



## African Swine Fever in Pigs: Recent emergence and Indian Perspectives

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### ABSTRACT

African Swine Fever disease is an economically important disease of pigs caused by *Asfivirus* of *Asfarviridae* family. The disease is characterized by acute haemorrhagic fever with a mortality rate of 100% in which pigs of all age groups are susceptible to the infection. Wild pigs and *Ornithodoros* soft ticks are the natural reservoir host transmitting the infection to the domestic pigs and also by direct contact with the infected animal. The three patterns of infection in ASF are sylvatic cycle, tick-pig cycle, domestic pig cycle and wild boar habitat cycle. ASF presents four clinical forms such as peracute, acute, subacute and chronic forms depending upon the virulence nature of the virus. Recently in the year 2020, India reported an ASF outbreak in two North-eastern states Assam and Arunachal Pradesh during which around 3701 deaths were evidenced. North eastern states of India contribute 9% of the pig population and also has a permissible border with other countries like China, Bhutan, Bangladesh, Nepal, Myanmar which permits the emergence of transboundary diseases. The possible reason or transmission route involved in the outbreak of ASF is still inconclusive. As no commercial drugs or vaccines are available for the control of ASF, improved sanitation and biosecurity measures will protect the swine population in developing countries like India in order to sustain the pig population which is the major economy for the people of North eastern states.

### 1. Introduction

African Swine Fever (ASF) is an acute, highly contagious, viral disease infecting pigs with a high mortality rate. ASF is an economically important disease affecting pigs has been listed as a notifiable disease and has been prioritized among any other diseases of pigs by OIE (Dixon *et al.*, 2005). The ASF is the only vector borne disease caused by DNA virus - African Swine Fever Virus (ASFV) belonging to the *Asfivirus* genus under the family *Asfarviridae* (Blome *et al.*, 2020). The vector involved in the transmission of ASFV is a soft tick, *Ornithodoros* spp. ASFV is an enveloped, linear, double stranded DNA virus with icosahedral symmetry whose genome length is 170-190 kbp (Dixon *et al.*, 2013). The target host cells for the virus are monocytes and macrophages in which they undergo cytoplasmic replication and are released by budding through the plasma membrane. Based on the capsid protein p72, a major structural protein, 24 different genotypes of ASFV had been described to date by sequencing the gene C-terminal B646L coding it (Bastos

*et al.*, 2003; Chapman *et al.*, 2008). The virus can be easily inactivated by heating at 60°C for 20 minutes/ 56°C for 70 minutes, exposing it to chemical agents like hypochlorites, NaOH, formalin, iodine and 3% ortho-phenyl phenol for 30 mins also inactivates the virus. At the same time, the virus can remain viable under low temperatures, in the blood, faeces and undercooked meat of infected animals for longer periods of time (Dutta *et al.*, 2019).

North Eastern states of India is highly populated with pigs as most of them consume pork. At the same time, North East India has a unique geographical location that shares international boundaries with Bangladesh, Bhutan, Myanmar, China favouring the permeability of transboundary diseases, ASF being one of them. The first report of ASF among the domestic pig population in India has been reported on May 2021 in Assam and Arunachal Pradesh with deaths numbered 3701 (Bora *et al.*, 2020; Patil *et al.*, 2020). Considering the economic importance of the disease in

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context to North Eastern India, this article will throw a light on this important disease.

## 2. Epidemiology

ASF is endemic in most of the sub-Saharan African countries. The pattern of infections of ASF is unique in different continents like Africa, Europe, Asia viz., sylvatic cycle, tick-pig cycle, domestic, and wild boar-habitat cycle involving wild pigs, domestic pigs, *Ornithodoros* ticks, pork and its products (Chenais *et al.*, 2018). In the sylvatic transmission cycle i.e. the warthogs, bush pigs, other wild suids like giant forest hogs and ticks, are infected but with no observant clinical signs as warthogs being natural reservoir hosts for the virus. This sylvatic cycle was first predicted in Africa (Plowright *et al.*, 1994; Guberti *et al.*, 2018). In the tick-pig cycle, soft ticks act as the biological reservoirs for the virus to multiply, maintains the infection and is then transmitted to the domestic pigs, in spite of little or no contact with wild boars, clearly stating the *Ornithodoros* ticks' role in disease transmission among the domestic pigs. Thus, an assumption that soft ticks are the actual hosts of ASFV and the domestic pigs would be the accidental hosts (Wilkinson 1984; Dutta *et al.*, 2020). In domestic cycle, the transmission and infection is among the domestic pigs and by other factors by pork (Chenais *et al.*, 2019). The wild boar-habitat cycle, was newly reported in Europe which involves direct transmission between the wild boars and also the indirect transmission through their habitat, their carcasses in which the virus is maintained and transmitted to the domestic pigs (Chenais *et al.*, 2018). It was hypothesised that this wild boar-habitat cycle might be the reason for the transmission of infection of ASFV from wild pig to domestic swine/feral pigs in new areas/regions like national parks or sanctuaries (Bora *et al.*, 2020).

## 3. Hosts

All the members of *Suidae* family are the susceptible hosts for ASF irrespective of age (Mulumba-Mfumu *et al.*, 2019). The host species considered as the target for ASFV are wild pigs, domestic pigs and *Ornithodoros* ticks. African warthogs (*Phacochoerus aethiopicus*/ *Phacochoerus africanus*) are considered to be an important reservoir host of ASFV in Africa. Other natural reservoir hosts which are primarily infected by ASFV in Africa are giant forest (*Hylochoerus meinertzhageni*), bush pigs (*Potamochoerus* sp) and *Ornithodoros* spp. ticks which maintain and transmit the infection to the susceptible hosts (Chenais *et al.*, 2019; Golnar *et al.*, 2019; King *et al.*, 2003; Sanchez-Vizcaino *et al.*, 2009). Vertebrate hosts in which the disease is actually demonstrated with clinical illness are American wild pigs, European wild pigs and domestic pigs (*Sus domestica*) (Dixon *et al.*, 2020). The disease transmission by ticks in India is not clearly

determined yet. Patil *et al.* (2020) has hypothesized that the infection in domestic pigs /outbreak in India would be due to movement of personnel or through fomites from the contaminated habitat and the role of ticks in disease transmission is yet to be investigated.

## 4. Transmission and Transboundary spread

The disease transmission occurs by direct contact of susceptible pigs with infected ones, by the bite of an infected *Ornithodoros* tick, and indirectly consumption of pork or pork products from an infected animal, when undercooked as the virus can survive and remain infectious for about 3-6 months period (Penrith and Vosloo 2009; Dutta *et al.*, 2020). The disease is also indirectly transmitted by blood, urine, faeces of an infected pig which contaminates the habitat and is then transmitted to the susceptible host pigs through fomites like personnel clothes, vehicles, implements etc., (de Carvalho Ferreira *et al.*, 2013a; 2013b; Gallardo *et al.*, 2015). The disease transmission to domestic pigs from the wild suids is mainly through the biological vector *Ornithodoros* spp. and is considered to be the most common route of transmission. The virus replicates in the tick vector and is transmitted among the tick population transovarially, transtadially and transsexually. The tick while feeding on the wild pigs (Warthogs, bush pigs) transmits the virus, leading to persistent infection in them and remain asymptomatic. Whereas, transient viremia may develop in young infected pigs without developing the disease in order to infect the new ticks (Plowright *et al.*, 1974; 1970; Endris and Hess 1994; Hess *et al.*, 1987; Kleiboeker *et al.*, 1999). The stable fly (*Stomoxys calcitrans*), are also found to be involved in the transmission of ASFV among the pig population. The stable fly acts as a mechanical vector, in which the virus can survive for up to 48 h after the virus is ingested by the bite of an infected pig (Mellor *et al.*, 1987).

Africa was the first country to record and report ASF, where the disease is endemic and is re-emerging (Montgomery 1921). The first transcontinental spread of ASFV occurred in Portugal and then to other European countries followed by spread to Russian Federations, Poland, Belgium and finally China (2018) which holds half of the world's pig population (Sanchez-Vizcaino *et al.*, 2012a; Biron *et al.*, 1987; Terpstra *et al.*, 1986; Swaney *et al.*, 1987; Wilkinson *et al.*, 1980; FAO 2018). From China, the top most producer of pork among the Asian countries, the virus disseminated to Vietnam, Mongolia, South Korea, Philippines and other Asian countries (OIE 2020). In history, ASF are not more common in Asian countries restricting the transboundary spread, because of strict rules and regulations in trades, improved sanitary measures, but the periodic outbreak of other swine diseases like hog cholera, swine flu and BSE has been reported (Costard *et al.*, 2013; Ozawa *et al.*,

2006). In recent years, many Asian countries like Hong Kong, Cambodia, Papua New Guinea, India (Penrith 2020; OIE 2020) reported the outbreak of ASF. In India, pork production is highly concentrated only in the NE states which is surrounded by international borders like China, Myanmar etc., creating a potential threat of transboundary spread of ASF. As the production of pork is only 9%, India imports the processed pork products from Italy, Belgium, Sri Lanka posing a threat for disease spread and further outbreaks (USDA 2016; DAHD 2020; Bora *et al.*, 2020). Thus, illegal trade of pork and pork products, men movement enables the entry of ASFV into new zones leading to transboundary transmission of ASF apart from the wild boar and tick involvement.

### 5. Pathogenesis

The virus ASFV makes entry via the oro-nasal route, viral attachment to the cells is mediated by structural proteins like p12, p72 and p54 and released inside the cell (Greig 1972; Gaudreault *et al.*, 2020). The virus replicates initially in the tonsils and regional lymph nodes followed by viraemic spread to other target organs through blood or lymph and they undergo cytoplasmic replication in macrophages and monocytes (Colgrove *et al.*, 1969; Salguero *et al.*, 2002; Gomez-Villamandos *et al.*, 2013). The virulence gene involved in pathogenesis is NL genes along with 8-DR protein which has the haemadsorption property (Nogal *et al.*, 2001). After infecting the cells and maturation of the virus, the virus is then released into the blood or lymphatic circulation by budding, further infecting the other organs. The complete ASFV infection cycle (from attachment to maturation and release) occurs within 24hpi (Gaudreault *et al.*, 2020; Munoz-Moreno *et al.*, 2015).

### 6. Clinical signs and Lesions

Though the ASF is asymptomatic with persistent infection in wild suids and soft ticks (reservoir host) in Africa, most of the ASFV isolates, cause severe infection with acute haemorrhagic fever in domestic pigs and wild boar, resulting in a 100% fatality rate (Blome *et al.*, 2013; Pietschmann *et al.*, 2015). The incubation period for ASF varies from 4-19 days depending on the route of infection. The disease occurs in four forms - peracute, acute, subacute and chronic forms depending on the virulence nature of the virus (Dutta *et al.*, 2020; Yoo *et al.*, 2020). The peracute form of the disease is caused by infection of pigs with highly virulent ASFV isolates leading to the death of the host within 4-10 days after infection without any clinical signs. Some animals may exhibit clinical signs like pyrexia, rapid breathing or hyperemia of the skin with cent percent morbidity and mortality (Sanchez-Vizcaino *et al.*, 2015; Mebus 1988). The highly virulent and moderately virulent strains of ASFV leads

to acute and sub-acute forms of the disease which are most commonly observed. The clinical signs observed in acute forms include anorexia, pyrexia (up to 42°C), lethargy, epistaxis, vomiting, cyanotic patches/haemorrhagic spots in the leg extremities, ears, abdomen, tail, abortion, diarrhoea, conjunctivitis, mucopurulent discharge from nose and eyes followed by death within 4-9 days of infection (Beltrán-Alcrudo *et al.*, 2017; Gallardo *et al.*, 2015; Salguero 2020). The sub-acute form of ASF shows clinical signs similar to that of acute forms, but the disease severity is less with 30-70% mortality. Animals surviving the infection recovers within 1 month and excrete the virus in its secretion up to 6 weeks (Beltrán-Alcrudo *et al.*, 2017; OIE 2019). The chronic form of the disease is caused by low virulent ASFV isolate and mild signs like emaciation, pneumonia, poor and stunted growth, arthritis, intermittent fever, skin lesions may be observed often resembling Classical swine fever which requires laboratory confirmation (Sanchez-Vizcaino *et al.*, 2015; Salguero 2020).

The pathological lesions in peracute forms are usually not observed. The lesions associated with the acute form of ASF include splenomegaly, extensive/ petechial haemorrhage of internal organs like lungs, kidneys, and mucous membrane of urinary bladder, larynx. Lymphoid tissue necrosis and multifocal haemorrhagic lymphadenitis are the important lesions observed in post-mortem with the lymph nodes showing marbled appearance due to multifocal haemorrhages. Intense haemorrhages and oedema are the lesions observed in subacute form. The lesions are observed in the chronic form may be due to secondary bacterial infections which include pleuritis and fibrinous pericarditis (Salguero 2020; OIE 2019; Gallardo *et al.*, 2015).

### 7. Economic importance

ASF is a highly contagious economically important, transboundary disease. In case of a large epidemic, there will be a drastic reduction of the pig population having a severe socioeconomic impact on trade, business and costs involved in the control of disease and obviously result in inflation of pork and pork products. The pig population in India is 9 million with more population in NE India whose staple food is pork. The total pork production during the year 2014-15 was 464.11 thousand metric tonnes as per reports by GOI (dahd.nic.in; USDA 2016). The pig farming sector in India is predominantly unorganized, low input driven and the backyard system of rearing is practiced by small and marginal farmers in which the pigs are usually fed with locally available feed materials like kitchen waste/swill feeding (Kumaresan *et al.*, 2009; Shyam *et al.*, 2016). In the North Eastern states of India, pigs are mostly reared under the scavenging system and those are the places where ASF outbreak has been reported (Talukder *et al.*, 2019; Chauhan *et*

*al.*, 2016). The recent outbreak of ASF in Arunachal Pradesh and Assam shows a report of 11 outbreaks with loss of 3701 pigs which is obviously a huge economical loss to the poor farmers of the NEH region (Patil *et al.* 2020).

### 8. Diagnosis

As the disease ASF shows clinical signs closely resembling CSF, it is important to undergo laboratory tests for confirmatory diagnosis. The laboratory techniques for diagnosis include isolation and identification of the virus, detection of antigen or antibody from the samples like blood, serum, lymph nodes, kidney, spleen (Yoo *et al.*, 2020). The current laboratory techniques used in the ASF diagnosis has certain limitations like lack of analytical sensitivity and specificity, inability to detect early acute and chronic infections, costly equipments and are time consuming. In spite of limitations, the best method of virus detection is molecular assays like PCR or real time PCR (Gaudreault *et al.* 2019). As no commercial vaccines are available, serological tests are also considered more relevant by detecting the circulating antibodies. There is a need for rapid, accurate diagnosis of ASF at field level in less time without any costly equipments would be highly significant. The list of diagnostic procedures in ASF are mentioned below in the Table 1.

### 9. Prevention and Control

Prevention and control of ASF outbreaks can be focussed by successful vaccination, early disease diagnosis, restricted movement of susceptible pig population and culling of infected herds. The control measures have to be directed towards understanding the epidemiology of the disease, the geographical distribution of potential disease spreaders namely wild pigs and soft ticks. Adopting integrated measures or development of countermeasures for effective control of disease spread and further epidemic outbreaks. The following are the preventive/control measures that can be adopted for ASF disease:

- Sanitation/ Disinfection of premises: ASF recovered pigs and the reservoir hosts i.e. wild suids can contaminate the environment through their excretions and secretions where the ASFV can remain stable for several days to months. 2-3% chlorine (calcium or sodium hypochlorite), 2% NaOH, iodine compounds, phenol substitutes and detergents can be used to disinfect the animal sheds and surrounding premises which inactivates the virus (FAO 2020; DADF 2020). Small scale or backyard piggery sectors can adopt the sanitation procedures by regularly cleaning and disinfecting the animal sheds, farm utensils, removal of litter and dust particles.

- Strict biosecurity measures like segregation of infected from healthy pigs, restricting the movement of personnel and other populations of pigs or pests inside the farm premises by proper fencing, following the quarantine procedures, proper disposal of the infected carcass by incineration or deep burial (Kouam *et al.*, 2020; Davies *et al.*, 2017)
- In case of an outbreak, the infected zones should be marked, movement of pigs should be restricted, culling or slaughtering the infected herds is vital considering all the biosecurity measures (Dutta *et al.*, 2020)
- Avoid scavenging system of pig rearing (FAO 2010)
- Disease diagnosis and surveillance by early detection, geographical distribution and investigation of soft ticks (biological vector) prevalence in the country (Gervasi *et al.*, 2020).
- Strict trade protocols to be followed for the import of pork and pork products will prevent illegal trade business and also limit the transboundary spread of the disease to vulnerable countries like India (Adkin *et al.* 2004).
- Creating awareness among the farmers about the disease, preventive measures and its management followed by timely reports to the veterinary officials (FAO 2010)
- No prophylactic treatment followed to date. But some antiviral agents targeting the viral replication has been successful in vitro but are validated under field conditions (Dixon *et al.*, 2020; Patil *et al.*, 2020)
- Vaccination: No commercial vaccines are available. Some of the vaccines developed but not in use due to some limitations are mentioned in Table 2.

**Table 1.** List of methods involved in ASF diagnosis

S.No	Approach	Diagnostics used	Significance and limitations	References
1	<b>Detection of ASFV</b>			
	Isolation of ASFV	Primary pig monocyte culture or porcine bone marrow cells	<ul style="list-style-type: none"> <li>• Gold standard method</li> <li>• Cytopathic effect seen</li> <li>• Time consuming (up to 7 days)</li> </ul>	Beltrán-Alcrudo <i>et al.</i> , 2017
	Identification of ASFV	Haemadsorption test (HAD) 8DR protein present in the ASFV has HAD property	<ul style="list-style-type: none"> <li>• Gold standard test for definitive diagnosis</li> <li>• The virus in the macrophages bind with the pig RBC's forming a rosette</li> <li>• Time consuming</li> </ul>	OIE 2019
2	Detection of viral antigen	Fluorescent antibody Test (FAT)	<ul style="list-style-type: none"> <li>• Presumptive diagnostic test application where virus isolation is not possible</li> <li>• Field samples or laboratory obtained viral culture can be used in this assay</li> </ul>	Sanchez-Vizcaino <i>et al.</i> , 2012b
		Antigen based ELISA	Low sensitivity and specificity	Gallardo <i>et al.</i> , 2015
		Lateral flow assays	Low sensitivity and specificity	Gallardo <i>et al.</i> , 2015
		Polymerase chain reaction (PCR)	<ul style="list-style-type: none"> <li>• Detection of ASFV gene (p72)</li> <li>• Best method to detect virus in clinical samples</li> </ul>	Agüero <i>et al.</i> , 2003
		Real time PCR (TaqMan/ Universal Probe Library (UPL) probes)	<ul style="list-style-type: none"> <li>• Highly sensitive and specific targeting p72 gene</li> <li>• Confirmatory diagnosis recommended by OIE</li> <li>• Used for screening in field</li> <li>• Less time consuming</li> </ul>	King <i>et al.</i> , 2003; Fernandez-Pinero <i>et al.</i> , 2013
		Linear isothermal amplification assay	Highly specific but not sensitive	Hjertner <i>et al.</i> , 2004
		LAMP assay combined lateral flow test	Target gene is viral topoisomerase II gene (P1192R) Highly sensitive	James <i>et al.</i> , 2010

		Recombinase polymerase amplification (RPA)	<ul style="list-style-type: none"> <li>• Rapid and highly sensitive targeting p72 gene</li> <li>• RPA combined with lateral flow dipstick showed robust sensitivity</li> </ul>	Wang <i>et al.</i> , 2017
3	Detection of antibody (Serological tests)	Indirect ELISA	<ul style="list-style-type: none"> <li>• Recommended test for international trade</li> <li>• Applicable in endemic areas for detection of circulating antibody</li> <li>• Positive samples should be re-confirmed in IFAT</li> </ul>	Gallardo <i>et al.</i> , 2019; Pastor <i>et al.</i> , 1990
		Indirect Immunoperoxidase test (IPT)	FAT and OIE recommended confirmatory test for the sera from endemic areas which has given inconclusive result in ELISA, sera from the areas free of ASF and that has shown positive in ELISA.	Sánchez-Vizcaíno 1987; Pastor <i>et al.</i> , 1989

**Table 2.** Details of vaccines developed for ASF

S.No	Experimented Vaccines	Limitations	Reference
1	Whole viral antigen inactivated vaccine		Forman <i>et al.</i> , 1982
2	Subunit, DNA and vector-based vaccines	Variable results with poor protective nature	Gaudreault and Richt 2019; Sunwoo <i>et al.</i> , 2019; Argilaguet <i>et al.</i> 2012
3	Attenuated MLV with gene deletion	Protection only against homologous strain	Borca <i>et al.</i> , 2020; Sanchez <i>et al.</i> , 2019
4	Live attenuated candidate vaccine	Under field trail	Teklue <i>et al.</i> , 2020

## 10. Conclusion

ASFV is a highly fatal contagious disease having a great impact on the economy to the farmers rearing pigs as there is no vaccine available to date for the disease. The only way of preventing the disease spread is by identifying the positive reactors and the in contact susceptible pig has to be culled which indirectly decelerate the pig population in the country and significant loss to the farmers. Farmers in developing countries like India especially in villages and remote areas are not aware of the biosecurity measures and so are not able to adopt effective preventive strategies to check the economic loss. DIVA strategy of diagnosis may be developed by properly understanding the nature of the virus, its replication, gene functions, disease pathogenesis and the host immune response which will be more rationale in preventing ASF. Thus, early disease diagnosis methods, effective vaccination,

strict biosecurity measures, restricted pig movement, control of disease spread among the wild pigs, feral pigs and soft ticks remain to be critical and significantly important to prevent and control ASF in domestic pigs.

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