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African Swine Fever: An Emerging Viral Disease in India – A Review

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ABSTRACT: African Swine Fever Virus (ASFV) is highly contagious and often lethal to domestic and wild pigs, has been around for decades. African Swine Fever Virus (ASFV) causes a serious swine disease that is endemic in Africa and Sardinia and spreading in Russia and neighboring countries, including Poland, Czech Republic. ASF was introduced into East Asian countries including China in 2018 and continued to propagate within East and Southeast Asia (Mongolia, Vietnam, Laos and the Philippines). Recently in India, around 2500 swine deaths reported in six districts of Assam due to the emergence of ASFV. The virus enters host cells by receptor-mediated endocytosis that depends on energy, vacuolar pH, and temperature. The specific receptors and attachment factors involved in the viral entry are still unknown, although macropinocytosis and clathrin-dependent mechanisms have been proposed. This uncontrolled dissemination is a worldwide threat, as no specific protection or vaccine is available. Prevention and controls are one of the most important treatments for the curing of this ASFV infection. This review imparts the updated information about virus morphology, etiopathology, transmission, symptoms, prevention, and control. This review also enlightens the numerous *in-vitro* therapeutic approaches studied against ASFV infection and provides future direction to the researchers working for ASFV infection treatment.

Keywords: ASFV; Viral Disease and Swine.

INTRODUCTION: Infectious diseases remain the major causes of animal morbidity and mortality leading to significant healthcare expenditure in India. The country has experienced the outbreaks and epidemics of many infectious diseases (Mourya et al., 2019). The components that affect the emergence and large-scale transmission of viral malady incorporate natural changes, sociological and economic changes, and changes in the ecology of vector, reservoir, and host species (Peters, 2014). Twelve virus diseases are known to affect domestic pigs (Scott, 1957).

African Swine Fever (ASF), first described in Africa in the 1920s, is caused by the African Swine Fever Virus (ASFV) (Penrith and Vosloo, 2009). ASF is a highly contagious viral disease affecting domestic and wild pigs; the disease is usually fatal (Penrith, 2004). It is listed as a "notifiable disease" by the World Organization for Animal Health (OIE), in part because of its high mortality rate. The virus (ASFV) is spread by direct contact (oronasal) with infected animals, ingestion of contaminated animal byproducts, indirectly by contaminated equipment, vehicles, footwear, feed, or clothing. The virus can also be spread by certain ticks [Ornithodoros sp. (soft ticks)] and possibly by biting flies (Plowright et al., 1969; Sánchez-Cordón et al., 2018). ASFV can be found in all tissues and body fluids of infected swine, with particularly high levels in the blood, which may lead to environmental contamination; the virus can persist for up to a month in contaminated pig pens and some pork products for over 4-1/2 months. ASF has primarily spread between countries through the feeding of uncooked garbage containing ASFV-infected pork scraps. ASF is endemic in most of sub-Saharan Africa, including the island of Madagascar, with the highest area of incidence seen from the Equator to northern South Africa. ASFV infection results in high morbidity and mortality in swine and has drastic implications for global domestic swine production (Brown and Bevins, 2018).

History of ASF Distribution: The first time, when ASF was identified as an independent disease entity, was in Kenya in 1910 (Montgomery, 1921). After its first detection, ASF was found to circulate in several African states until it was introduced into Portugal in 1957. After successful eradication in Portugal, the disease was reintroduced in 1960 and spread to several European countries. Before it was finally eradicated in 1995, ASF stayed endemic on the Iberian Peninsula (Penrith and Vosloo, 2009; Sanchez-Vizcaino et al., 2012; Costard et al, 2009). Since the virus was newly introduced into Sardinia in 1978, ASF has remained endemic in several parts of Sardinia (Mur et al., 2016). The disease did not only reach Europe, but also different countries in South and Central America, from where it was successfully eradicated. For many years, ASF could be found endemic only in African states and Sardinia (Costard et al, 2009). However, in 2007 ASF was again detected in Europe, namely in Georgia, from where it spread to neighboring states Armenia, Azerbaijan and the Russian Federation (Rowlands et al., 2008; Sanchez-Vizcaino et al., 2013). In 2012 and 2013, Ukraine and Belarus additionally revealed an ASF episode (Sanchez-Vizcaino et al., 2015). In 2014, ASF arrived at the European Union, where flare-ups in Lithuania, Latvia, Estonia and Poland were affirmed (Sanchez-Vizcaino et al., 2015; Smietanka et al., 2016; Wozniakowski et al., 2016; Gavier-Widen et al., 2015). The first ASF case in China was accounted for on August 3, 2018. As of January 19, 2019, in any event, 100 ASF cases had happened in 23 areas or regions across the country (Dongming et al., 2019).

ASF Outbreak in India: The news published in Indian newspapers revealed that so far more than 2.5 thousand pigs have died due to the African swine flu in about 306 villages in six districts of Assam viz. Sivasagar, Dhemaji, Lakhimpur, Dibrugarh, Jorhat, and Biswanath (Figure 1) (https://www.amarujala.com; https://navbharattimes.indiatimes.com).

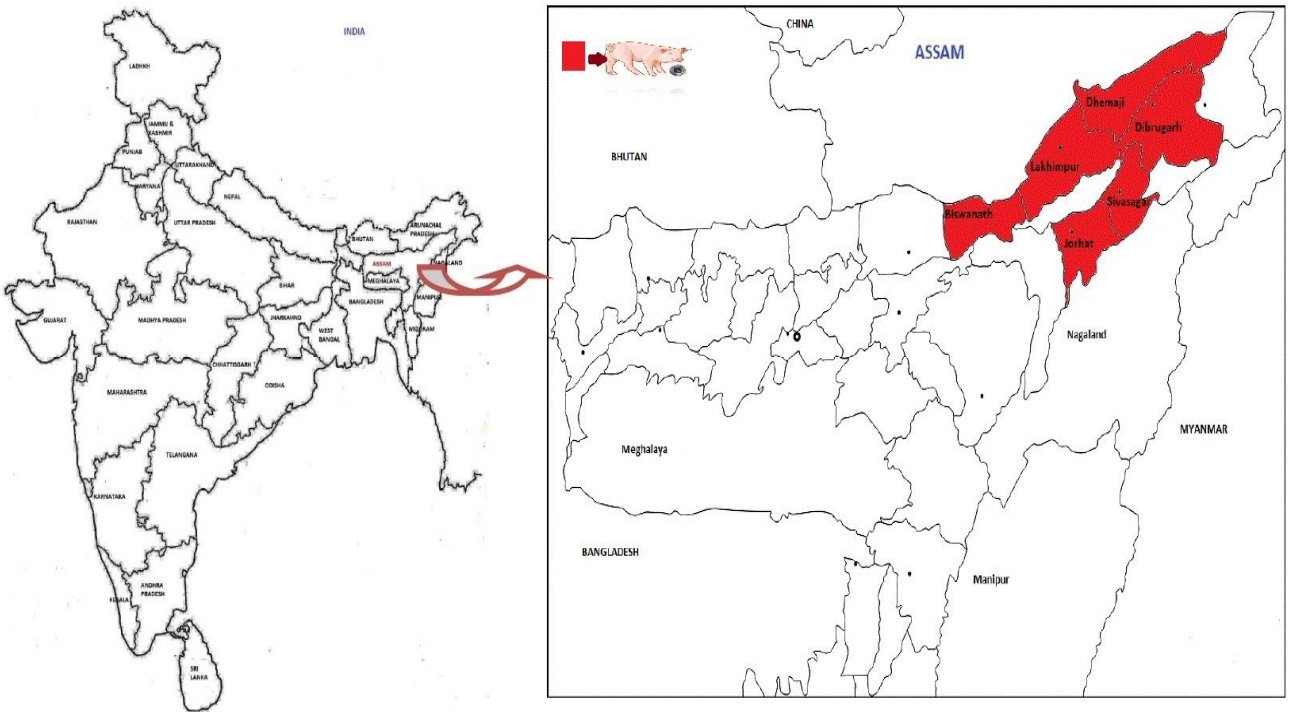

Figure 1: Death reported due to ASF in highlighted six districts of Assam state of India.

African Swine Fever Virus Description: African swine fever virus is a large enveloped double-stranded DNA virus that is the sole member of the genus Asfivirus within the family Asfarviridae. Viruses in the families Asfarviridae and Iridoviridae are taxonomically and biologically distinct, but both families include large viruses with highly complex genomes of double-stranded DNA that are distantly related to one another and to other "nucleocytoplasmic" large DNA viruses. African swine fever virus in the family Asfarviridae is the cause of African swine fever, an important disease that remains a serious threat to swine industries throughout the world. African swine fever virus infects domestic swine and other members of the family Suidae, including warthogs (*Potamochoerus aethiopicus*), bushpigs (*Potamochoerus porcus*), and wild boar (*Sus scrofaferus*). In domestic pigs, the ASFV replicates, preferentially, in cells of the mono-

cyte lineage causing a broad range of symptoms and lesions, ranging from hyperacute to chronic forms of the disease, with mortality rates up to 100%. Therefore, ASF leads to devastating effects on pig production and animal trade with high economic and social costs to affected areas (Penrith and Vosloo, 2009; Costard et al, 2009).

Currently, ASFV is the only known DNA arbovirus. The vectors for ASFV are soft ticks in the family Argasidae classified within the genus *Ornithodoros* spp. The virus was initially discovered to infect *Ornithodorus erraticus* ticks following introduction of ASFV genotype I from Angola to the Iberian Peninsula (Penrith and Vosloo, 2009) and subsequently confirmed in Africa in *Ornithodorus moubata* ticks present in warthog (*Phacochoerus aethiopicus* and *P. africanus*) burrows. Susceptible animals are infected through either the sylvatic or the domestic cycles (Okoth et al., 2013). In the sylvatic cycle, the ASF virus circulates between African wild pigs particularly warthogs and bush pigs which are wildlife reservoirs and soft ticks of the *Ornithodoros* spp, without any apparent clinical sign of disease in these African wild pigs (Costard et al., 2009). The domestic cycle occurs when the virus is transmitted directly from one domestic pig to another or from pig products to domestic pigs, without the involvement of sylvatic hosts or arthropod vectors (Costard et al., 2009; Jori and Bastos, 2009). In addition, several studies reported a direct transmission between infected bush pigs and domestic pigs, and between pigs-to-pigs in domestic the cycle through contact (Costard et al., 2009; Penrith et al., 2004a).

Morphology of ASFV: ASFA virus virions are enveloped, approximately 200 nm in diameter, and possess a nucleocapsid core that is surrounded by internal lipid layers and a complex icosahedral capsid. The genome consists of a single molecule of linear double-stranded DNA, 170-190 kbp in size, depending on the virus strain. African swine fever virus is thermolabile and sensitive to lipid solvents. However, the virus is very resistant to a wide range of pH, and survives for months and even years in refrigerated meat. The virus introduced in 2007 into the Caucasus belongs to genotype II, while an infection that has been endemic in Sardinia since the 1960s is of genotype I (Salas and Andrés, 2013; Galindo and Alonso, 2017).

Recently Liu and his co-workers depicted the cryogenic electron microscopy (cryo-EM) structure of the icosahedral capsid of ASFV at 4.6 Å. The ASFV molecule comprises 8,280 duplicates of the significant capsid protein p72, 60 duplicates of the penton protein, and in any event 8,340 minor capsid proteins, of which there may be 3 distinct sorts. Like other nucleocytoplasmic large DNA viruses, the minor capsid proteins structure a hexagonal system beneath the external capsid shell, working as stabilizers by "sticking" neighboring capsomers together (Liu et al., 2019).

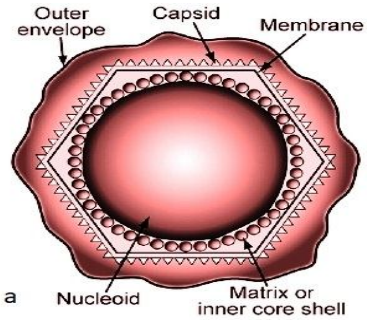

Figure 2: Extracellular morphology of ASFV.

Transmission and Spread: Transmission of ASFV occurs via contact among infected animals, intake of infected material, and/or soft tick vectors (Ornithodoros) (Boinas et al., 2004; Sanchez-Vizcaino et al., 2012). Three transmission cycles have been reported in endemic areas: (i) domestic pig / pig cycle, which does not involve other vertebrate or invertebrate hosts, (ii) a domestic pig/tick/wild pig cycle, and (iii) a domestic pig/ tick cycle without warthog involvement (Guinat et al., 2016; Gallardo et al., 2015; Wilkinson et al., 1977).

Entry and translation of virus: Early studies described ASFV cell entry as temperature, energy, cholesterol, and low pH-dependent procedure, which involves receptor-mediated endocytosis. Through the application of various pharmacological inhibitors and specific protein constructions inducing a dominant-negative effect against key protein players in virus entry, it has been demonstrated that ASFV entry in Vero and porcine IPAM cells is mainly achieved by macropinocytosis, in a process that requires sodium/proton exchangers (Na+/H+), activation of EGFR, phosphorylation of PI3K and Pak1 kinases, together with the activation of the small Rho-GTPase Rac1, resulting in actin-dependent blebbing/ruffling perturbations of the cell membrane, allowing virions to get access to the host cell (Carrascosa et al., 1999; Cuesta-Geijo et al., 2012; Hernaez et al., 2010).

Pathogenesis and Pathology: African swine fever virus infection of domestic swine results in leukopenia, lymphopenia, thrombocytopenia, and apoptosis of both lymphocytes and mononuclear phagocytic cells. Ability of the African swine fever virus to efficiently induce cytopathology in macrophages is a

critical factor in viral virulence. In infected macrophages, the virus effectively inhibits the expression of proinflammatory cytokines such as tissue necrosis factor, type 1 interferon, and interleukin-8, but induces expression of transforming growth factor β. In contrast, increased expression of TNF has been also reported after African swine fever virus infection *in-vitro* and *in-vivo*.

Importantly, African swine fever virus strains with different virulence phenotypes differ in their ability to induce expression of pro-inflammatory cytokines or interferon-related genes early in the infection of macrophages. If an infection is acquired via the respiratory tract, the virus replicates first in the pharyngeal tonsils and lymph nodes draining the nasal mucosa, before being disseminated rapidly throughout the body via a primary viremia in which virions are associated with both erythrocytes and leukocytes. A generalized infection follows, with very high virus titers, and all secretions and excretions contain large amounts of infectious virus. Swine that survive the acute infection may appear healthy or chronically diseased, but both groups may remain persistently infected. Indeed, swine may become persistently infected without ever showing clinical signs. The duration of the persistent infection is not known, but low levels of 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).

Incubation Period: The incubation period is reported to be 4 to 19 days in naturally-acquired cases (Sánchez-Cordón et al., 2018).

Clinical Signs and symptoms: The illness indications incorporate peracute, acute, subacute, and chronic forms. In the peracute type of ASF, pigs die within 4 d pi without gross lesions. The acute form can result in the death of infected pigs with the mortality rate of 90%–100% within the 4–21 d pi range. In acute form pigs show characteristic pathological changes identified with vasculitis, for example, skin erythema, aspiratory edema, hyperemic splenomegaly, hemorrhagic lymphadenitis, and petechial hemorrhages in the lungs, urinary bladder, and kidneys. The subacute type of ASF is related to respectably harmful segregates, and the death rate falls inside the 30%–70% territory. The subacute structure brooding period is longer with pigs passing on after 20 d pi, and their clinical signs will in general be less exceptional; be that as it may, vascular changes, for example, hemorrhages and edema are more extreme than those detailed in intense structure ASF. Low harmfulness disengages by and large reason a ceaseless type of the sickness, which is portrayed by the nonattendance of vascular injuries and a low death rate yet with indications of deferred development, skinniness, joint growing, skin ulcers, and sores related with auxiliary bacterial diseases (Sánchez-Vizcaíno et al., 2015).

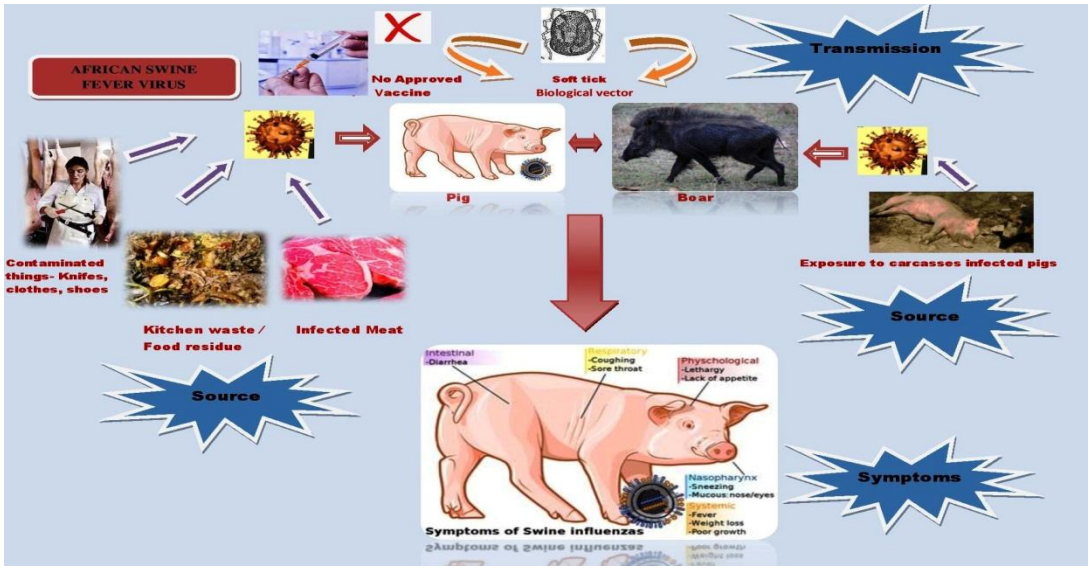

Figure 3: **ASF- Source, transmission, sings & symptoms.**

Diagnostic Tests: Because of the absence of vaccines that are effective against ASFV (Galindo and Alonso, 2017), the focal point of anticipation and control of ASF is presently still on early diagnosis and episode control; thusly, research facility finding is of crucial significance. The current methods for early diagnosis of ASF include an immunoblotting assay (Pastor et al.; 1989), sandwich enzyme-linked immunosorbent

assay (ELISA) (Hutchings and Ferris.; 2006), a polymerase chain reaction (PCR) assay (Agüero et al.; 2003), nested PCR assay (Basto et al.; 2006), TaqMan®PCR assay (King et al.; 2003), hot-start multiplex PCR (Giammarioli.; 2008), real-time PCR (Haines et al.; 2013), cross-priming amplification (CPA) assay (Frączyk et al.; 2016), polymerase cross-linking spiral reaction (PCLSR) assay (Woźniakowski et al.; 2016), and a loop-mediated isothermal amplification (LAMP) assay (James et al.; 2010).

Loop-mediated isothermal amplification (LAMP) has been developed to amplify nucleic acids under isothermal conditions (Notomi et al.; 2000), and it is more specific, sensitive, cost-effective and rapid than real-time PCR assays (Nagamine et al.; 2002; Wang et al.; 2010; Wang et al.; 2011).

Recently Wang and his co-workers developed the real-time LAMP assay and visual assay for early diagnosis for ASF in the less developed areas (Wang et al., 2019).

The study performed by Petrovan and his co-workers suggested that along with other immunogenic proteins, p54 is a good serological target for conducting ASF detection and surveillance (Petrovan et al., 2020).

Control and Therapeutic Prospects: Since the principal antiviral medication, idoxuridine, affirmed in 1963, incredible accomplishments have been made in the field of antiviral medication disclosure. Today in excess of 90 antiviral medications classified into 13 functional groups are accessible for the treatment of human irresistible illnesses, for example, HIV and hepatitis B infection contaminations. In any case, there is still no antiviral medication for more than 200 infections influencing human populaces overall (De Clercq and Li, 2016). Additionally, control of some creature infections like ASFV by methods for an antiviral treatment gives off an impression of being an appealing methodology because of the absence of other control measures. In this way, the advancement of antiviral specialists for mass application in veterinary is as much significant as the improvement of antiviral medications against human infections. There is no treatment for African pig fever, other than steady consideration.

Antiviral drug Iododeoxyuridine belong to class nucleoside analogue exhibited its *in- vitro* inhibitory potential against ASFV infection at 100 µg/ml concentration in Vero cells (Haag et al., 1965; Berry and Kinsella, 2001; Gil-Fernandez et al., 1979). Another broad spectrum antiviral agent (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) (HPMPA) adenine demonstrated its *in-vitro* inhibitory effect against ASFV infection along with nucleosides like pyrazofurin and ribavirin at 50 µg/ml concentration (Gil-Fernandez and De Clercq, 1987). HPMPA may inhibit ASFV infection by disrupting viral DNA synthesis (Arzuza et al., 1988).

Earlier researches revealed that naturally occurring plant flavones such as genistein, apigenin and genkwanin (Figure 4) also exhibiting their *in-vitro* inhibitory potential towards ASFV (Zakaryan et al., 2017; Xu et al., 2017; Kaihatsu et al., 2018). Genistein exhibited their inhibitory potential towards ASFV by inhibiting type II topoisomerase activity (Chae et al., 2019). Type II topoisomerase has an essential role during viral genome replication and transcription, suggesting that it could be a possible target for antiviral drugs including genistein (Coelho et al., 2016; Freitas et al., 2016). Molecular docking study suggested that genistein may act as an ATP-competitive inhibitor in ASFV infection because it interacts with four residues of the ATP binding site of viral topoisomerase, Asn-144, Val-146, Gly-147 and Leu-148 (Arabyan et al., 2019). Genistein also exhibited its therapeutic potential towards DNA and RNA viruses including HSV-1, Ebola virus, HIV and rotaviruses (Lyu et al., 2005; Kolokoltsov et al., 2012; Sauter et al., 2014; Huang et al., 2015). While apigenon and genkwanin both are potent inhibitors of tubulin polymerization by targeting the colchicine-binding site thereby disrupting the viral entry and egress (Choudhury et al., 2013; Arabyan et al., 2019).

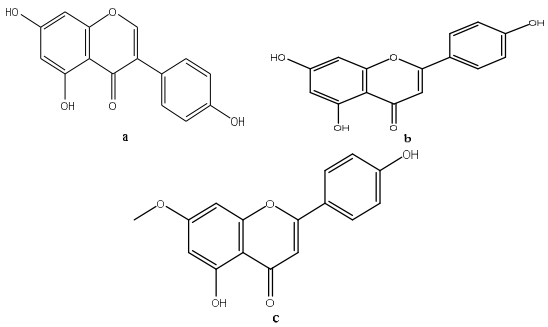

Figure 4: Structure of some naturally derived flavones. [a. Genistenin; b. Apigenin; c. Genkwanin]

Silva et. al. studied the *in-vitro* antiviral potential of 28 African traditional medicinal plants extract on ASFV (Strain Lisbon 60) using cytotoxicity assay. The results of this study revealed that four extracts (*Adansonia digitata* fruit; *Cochlospermum angolense* root; *Lippia chevalieri* root and *Piliostigma thonningi* stem bark) inhibited ASFV yield in the host cells (.80%) and only six extracts (*Cassia sieberiana* aerial

part; *Gardenia ternifolia* root; *Pavetta oblongifolia stem* and root; *Rhamnus glandulosa* leaf and *Sarcocephalus latifolius* root) have virucidal activity (80%) against this virus. Extracts from five plants (*Cassia sieberiana*, *Guiera senegalensis*, *Pavetta oblongifolia*, *Rhamnus glandulosa* and *Sarcocephalus latifolius*) had a virucidal effect on virus, and four plant extracts (*Cochlospermum angolense*, *Lippia chevalieri*, *Pavetta oblongifolia* and *Piliostigma thonningii*) inhibited the replication in virus (Silva et al., 1997).

CONCLUSION AND FUTURE DIRECTIONS:

African Swine Fever (ASF) is one of the most deadly illnesses among domestic pigs and wild boars, detailed by OIE. ASF is a staggering haemorrhagic irresistible sickness portrayed by severe depression, with casualty rates moving toward 100% with no immunization: it is brought about by the ASF virus. The determination of ASFV disease in pig is accomplished by isolation of virus or by the demonstration of explicit ASF antibodies. Control of ASF spread is by identification, quarantine, and butcher of contaminated and uncovered animals. ASFV causes a deadly pig hemorrhagic sickness and is as of now liable for across the board harm to the pork business in Asia. Neither immunizations nor antivirals are accessible and the molecular portrayal of the ASFV particle is remarkable. The further *in-vitro* study should be conducted on the plants reported positive therapeutic potential towards ASFV infection. Depth research should be conducted in the direction of the development of an effective vaccine against ASF. Further *in-vivo* researches on naturally occurring flavones are warranted.

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