories of the country. The disease might have been introduced from an ASF-affected Eastern European country via smuggled pork or offals (Wang *et al.*, 2018). ASF has also spread beyond China within East and Southeast Asia (Normile, 2019). According to FAO's Emergency Prevention System for Animal Health, the disease had been reported in Vietnam, Cambodia, Mongolia, where millions of pigs were culled (FAO, 2019). At the end of 2018, Mongolia counted 27,819 domestic pigs (Heilmann *et al.*, 2020). In Mongolia, the disease was reported in January 2019, with 11 outbreaks in 6 provinces involving 105 farms/ households. More than 10 per cent of the total pig population (3,115)

in Mongolia died due to the ASF outbreaks (FAO, 2019).

The Ministry of Agriculture and Rural Development (MARD) confirmed ASF outbreaks in Vietnam during February 2019, a total of 26 provinces reported outbreaks and more than 89,600 pigs were culled (FAO, 2019). Twenty-four genotypes of ASFV have been described to date, and all of them have been described in Africa. The causative strain belonged to p72 genotype II and was similar to the viruses identified in China and Georgia (Le *et al.*, 2019). In April 2019 the Ministry of Agriculture, Forestry and Fisheries confirmed the first ASF outbreak in Cambodia, where more than 2400 pigs died or had been culled (FAO, 2019). The disease was also spread to the Philippines, Myanmar and Indonesia (OIE, 2020). ASF was first reported in South Korea in September 2019, and the virus strain (Korea/Pig/Paju1/2019) belonged to the p72 genotype II with an intergenic region II variant, similar to those strains identified in Europe and China (Kim *et al.*, 2020). In South Korea, the disease was spread in Eurasian wild boar populations like in Europe (Jo and Gortázar, 2020).

Outbreak in India

African swine fever has been first reported in the country in May 2020. There were 11 (7 from Assam and the remaining from the Arunachal Pradesh) outbreaks in North Eastern states bordering China (<https://rr-asia.oie.int/en/projects/asf/situational-updates-of-asf/>) (OIE). Mortality of the pig was noticed in both Assam and Arunachal Pradesh reaching 100% of the affected population and 1,199 pigs were found positive with ASFV out of 10,920 susceptible pigs. OIE referral laboratory, ICAR-National Institute for High Security Animal Diseases (NIHSAD), Bhopal confirmed the ASF by both Real-time PCR and nucleotide sequencing, further confirming the reported ASFV variant to be of genotype II origin (Abedin *et al.*, 2020). In order to control the disease, the interaction between wild and domestic pig has been prevented. To keep the wild boars

population safe from ASF infection, a trench of 2 km long and 6 feet deep was made near the World Heritage Site of Kaziranga National Park. A supervised zone circling the infected areas was created to restrict the movement of the pigs. Pig population in a 1 km radius around the infected area was tested and gradually culling was done. Transportation of pigs from other states like Meghalaya and Arunachal Pradesh was banned. Sanitization and awareness programs were conducted in different hot spots which were identified as core region for ASF (Bora *et al.*, 2020). According to the 20th Livestock Census, there are 9.06 million pigs in the country, which is 1.7% of the total livestock in the country. North Eastern region has 46.85% of the pig population in the country. According to the OIE report on May 21, 2020, a total of 3701 pigs died due to ASF.

Transmission

ASF could be transmitted through direct contact with affected animals; indirect contact is also possible through contaminated food waste, feed garbage or fomites (Niederwerder *et al.*, 2019) and also by biological vectors (soft ticks of the genus *Ornithodoros*). Experimentally the disease was transmitted through the intramuscular and oronasal route (Blome *et al.*, 2013).

The natural sylvatic cycle, domestic pig-tick cycle and domestic cycle are the three different epidemiological cycles that were described for ASFV transmission (Penrith *et al.*, 2019). The wild boar-habitat cycle, which involves wart hogs (*Phacochoerus africanus*), was recently described in Europe (Chenais *et al.*, 2018). Transmission of virus between the common warthog and argasid ticks (*Ornithodoros moubata* complex) is described under the natural sylvatic cycle (Chenais *et al.*, 2018). The tick feeding on the blood of the warthog transmits the virus. It has been well documented in Southern and Eastern Africa. In the domestic cycle, transmission can occur through direct contact between infected and susceptible pigs, consumption of contaminated

meat products and contact with fomites as well as when infected *Ornithodoros* ticks fed on them (Penrith *et al.*, 2019). Warthog involvement is not there in the domestic cycle (Guinat *et al.*, 2016).

Clinical signs

Domestic pigs and European wild boars showed a wide range of clinical signs from acute to chronic, where African wild pigs did not show any specific clinical signs (Sánchez-Vizcaíno *et al.*, 2019). In natural infection, incubation period ranges from 4 to 19, depending on the virus dose and inoculation route (Mebus *et al.*, 1981). Depending on the virulence of the isolate, exposure dose and route of infection, clinical manifestations vary. Highly virulent ASFV isolates may induce peracute and acute forms of the disease where moderately virulent isolates can produce acute, subacute and chronic or inapparent forms of the disease. Low virulent isolates may generate subacute or chronic or inapparent disease (Sánchez-Vizcaíno *et al.*, 2019)

Peracute form: The peracute form of ASF is characterized by inappetence, body temperature $>41^{\circ}\text{C}$, depression, and cutaneous hyperemia. Death usually occurs within 1-4 days after appearing clinical signs and symptoms (Sánchez-Vizcaíno *et al.*, 2019).

Acute form: Affected animal shows high fever, fatigue and anorexia along with non-specific respiratory tract infection. Haemorrhages are observed on the skin around the ears, abdomen and legs. Diarrhoea along with other gastrointestinal symptoms are seen and death occurs within 6-13 days (or up to 20 days). Mortality rates may be as high as 100%. Pregnant animals may abort (Sandra *et al.*, 2020). In the final stages, rapid, labored breathing may be observed, as well as serous or seromucous nasal secretions caused by pulmonary edema (Sandra *et al.*, 2020).

Apart from Sardinia, causative virus strains in Europe and Asia show high virulence for both domestic pigs and European wild boar under experimental conditions (Nurmoja *et al.*,

2017). In Sardinia, surviving animals were described with anti ASFV antibodies, intermittent viremia or subacute disease, as well as survivors did not show any clinical signs (Mur *et al.*, 2016).

Chronic forms: This clinical form is characterized by multi-focal necrosis in the skin and arthritis, growth retardation, emaciation, respiratory distress and abortion, these signs are mainly associated with secondary bacterial infections inducing fibrinous polyserositis, chronic pneumonia, necrosis of skin, tongue or tonsils (Sánchez-Vizcaíno *et al.*, 2015).

Lesions

Depending on the virulence factor of the virus, variation in the lesions may be observed in animals infected with ASFV. The important gross lesions are found in the kidney, spleen, lymph node and heart (Botija, 1982). The spleen may be enlarged, infarcted, friable and also darkened. The lymph nodes are hemorrhagic and it appears as red dark hematoma. Kidneys usually have petechial hemorrhages on cortical surfaces and cut surfaces as well as in the renal pelvis (Sandra *et al.*, 2020).

Sub-acute form - it is a mild form of acute form. Hemorrhages are noticed in lymph nodes, kidneys and spleen, congested and edematous lungs. Sometimes interstitial pneumonia is also noticed (Mebus *et al.*, 1981)

Chronic form - Fibrinous pericarditis, pleuritis, enlarged lymph nodes and spleen are the lesions found in chronic form. Caseous focal necrosis and mineralization are found in some cases (Mebus *et al.*, 1981)

Treatment options

Till now no commercial vaccines are available, several antiviral agents are there. It is useful for early control of virus spread after an outbreak. Antivirals that are used against ASFV include resveratrol and oxyresveratrol, these antivirals achieved a 98-100% reduction in viral titers. Resveratrol at a noncytotoxic concentration of 5 $\mu\text{g}/\text{mL}$ decreases viral

production by 100%. Oxyresveratrol at concentrations of 30 µg/mL decreases the viral production 98.5% for synthetic and 99.15% for natural oxyresveratrol. Both compounds permit early protein synthesis but prevented viral DNA replication, late viral protein synthesis and viral factory formation (Galindo *et al.*, 2011).

Fluoroquinolones are an antibacterial agent but having antiviral activity. When ASFV-infected vero cells exposed to six different fluoroquinolones or their combinations, there was a severe reduction of the cytopathic effect from an early phase of infection (Mottola *et al.*, 2013).

Cholesterol-lowering drugs or inhibitors of cholesterol are also antiviral agents. Accumulation of cholesterol in endosomes impairs fusion, resulting in the retention of virions inside endosomes (Cuesta *et al.*, 2015)

Control

Many works have been done for developing a vaccine against ASF, attempts like vaccinate animals using extracts of infected cells, supernatants of infected pig peripheral blood leukocytes, inactivated and purified virions, infected glutaraldehyde-fixed macrophages, or detergent-treated infected alveolar macrophages have failed to induce protective immunity (Mebus, 1988). Currently, there are several reports of the protection elicited by experimental vaccines based in live attenuated ASFV (LAV) containing single or double gene deletions in the genome (O'Donnell *et al.*, 2017), their different approaches including DNA vaccines (Reis *et al.*, 2016). Some of them might turn into successful vaccines in future.

Countries which are free of disease followed prevention method like safe import policies (make sure to avoid illegal imports of live pigs and pork products, appropriate discarding of waste food from aircraft, vehicles or ships coming from infected countries) and biosecurity.

Control measures during an outbreak will be difficult and must be adapted to the specific

epidemiological situation. Sanitary measures may be employed including early detection and humane killing of animals (with proper disposal of carcass and waste); thorough cleaning and disinfection; zoning/compartmentalisation and movement controls, surveillance and detailed epidemiological investigation; strict biosecurity measures on farms (OIE, 2020). In some places like Europe and Asia, a large number of wild boar population and unhygienic pig production are the main reason for the transmission of the disease. A good understanding and management of the wild boar population and good strategy among the veterinary services, wildlife and forestry authorities are required to successfully prevent and control ASF. The involvement of the soft tick vector, depending on the epidemiological situation should also be considered in the control program (OIE).

Conclusion

African swine fever disease has a consequential threat to the income and food security of huge numbers of people depending on the production and processing of pigs (FAO 2019). The disease has a high possibility for spreading into new areas where it can quickly develop and endure if initial detection and stern control measures are not applied. The disease distribution and recent spread to the Asian countries including India illustrate well the threat ASF poses to the pig production sector worldwide. ASF is having complicated epidemiology with the presence of different patterns, which depend on host and vector species densities and interrelationships, and are thus influenced by livestock production system characteristics and ticks and wild pig habitat. For that reason, prevention and control of the infection is needed along with a better understanding of its epidemiology, so that measures can be initiated.

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