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African swine fever (ASF) outbreak in India: A review of literature about the virus and its control measures

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Abstract

African swine fever (ASF) is a highly contagious viral disease of swine causing high mortality in domestic pigs with mortality rates approaching 100%. The disease is caused by a double stranded DNA virus called as ASF virus (ASFV) and is believed to be the only member of the *Asfarviridae* family and genus *Asfivirus*. Viral entry into the host is through receptor mediated endocytosis. Although the attachment factors and specific receptors involved in the process are still unknown, macro-pinocytosis and clathrin- dependent entry mechanisms have been proposed. ASFV replicates predominantly in the cytoplasm of macrophages. ASFV infection in its natural hosts is usually asymptomatic and the host remains persistently infected (PI). In contrast, ASFV infection in domestic pigs leads to lethal haemorrhagic fever and death. Wild suids and arthropod vectors of the genus *Ornithodoros* spp are believed to be the natural hosts of the virus. Pigs acquire the infection either through the sylvatic cycle or the domestic cycle. ASFV is endemic to Africa but due to transboundary movement, the virus has spread to other continents. India reported its first outbreak of the virus recently in the Northeastern regions of the country i.e in Assam and Arunachal Pradesh. Till the month of June, ASF has killed over 17,000 pigs in Assam and an unknown number in Arunachal Pradesh. This review throws a light into the nature of the virus, epidemiology, transmission cycle, viral entry mechanism, signs and symptoms, treatment options and preventing the spread of the disease.

Keywords: ASF, pigs, biosecurity, control, pathophysiology

Introduction

India being in the tropical region has abundance of livestock and majority of the marginalized population earn their livelihood through agriculture and animal rearing practices. Important components of the Indian livestock sector are the pigs. Generally raised by the weaker sections of the society, pigs provide them with nutritional support and also serve as a source of livelihood. According to the 19th Livestock census, pig population comprises around 2.01% of the total livestock population. India ranked 5th in the world in terms of its pig population which was estimated to be around 10.29 million. The tribal community people, particularly in Assam are found engaged in pig rearing. Pig rearing has got enormous potential in uplifting the socio-economic and backward section of the society and also poor and marginalized farmers to attain stability at a comparatively low expenditure, It can also serve as a sustainable source of income by fulfilling the ever increasing demand for pork and pork products. According to the latest Livestock census, Assam has got the highest pig population in the country (15.89%) followed by Uttar Pradesh (12.96%) and Jharkhand (9.35%). Among the indigenous breed the Doom breed (Assam) has the highest population of around 2 lakhs. Bacterial and Viral infectious diseases remain the major causes of animal morbidity and mortality in India. Natural, sociological, economic, changes in the ecology of vector, reservoir, and host species are the major components that affect the emergence and large-scale transmission of viral malady (Peters, 2014) [43]. African Swine Fever (ASF) was first described in the African subcontinent in the 1920s. The virus responsible for this disease is caused by the African Swine Fever Virus (ASFV) (Penrith and Vosloo, 2009) [41]. This highly contagious viral disease affects both domestic and wild pigs, the disease is usually fatal with mortality reaching almost 100% (Penrith *et al.*, 2004) [42]. It has been listed into the category as “notifiable disease” by the World Organization for Animal Health (OIE). Transmission of the virus to healthy animals is by direct contact (oronasal) with infected animals, ingestion of contaminated animal byproducts, indirectly by contaminated equipment, vehicles, footwear, feed, or clothing. Certain species of soft ticks (*Ornithodoros* species) and biting flies are also

the transmission vectors (Plowright *et al.*, 1969) [45], (Sánchez-Cordón *et al.*, 2018) [51]. The persistence of ASFV after infection has been demonstrated in all tissues and body fluids of infected swine, with particularly higher levels in the blood. The virus can persist for up to a month in contaminated pig pens and some pork products for over 4-1/2 months (Kabara *et al.*, 2020) [32]. ASF has primarily spread between countries through the feeding of uncooked garbage containing ASFV-infected pork scraps and pork sausages. ASF is endemic in most of sub-Saharan Africa. The recent outbreak of this disease in the Indian subcontinent especially the Northeastern region i.e in Assam and Arunachal Pradesh has particularly raised various concerns among the pig farmers with the authorities curbing sale and transport of pig or pork products across the states. This review throws a light into the nature of the virus, its pathophysiology and the different managerial practices to be adopted at the farm level to prevent further spread.

African swine fever virus (ASFV) description

ASFV is a large enveloped double-stranded DNA virus of the genus *Asfivirus* within the family *Asfarviridae*. These viruses are larger with highly complex genomes of double-stranded DNA that are distantly related to one another and to other “nucleocytoplasmic” large DNA viruses. ASFV mostly infects domestic swine and other members of the family Suidae such as warthogs (*Potamochoerus aethiopicus*), bushpigs (*Potamochoerus porcus*) and wild boar (*Sus scrofaferus*). Viral replication in domestic pigs has been linked to the cells of the monocyte lineage causing a wide range of symptoms ranging from hyperacute to chronic with mortality rates soaring up to 100% (Penrith and Vosloo, 2009) [41], (Costard *et al.*, 2009) [11]. Currently, ASFV is the sole known DNA arbovirus. Vectors responsible for transmission include soft ticks of the genus *Ornithodoros* spp. The virus was initially discovered to infect *Ornithodoros erraticus* ticks (Penrith and Vosloo, 2009) [41] and subsequently the infection was confirmed in Africa in *Ornithodoros moubata* ticks present in warthog burrows (*Phacochoerus aethiopicus* and *P. africanus*). Pigs are infected through either the sylvatic or the domestic cycles of transmission (Okoth *et al.*, 2013) [39]. In the sylvatic cycle, the virus circulates between African wild warthogs and bush pigs as reservoirs and soft ticks of the *Ornithodoros* spp without manifesting any apparent clinical sign of disease in these African wild pigs (Costard *et al.*, 2009) [11]. The domestic cycle is seen when the virus gets transmitted directly from one domesticated pig to another or from pig products to domestic pigs, without the involvement of arthropod vectors or sylvatic hosts (Costard *et al.*, 2009) [11], (Jori and Bastos, 2009) [31]. In addition, several studies reported a direct transmission between infected wild pigs and domestic pigs, and in between pigs-to-pigs in domestic cycle through direct contact (Costard *et al.*, 2009) [11], (Penrith *et al.*, 2004) [42].

Epidemiology

ASF disease was first identified in Kenya in the 1920s (Montgomery, 1921) [35]. It was confined to the African subcontinent until it spread to Europe in the middle of the last century, and later to South America and the Caribbean nations. Drastic control and eradication programs resulted the disease being eradicated from Europe (except Sardinia) in the 1990s. Transboundary movements led to the disease spread out of Africa into Georgia, and in 2014 it reached the eastern

boundary of the European Union. The latest reports of the disease include an increasing list of European countries such as Poland and Moldova (Galindo and Alonso, 2017) [17]. The latest outbreak of the virus in Asia in 2020 being the North eastern states of Assam and Arunachal Pradesh of the Indian subcontinent, causing widespread mortality in local and wild boars. Due to the absence of vaccines with protective efficacy, ASF represents a serious threat to all European countries and especially developing countries like India. The epidemiological complexity of ASFV has already been documented in eastern and southern African regions, where 22 genotypes of the virus have been revealed when genetic characterization of ASFV was done based on sequence variation in the C-terminal region of the B646L gene encoding for the major capsid protein p72. (Boshoff *et al.*, 2007) [9], (Bastos *et al.*, 2003) [8].

Mechanism of entry of ASFV into the host

The infectious cycle of ASFV commences with its adsorption and entry into the host cell. This entry event of the virus may be a low pH and temperature-dependent process with specific receptor-mediated endocytosis (Alcami *et al.*, 1990) [2]. However, the receptor(s) for ASFV is still under debate. The most favorable pathway for viral entry may be receptor-dependent mechanisms, such as clathrin-mediated dynamin-dependent endocytosis (Galindo *et al.*, 2015) [18], (Hernaez and Alonso, 2010) [25]. There are also evidences that this virus exploits other mechanisms such as phagocytosis and macropinocytosis (Sanchez *et al.*, 2012) [50]. The presence of cholesterol is a prerequisite for successful entry of the virus. Such mechanisms may occur both in the macrophage target cell and in vero cells. Also, several studies suggest other entry mechanisms of the virus such as p30, which is important for internalization of the virion particles. Other cellular proteins such as p12 and p54 have also been illustrated as potential viral attachment proteins (Gomez-Puertas *et al.*, 1998) [22], (Angulo *et al.*, 1992) [4], (Angulo *et al.*, 1993) [5].

1. ASF enters the endosomal pathway

After entry through any of the above mentioned pathways ASF virus should finally reach the endocytic pathway (Cuesta-Geijo *et al.*, 2012) [13]. Once into the endocytic pathway, it must pass through different endosome populations to be successful for infection. Proteins and lipids that are recruited to the endosomal membrane carefully design the process of endocytic pathway maturation. It was identified that the major regulator of the endosomal maturation pathway happens to be the Rab GTPase protein family (Stenmark, 2009) [53]. In fact, complete encapsulated virions are only found at the level of early endosomes (EE) but not in other mature acidic compartments as incoming viruses are found in EE labeled with Rab5 (Cuesta-Geijo *et al.*, 2012) [13].

2. ASFV gene expression and DNA replication

The replication site of the ASFV virions is the perinuclear area close to the microtubule organizing center (MTOC) (Alonso *et al.*, 2001) [3]. Just before the onset of DNA replication early genes are expressed here. The transcription of intermediate and late genes begins after DNA replication. To encode genes involved in the transcription and modification of the mRNA fragments, the ASF virions commits approximately 20% of its genomic material. This transcriptional machinery imparts the ASF virus an accurate positional and temporal control of its gene expression, being

relative independence from its host (Rodriguez and Salas, 2013)^[48].

3. Formation of the viral factory

Replication of the virus takes place in the perinuclear area where the microtubules aid in the transport of the virus. So, structural and functional integrity of the microtubules is a prerequisite for the formation of viral factories (Hernaiz *et al.*, 2006)^[26] and reports suggest that virus particles are found in association to stabilized microtubules at the point of its entry (Quetglas *et al.*, 2012)^[46]. Microtubule-mediated viral transport by the interaction of p54 structural protein with the dynein motor protein during infection might constitute a molecular mechanism for its transport (Alonso *et al.*, 2001)^[3]. Assembly of the icosahedral capsid and the core shell domain results into icosahedral intermediates and are thought to be the precursor of viral factories. The last step of morphogenesis of the virion is the encapsidation of its DNA giving rise to mature virions. Finally, the newly formed virus leaves the viral factory and with the support of kinesins and is transported to the cell surface where it is released by the process of budding (Stefanovic *et al.*, 2005)^[52]. Extracellular viral materials are covered by an additional external envelope which may be acquired during this budding process (Breese and de Boer, 1966)^[10].

4. ER stress and unfolded protein response

The virus modifies itself and interacts with the various cellular pathways in response to infection. Majority of the steps in ASF virus replication and maturation takes place in the endoplasmic reticulum (ER) and a major portion of the viral proteins are synthesized in infected cells gets accumulated in the ER during the viral life cycle. This process triggers ER stress (Galindo *et al.*, 2012)^[16]. ASFV infection favours the upregulation of molecular chaperones, calnexin and calreticulin, but not ERp57 (Galindo *et al.*, 2012)^[16] or BiP/Grp78 (Galindo *et al.*, 2012)^[16], (Netherton *et al.*, 2004)^[38]. Moreover, the transcription factor 6 (ATF6) signaling pathway of the UPR is selectively induced by the virus, but not the protein kinase-like ER kinase (PERK) or the inositol-requiring enzyme 1 (IRE1) pathways.

Transmission and spread of ASF virus

ASF virus can be transmitted through direct contact between infected and susceptible pigs. Other modes of spread include consumption of the meat from infected pigs, by the bites of infected ticks (*Ornithodoros* spp.) and by contact with bedding materials, feed, equipment, clothes, footwear, vehicles contaminated with virus-containing materials such as blood, faeces, urine or saliva from infected pigs. Although wild warthogs are the natural hosts of the virus, but it has been demonstrated that they are unable to transmit the virus directly to domestic pigs. On the other hand, bush pigs were able to transmit the virus to domestic pigs under experimental conditions. Several studies delineate that ASF virus can only be airborne over short distances, not much more than 2 metres (Mellor *et al.*, 1987)^[34]. Apart from soft ticks, the only known arthropod that is capable of maintaining ASF virus for a reasonable period (up to 48 hours) and transmitting it to pigs are stable flies (*Stomoxys* spp.) (Mellor *et al.*, 1987)^[34]. Other potential sources of virus transmission to health herd are unlikely through water sources (the virus gets diluted rapidly in water and therefore unlikely to be present in infective doses required for spreading the virus), rodents and birds, but such

things have only been demonstrated experimentally and are yet to be proven (Penrith *et al.*, 2004)^[42]. There is no reliable evidence relating transplacental transmission from sows to fetuses during pregnancy (Penrith *et al.*, 2004)^[42]. Sexual transmission in pigs has not yet been reported, but ASF virus is shed in genital secretions so as to be on the safe side it should be ensured that semen sample used for Artificial insemination (AI) or for cryopreservation should be free of ASF virus.

Incubation Period

The incubation period of the disease reported to be around 4 to 19 days in naturally-acquired cases (Sánchez-Cordón *et al.*, 2018)^[51].

Pathophysiology

Thrombocytopenia, leukopenia, lymphopenia and apoptosis of both lymphocytes and mononuclear phagocytic cells are the major manifestation of ASFV infection. In infected macrophages of the infected cells, the virus inhibits the expression of pro-inflammatory cytokines such as TNF, type 1 interferon and IL-8, but induces expression of TGF- β . Importantly, ASFV strains with potentially different virulence phenotypes differ in their ability to induce the expression of pro-inflammatory cytokines or interferon-related genes in the early stages. If an infection happens to be via the respiratory tract, replication of the virus is first exhibited in the pharyngeal tonsils and lymph nodes which drains the nasal mucosa, then get disseminated rapidly throughout the body producing primary viremia in which virions are associated with both erythrocytes and leukocytes affection. If the case is of generalized infection with very high viral titers, all secretions and excretions of the infected animal contain large amounts of infectious virus. Swine that survive the acute infection may appear healthy or chronically diseased, but both groups tend to remain persistently infected (PI). Indeed, swine may become PI without ever showing clinical signs. The duration of the persistent infection is not known, but low titre of the virus have been detected in tissues more than a year after exposure. In acutely fatal cases in domestic swine, gross lesions are most prominent in the lymphoid and vascular systems (Salas, 1999)^[49].

Clinical signs

Clinical presentation of the disease are most likely to be peracute or acute. Even with lower viral titre, infected pigs start showing symptoms from two to three days after infection, but can also take 15 days to develop with very low virus doses. Increase in body temperature ($>41^{\circ}\text{C}$), loss of appetite, dullness, nestling together, reluctance to move, red-purple skin discolouration especially in the extremities (e.g. ears) and ventral body surfaces, visible haemorrhages are sometimes manifested, but pigs may die without showing external signs of haemorrhage. Clinical signs that are more variable and may not be present in all pigs or in all groups of pigs include gastrointestinal signs such as vomiting and diarrhoea/dysentery. Nervous signs such as ataxia and other signs - oculonasal discharges, epistaxis, dyspnea and coughing are also manifested (Animal and Plant Health Agency, The Pirbright Institute, UK)^[57].

Post Mortem lesions

Post mortem lesions in the affected animals vary considerably and may not be visible in each pig or in all groups of pigs.

Haemorrhages in the skin, lymph nodes, bladder, larynx, heart, lung, liver, serosal & mucosal surfaces. The most distinct lesions are seen in the kidney with the cortex getting stripped off and “turkey-egg” appearance of the organ. Lymph nodes are enlarged appear deep reddened (almost black). Splenomegaly is eminent and the organ becomes more friable. Blood-stained effusions are also seen in the pericardium, abdomen and thorax (Animal and Plant Health Agency, The Pirbright Institute, UK) ^[57].

Outbreak in India

India has had a culture of backyard pig-rearing since ages, mainly in the Northeastern region. Pigs are also reared under scavenging and semi-scavenging systems by some tribal communities. Although rearing pigs is considered as dirty and unhygienic, these intelligent animals are efficient converter of poor quality household and kitchen wastes into tasty meat. According to the 2019 Livestock census, Assam's pig population was estimated to be around 21 lakh. Due to the ASF outbreak which is first of its kind in the country, thousands of pig deaths were reported from Dhemaji, Sivasagar, North Lakhimpur, Biswanath, Dibrugarh, and Jorhat districts of Assam and in West Kameng, Lower Siang, Papum-Pare, Upper Siang, East Siang, Lohit, Lepa-Rada, Namsai, Changlang districts of Arunachal Pradesh. Since its outbreak in February 2020, African swine fever (ASF) has killed over 17,000 pigs in Assam and an unknown number in Arunachal Pradesh. ASF may also spread to populations of wild boar in the forests and wildlife sanctuaries. As an extremely contagious transboundary disease, it can swiftly cross national borders, spreading through live or dead bodies, even through packaged pork products. This is the first time India has reported the disease. Poor and marginal farmers afraid of having sick animals die on their hands dump them in the market, leading to distress-selling and more disease transmission. Carcasses have also been dumped in local rivers and ponds, leading to the rapid spread of contagions downstream of the river bed. The banks of the mighty Brahmaputra river bears mute testimony to the pig carcasses floating in it at present (<http://www.economicstimes.com>)^[55], <http://www.science.thewire.in>)^[56].

Diagnostic Tests

The focal point of anticipation and control of ASF is still on early diagnosis and control due to absence of effective vaccines in controlling the disease (Galindo and Alonso, 2017) ^[17]. Methods for early diagnosis of the disease include an immunoblotting assay (Pastor *et al.*, 1989) ^[40], sandwich ELISA (Hutchings and Ferris, 2006) ^[29], PCR assay (Agüero *et al.*, 2003) ^[1], nested PCR assay (Basto *et al.*, 2006) ^[7], TaqMan[®]PCR assay (King *et al.*, 2003) ^[33], hot-start multiplex PCR (Giammaroli *et al.*, 2008) ^[20], real-time PCR (Haines *et al.*, 2013) ^[24], cross-priming amplification (CPA) assay (Frączyk *et al.*, 2016) ^[15], polymerase crosslinking spiral reaction (PCLSR) assay (Woźniakowski *et al.*, 2016) ^[54] and a loop-mediated isothermal amplification (LAMP) assay (James *et al.*, 2010) ^[30]. A real time LAMP and visual assay for early diagnosis for ASF in the less developed areas has also been successful in confirmatory detection of the virus. Studies demonstrate that p54, an immunogenic proteins is a good serological target for conducting ASF detection and surveillance (Petrovan *et al.*, 2020) ^[44].

Prevention and control

As of present, there is no particular vaccine or treatment regime for the disease. The most adequate preventive measure has to be by checking and stopping the spread of the virus. The demographic location of the vectors such as ticks and flies should be taken into account while mapping control measures. The changing climatic and habitat patterns on the distribution of the vectors also needs to be stressed on. Firstly, the control and preventive measures are mostly aimed at preventing contact between warthogs/bushhogs and domestic pigs. Secondly, it should also be ensured that infected pigs and bedding material do not leave the area of the farm premises where outbreak has been confirmed. However the cases reported in North eastern Indian states of Assam and Arunachal Pradesh can be potentially linked to international movement and trade. It is possible to prevent the spread of the virus by implementing strict biosecurity measures since without human assistance ASF virus cannot be transmitted over long distances. In this context, limiting the access for people and vehicles in and around the farm premises and in areas where pigs are reared can be of great help in containing the spread of the disease. Ensuring that farm workers and other essential visitors such as veterinarians are disinfected properly before entering the farm premises and entry should be based on protective clothing and other PPE gears. Feeding of leftovers should be strictly avoided, no human food should be allowed into the pig facility and biosecurity guidelines to be adhered strictly. Disinfectant foot baths for visitors and working personnel's can also be installed but generally are not much effective in terms of destroying the virus, but should be included as part of the biosecurity plan for the farm. In majority of the ASF control zone, OIE designed concept of compartmentalization and many of its principles, for obtaining recognition of freedom from disease have already been applied and promulgated efficiently. The guidelines for compartments are still under development, but majority of these guidelines are based upon maintaining strict biosecurity measures throughout the whole production chain, so as to prevent the entry of specific diseases, taking into account all the ways in which they are transmitted. Early detection and humane killing of the pig population with proper disposal of carcasses and weight with surveillance and detailed epidemiological investigation are deemed to be ensured (OIE).

Potential antivirals that can be incorporated in the treatment regime

Until researchers develop an appropriate vaccine, the need of the hour is incorporating antiviral drugs in the symptomatic treatment regime. Antiviral approaches can be equally effective in case of animal subjects too as in human subjects. For effective protection against the disease and to elicit an effective immune response against the virus antivirals should be effectively combined with vaccination protocols. Several researches suggests that neutralizing antibodies to proteins such as p30, p54 and p72 can significantly provide full protection against the virus (Barderas *et al.*, 2001) ^[6], (Gomez-Puertas *et al.*, 1998) ^[22], (Gomez-Puertas *et al.*, 1996) ^[23]. In contrast, in another finding such claims have been turned down where it was demonstrated that neutralizing antibodies to the aforementioned proteins were unable to mediate protection (Neilan *et al.*, 2004) ^[37]. Antivirals frequently used against ASFV treatment include resveratrol and oxyresveratrol (Galindo *et al.*, 2011) ^[19], microalgae

(Fabregas *et al.*, 1999)^[14], cholesterol lowering drugs (Quetglas *et al.*, 2012)^[47] or inhibitors of cholesterol transport (Cuesta-Geijo *et al.*, 2015)^[12], valproic acid (Hurtado *et al.*, 2008)^[28], dynamin inhibitors (Cuesta-Geijo *et al.*, 2012)^[13], fluoroquinolones (Mottola *et al.*, 2013)^[36], serine protease inhibitors (Galindo *et al.*, 2012)^[16], and specific peptides (Hernaiz *et al.*, 2010)^[27].

Conclusion

ASF is one of the dreaded disease prevalent among domestic pigs and wild boars. In the affected animals the ASF virus brings about sickness portrayed by severe depression with mortality rates touching in and around 100%. Detection of the virus is by isolation or demonstration of specific ASF antibodies. Identification of the diseased, quarantine and culling of contaminated and uncovered animals can be adopted in control and preventive measures. In the short term, various categories of viral drugs and symptomatic therapy can provide technical fixes to such epidemics. In the long term, veterinarians, researchers and everyone concerned with animal and human health should collaborate to work along with small pig farmers to study ASF and help in making pig farming practices more secure. Further research should be directed towards *in vivo* and *in vitro* trials on therapeutic plants and antivirals effective against the virus. Nevertheless, the control and spread of serious transboundary diseases such as ASF will officially belong to the veterinary authorities cooperating hand in hand with the pig farmers, but one cannot rule out the possibility that outbreak of the disease is unlikely until pig producers understand the disease transmission pattern and take necessary precautions and ensure that their own herd will not get the disease.

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