DHS SCIENCE AND TECHNOLOGY

Master Question List for African Swine Fever Virus (ASFV)

3 April 2025

For comments or questions related to the contents of this document, please contact the DHS S&T Hazard Awareness & Characterization Technology Center at HACTechnologyCenter@hq.dhs.gov.

Science and Technology

DHS Science and Technology Directorate | MOBILIZING INNOVATION FOR A SECURE WORLD

African Swine Fever Virus (ASFV) – Master Question List

Table of Contents

Page

Foreword **1**
Situation Overview **1**
Major Findings by Topic Area **4**
Infectious Dose 9
Transmissibility 10
Susceptible Species and Vectors 11
Incubation Period 13
Clinical Presentation 13
Biosurveillance and Clinical Diagnosis 15
Veterinary Medical Countermeasures 16
Virus Introduction and Spread 17
Viral Persistence and Environmental Stability 19
Environmental Detection 20
Decontamination 21
Personal Protective Equipment (PPE) 22
Depopulation and Carcass Disposal 23
Swine Wastewater Treatment 24
Genomics 25
Commonly Used Acronyms and Abbreviations **27**
References **29**

Foreword

The following Master Question List (MQL) was developed by the U.S. Department of Homeland Security (DHS) Science and Technology Directorate (S&T) to provide government decision makers with up-to-date information, which will enable them to appropriately respond to a possible African Swine Fever Virus (ASFV) outbreak. This MQL summarizes what is known and what knowledge gaps exist to address fundamental questions such as, “What is the infectious dose?” and “How long does the virus persist in the environment?” The information provided is a succinct summary to facilitate structured and scientifically guided discussions across the Federal Government without burdening them with the need to review scientific reports, and to prevent duplication of efforts by highlighting and coordinating research.

Situation Overview

ASFV is the causative agent of African Swine Fever (ASF), a highly infectious and deadly disease in domestic pigs. The virus originated in Africa, where native hosts include warthogs and bush pigs, neither of which exhibit clinical disease. Domestic pigs are non-native hosts that exhibit signs of fever, respiratory distress, diarrhea, abortion, and sudden death. Of 24 known ASFV genotypes, only genotypes I and II have spread outside of Africa. ASFV began spreading in Eastern Europe in 2007 then spread quickly in Western Europe and China in 2018. ASFV is not a human pathogen; however, an outbreak in the U.S. would result in disruption of the swine industry with the potential for billions of dollars in economic impact due to loss of meat products and culling expenses. In 2021, ASFV was detected in the Dominican Republic and spread to Haiti. According to the World Organisation for Animal Health (WOAH or OIE), the outbreak is still ongoing as of 04 December 2024. ASFV has not been detected in the U.S., but the U.S. Department of Agriculture (USDA) and U.S. Customs and Border Protection (CBP) have increased precautions to prevent importation of the virus. The proximity of this outbreak has caused concern for further spread and motivates evaluation of the threat of ASFV to the U.S.

The cutoff date for information gathering related to this document was 04 December 2024.

The U.S. Department of Homeland Security's Science and Technology Directorate is committed to providing access to our web pages for individuals with disabilities, both members of the public and federal employees. If the format of any elements or content within this document interferes with your ability to access the information, as defined in the Rehabilitation Act, please contact the Hazard Awareness & Characterization Technology Center for assistance by emailing HACTechnologyCenter@hq.dhs.gov. A member of our team will contact you within 5 business days. To enable us to respond in a manner most helpful to you, please indicate the nature of your accessibility problem, the preferred format in which to receive the material, the web address or name of the document of the material (Master Question List for African Swine Fever Virus) with which you are having difficulty, and your contact information.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
BACKGROUND	• ASFV is a large double-stranded DNA virus with a 170-180 kilobase (kb) genome, and the sole member of its family (*Asfarviridae*). • There are 24 known genotypes of ASFV, but only two (genotypes I and II) occur outside the natural range in Africa. • ASFV is the causative agent of ASF.
INFECTIOUS DOSE	• The median infectious dose (ID_{50}) of ASFV in domestic swine ranges from ~10-10,000 viral particles, but depends on age, body weight, inoculation route, and virus strain. • Wild boar and domestic pigs can acquire ASFV infection after exposure via ingestion, intramuscular injection, and respiratory routes.
TRANSMISSIBILITY	• ASFV can spread between swine by direct or indirect contact, as well as through vectors such as soft-bodied ticks. • ASFV is highly transmissible, with infected domestic swine each infecting an average of 2.8 other individuals sharing a pen (also called the basic reproduction number or R_0), although this varies by genotype.
SUSCEPTABLE SPECIES AND VECTORS	• ASFV is native to Africa, where hosts include warthogs (*Phacochoerus* spp.) and bush pigs (*Potamochoerus* spp.). Neither warthogs nor bush pigs exhibit clinical disease signs. • Outside of Africa, domestic pigs (*Sus scrofa domesticus*), wild bearded pigs (*Sus barbatus*) in Borneo, Sulawesi warty pig (*Sus celebensis*) in Asia, and wild boar/feral hogs (*Sus scrofa*) are non-native hosts that can acquire fatal illness. • African warthogs imported into Texas have the potential to become infected without clinical signs. Warthogs in combination with tick vectors have the potential to create a setting for endemic ASFV transmission. • Soft-bodied ticks (genus *Ornithodoros*) are a natural vector in Eastern Africa and Europe. Ticks are required for transmission between warthogs, but not between domestic and/or feral pigs. • There is limited evidence for transmission via non-tick vectors such as stable flies and leeches.

INCUBATION PERIOD	• In natural infections, the time from exposure to development of clinical signs ranges from 3-21 days; domestic and wild swine have incubation periods around 15 days. • Less virulent strains produce mild signs, while more virulent strains have a shorter incubation period (around 3 days) and may result in death before clinical signs are observed. • In experimental studies, the incubation period depends on exposure dose, exposure route, and virus strain, but clinical signs generally occur within 4 days, with a wide range of 1-28 days
CLINICAL PRESENTATION	• Clinical signs vary, depending on host species, in domestic pigs signs include fever, loss of appetite, dull/depressed attitude, red/purple skin lesions, respiratory distress, vomiting/diarrhea/bloody diarrhea, high mortality, sudden death, and abortion. • Clinical forms of ASFV include acute, subacute, and chronic. Morbidity is high for all forms of disease; mortality rates vary but can reach 100% in peracute/acute infections.
BIOSURVEILLANCE AND CLINICAL DIAGNOSIS	• The United States has ongoing surveillance efforts in certain geographic areas. • Rapid ASFV point-of-care tests and detection methods are being researched to improve early detection and response, and there are multiple field-deployable genetic tests to detect ASFV under study. • Signs and symptoms of ASF are similar to other pig diseases such as classical swine fever (CSF) and necessitate diagnostic tests to establish causal disease agent. ASF should be considered in swine with febrile disease, disseminated hemorrhage, and high mortality. • Differential diagnosis should include CSF and porcine reproductive and respiratory syndrome, porcine dermatitis and nephropathy syndrome, erysipelas, Aujeszky's disease, salmonellosis, other septicemic conditions, and poisoning. • PCR is the most sensitive technique and can detect ASFV DNA at early stages of infection (within 3-4 days). Enzyme-linked immunosorbent assay (ELISA) can detect antibodies 7-14 days-post-infection (dpi), with utility for confirmation of clinical cases; antibodies may last for months to years.

VETERINARY MEDICAL COUNTERMEASURES	• There is no approved treatment for ASF. • There is no vaccine approved for use in the U.S. However, there are ASF vaccines in use internationally, including NAVET-ASFVAC in Vietnam. • A number of vaccine candidates have been investigated internationally, including inactivated vaccines, live attenuated vaccines, and subunit vaccines, but protection and side effects vary. • WOAH warns that even with efficacious vaccines, vaccination alone is not a sufficient disease control measure. Rather, vaccines are part of a robust prevention program that includes vaccinations, strict biosecurity measures, and importation and movement controls.
VIRUS INTRODUCTION AND SPREAD	• In 2021, ASFV was detected in the Dominican Republic and spread throughout the country and to neighboring Haiti. According to WOAH, the outbreak is ongoing as of 04 December 2024. • As of 04 December 2024, ASFV has never been detected in the United States, however the presence of ASFV in the Caribbean is concerning. • Infected animals are the primary source of ASFV, but infection can occur via ingestion of waste food that contains infected pig meat products or unprocessed pig meat. • Routes of concern are legal and illegal entry of infected animals or animal products and intentional release of ASFV.
VIRAL PERSISTENCE / ENVIRONMENTAL STABILITY	• ASFV has high environmental persistence and can be transmitted via fomites including shoes, clothes, vehicles, glass, metal, rubber, paper, boards, bricks, and equipment. Of note, infection of domestic pigs via fomites is not well characterized. • ASFV is stable in raw and processed pork/meat products, showing viability from 16-155 days in room temperature and chilled temperatures; frozen products have shown ASFV viability from 103-118 days, with estimates as long as 1,000 days. • Stability of ASFV within carcasses and potential contamination of surrounding area varies by environmental conditions (temperature, time, soil type) and disposal method. • Three *Ornithodoros* species of ticks native to the United States have been identified as possible vectors of ASFV; *O. coriaceus*, *O. turicata*, and *O. puertoricensis*. These species, combined with three competent vertebrae hosts, domestic pigs (*Sus scrofa domesticus*), feral hogs (*Sus scrofa*), and common warthogs (*Phacochoerus africanus*), could severely complicate response(s) and extend the length of any outbreak, should the virus be introduced into the United States.

ENVIRONMENTAL DETECTION	• Most field diagnostic kits are based on animal samples and are not made for environmental sampling or even testing animal samples other than recent carcasses. • Environmental sampling presents challenges of low concentration as well as microbial or organic contaminants naturally existing in soil or on farm surfaces, which can degrade viral nucleic acid. Ongoing research is focused on environmental detection sampling techniques for increased sensitivity, including the use of pre-moistened swabs for sample collection, inhibition of sample detection inactivators, as well as improved eluants.
DECONTAMINATION	• Disinfectants listed by the Federal Insecticide, Fungicide, and Rodenticide Act Section 3 labels claims for use against ASFV, are registered by the U.S. Environmental Protection Agency, and should be used for cleaning and disinfection of infected premises. If a suitable commercially available registered disinfectant is unavailable, a disinfectant approved for use against ASFV under the Federal Insecticide, Fungicide, and Rodenticide Act Section 18 may be used. The labels and Environmental Protection Agency approval letters from the current Section 18 exemptions are available online from U.S. Department of Agriculture Animal and Plant Health Inspection Service. • The use of effective disinfectants for cleaning infected premises, trucks, and fomites is an important measure for preventing introductions of ASFV to new areas.
PERSONAL PROTECTIVE EQUIPMENT (PPE)	• The guidance for PPE follows Occupational Safety and Health Administration Level C, which covers most non-zoonotic Foreign Animal Disease events and includes National Institute for Occupational Safety and Health approved respirators, chemical resistant clothing, inner and outer gloves, and boots. • PPE guidelines will differ for various tasks associated with ASFV. For instance, the protection required for surveillance and depopulation could differ from the protection required for chemical decontamination, or for working on an infected or uninfected area.
DEPOPULATION AND CARCASS DISPOSAL	• Depopulation of ASFV-infected domestic swine must follow guidelines established by the American Veterinary Medical Association (AVMA) for the Depopulation of animals. • There are several recommended methods for effective depopulation of confirmed or suspected ASFV-infected swine in an agricultural setting. • Carcass disposal and environmental regulations differ by setting and state. A comprehensive list of State-approved methods and regulatory considerations is available from the U.S. Veterinary Compliance Assistance Group.

SWINE WASTEWATER TREATMENT	• There is minimal information about ASFV persistence in farm wastewater systems, though transmission via contaminated water (e.g., during seasonal rains) is suspected in several African communities. • Chemical disinfectants and heat can inactivate ASFV in pig slurry, though effectiveness in wastewater systems needs to be determined.
GENOMICS	• The ASFV genome is variable in size and can include more than 150 distinct open reading frames (ORFs) encoding multiple proteins via the production of multiple transcripts from the same gene. The functions of some proteins are known, and several are involved in evasion of host defenses. • Multiple genetic evaluations have shown that removal of specific genes reduces the virulence of the virus. However, changes and deletions of other genes allow the virus to still retain virulence.

Note: In this MQL, **biosecurity** is defined as a series of management practices designed to reduce the risk of disease agents being introduced and spread within animal populations. In broad terms, it refers to anything designed to prevent the transfer of disease-causing pathogens.

Infectious Dose
How much agent will make a healthy pig ill?

What do we know?

The median infectious dose (ID_{50}) of ASFV in domestic swine ranges from ~10-10,000 viral particles, but depends on age, body weight, inoculation route, and virus strain.

- The preferred method for quantifying ASFV infectivity is the median hemadsorption infectious dose (HAD_{50}). The HAD_{50} is the dose (titer) of virus, measured as hemadsorbing units (HAU), that it would take to infect 50% of inoculated pigs and/or cells *in vitro*. Other common virological methods like median tissue culture infectious dose ($TCID_{50}$) or immunofluorescence assays may be used for ASFV characterization, but these laboratory methodologies are more challenging and time consuming to perform.[1-3] The hemadsorption assay takes advantage of the classical feature of ASFV infected swine monocytes attracting erythrocytes (red blood cells) in a rosette around the monocytes.[1] However, not all ASFV strains are hemadsorbing.

Some domestic pigs can acquire ASFV infection after exposure to only a few viral particles. The infectious dose varies based on genotype, strain, and route of infection.

Ingestion

- The ID_{50} in approximately 8-week-old, cross-bred pigs for a genotype II strain through ingestion of contaminated liquid was about 10 $TCID_{50}$ units.[4]
- For contaminated dry feed, the ID_{50} was considerably higher at $10^{6.8}$ $TCID_{50}$ units.[4] Additional studies in which 4-5 week old pigs were fed dry feed spiked with genotype II-contaminated plasma for 14 days did not result in infection at doses of 10^5.[5]

Intramuscular

- The infectious dose for a genotype II strain in 7-week-old domestic swine inoculated via intramuscular injection was ≤ 10^2 HAD_{50} units.[6] Other studies have shown the dose can be as low as 5 HAD_{50} units for 5-6 weeks old[7-8] and 0.1 HAD_{50} units in 8 -week old pigs.[9] More recent studies on a genotype II strain isolated from wild boar in Asia and inoculated by the intramuscular route into 7 week old Bama minipigs determined the ID_{50} to be < 0.1 HAD_{50} units.

Oronasal and intranasal

- Doses for infection via oronasal inoculation were ≤10 HAU for approximately 8-week runts (smallest or weakest of the litter at birth).[11]
- For genotype I strains, Malta'78, and Netherlands'86, the ID_{50} was estimated to be ≤ 3.5 $\log_{10}$ $TCID_{50}$ units by intranasal inoculation, while the median lethal dose (LD_{50}) was estimated to be ≥ 3.5 $\log_{10}$ $TCID_{50}$ units .[12] Another genotype I strain, Brazil'78, has a reported ID_{50} of -0.25 $\log_{10}$ $TCID_{50}$ units.[12]

European wild boar can acquire ASFV infection after exposure via multiple routes.

- Studies in wild boars of various ages (9 weeks-10 years) with a genotype II strain was equally lethal at high doses by oral and intramuscular inoculation, suggesting an infectious dose ~10^6 $TCID_{50}$ units.[13-14] Lower ranges still need to be defined.
- Doses for infection via oronasal inoculation were ≤10 HAU for approximately 4-month-old runts.[11]

What do we need to know?

- What are the infectious doses for different breeds of pigs, specifically domestic U.S. breeds

to genotype II strains? Outside of pig production facilities, there are many small farms with various specialty breeds; do these different breeds have the same susceptibility or possess any immunity to genotype II strains?

- What are the differences in infectious dose for various ages of wild boar or warthogs?
- Which infectious dosage and route have relevance to natural disease transmission and best inform policy?

Transmissibility
How does it spread from one host to another? How easily is it spread?

What do we know?

ASFV can spread between swine by direct or indirect contact, as well as through vectors such as soft-bodied ticks.

- Transmission can occur through:
  - Direct contact with blood, feces, and oronasal excretions,[11, 13-16] including contact between infected and susceptible wild or domestic pigs;[15, 17-19]
  - Ingestion of contaminated pig products;[4, 20-21]
  - Indirect contact through people, vehicles,[22] fomites (e.g., virus transported on shoes, tools, or PPE), aerosols,[23] and contaminated pork products;[15, 24-27]
  - Limited transplacental transmission;[28-30]
  - Vector transmission by ticks in the genus *Ornithodoros*;[31-38]
  - And through ingestion of infectious stable flies (*Stomoxys calcitrans*).[39-42]
- Transmission among soft ticks can occur through many routes of transmission and some can preserve the virus for years.[43] However, their role in the current epidemic is questionable as they do not appear to be involved in transmission over long distances.[44]
- There is no evidence that mosquitoes play a role in ASFV transmission.[45-46]

ASFV is highly transmissible, with infected domestic swine each infecting an average of 2.8 other individuals sharing a pen (this is the basic reproduction number or R_0), although this varies by genotype.[17] ASFV is less transmissible than foot and mouth disease in swine.[47]

Evidence-based

- The estimated R_0 for genotype I virus can vary from 1.54 – 18[48 49].
- The estimated R_0 of genotype II virus can vary from 0.68 – 2.8 within pens and 0.82 – 2.13 between animals in adjacent pens[17, 50-51]
- Aerosol transmission between an infectious pig and a nearby healthy pig with no direct contact is possible, but the role of aerosol transmission in outbreaks is not well-established.[23, 52]
- Animals that recover from initial infection remain persistent carriers, with evidence suggesting they could transmit the virus, including asymptomatically, up to 70 dpi.[12, 53-55]

Model-based

- It is not known what an outbreak in the U.S. would look like but various models have been built to predict outbreak dynamics.[56-61]

- Utilization of field data to create a mathematical model for transmission in Vietnamese pig farms estimated R_0 values of 1.49 (1.05-2.21), 1.58 (0.92-2.56), and 1.46 (1.38-1.57) based on the exponential growth, maximum likelihood, and attack rate methods, respectively. This modeling also suggests that a 80% vaccination rate is necessary to prevent spread of disease.[62]
- Further studies to evaluate the R_0 in Vietnam between farms using indoor production facilities determined a range of 1.41-10.8 depending on the infectious period of 15, 19, or 30 days.[63]
- A study that similarly used field data to create a model for outbreaks in Lao People's Democratic Republic estimated the R0 to be between 3.08 and 7.80, with transmission rates ranging from 1.10 to 1.66.[64] To effectively inhibit ASF outbreaks, pig movements between farms need to be reduced by 50-75%.[65]

What do we need to know?

- Is there a threshold for virus or time necessary to transmit between domestic pigs?
- In the wild boar and feral swine population, how long can the transmission chain extend?
- How much are ticks and other vectors involved in transmission in pig farms and among wild boar?[45, 66-67]
- What is known about the presence of ASFV-competent tick vectors in the United States?
- To what extent do scavengers play a role in transmission?[18-19, 68]

Susceptible Species and Vectors
How many species does it infect? Can it transfer from species to species?

What do we know?

ASFV is native to Africa, where hosts include warthogs (*Phacochoerus* spp.) and bush pigs (*Potamochoerus* spp.).[69] Neither warthogs nor bush pigs exhibit clinical disease signs.[69] Outside of Africa, domestic pigs (*Sus scrofa domesticus*),[70] wild bearded pigs (*Sus barbatus*) in Borneo,[71] Sulawesi warty pig (*Sus celebensis*) in Asia,[71] and wild boar/feral hogs (*Sus scrofa*)[70] are non-native hosts that can acquire fatal illness.

- Peccaries are thought to be relatively resistant to ASFV,[72-73] but additional work is required to characterize them as a potential reservoir in the United States.
- Along with warthogs (*Phacochoerus africanus*) and bushpigs (*Patamochoerus larvatus*), red river hogs (*Patamochoerus porcus*) can be infected with ASFV and not display any clinical signs, and act as natural reservoir hosts.[74] African warthogs were imported into Texas for hunting purposes, escaped captivity, are reproducing in Texas, and have the potential to become infected without clinical signs. These animals along with tick vectors could create a setting conducive to endemic ASFV transmission.[75]
- There is currently no evidence that non-suid mammals act as a reservoir for ASFV.[10, 76-78]

Soft-bodied ticks (genus *Ornithodoros*) are a natural vector in Eastern Africa and Europe.[79] Ticks are required for transmission between warthogs, but not between domestic and/or feral pigs.[76]

- In the United States, there are at least three species of potentially competent tick vectors (*Ornithodoros coriaceus*, *Ornithodoros turicata*, and *Ornithodoros puertoricensis*).[76, 80] In one study from 1987, *Ornithodoros coriaceus* collected in California were shown to harbor and transmit ASFV for more than 440 days, but resulted in 40% of ticks dying and trans-ovarial passage was not demonstrated. *Ornithodoros turicata* collected in Florida were shown to

transmit ASFV to pigs 23 days post feeding. *Ornithodoros puertoricensis* collected in the Dominican Republic and Haiti were shown to become infected and transmit ASFV to pigs up to 239 days post feeding and trans-ovarial transmission was demonstrated. [81]

- Three *Ornithodoros* species of ticks native to the United States have been identified as possible vectors of ASFV; *O. coriaceus, O. turicata, and O. puertoricensis*. These species, combined with three competent vertebrae hosts, domestic pigs (*Sus scrofa domesticus*), feral hogs (*Sus scrofa*), and common warthogs (*Phacochoerus africanus*), could lead to outbreaks in the United States if the virus is introduced.[76, 82-84]
- Different tick species within *Ornithodoros* appear to have different capacities to transmit individual strains of ASFV.[33] The ASFV Uganda strain has an extremely low infectious dose in 70-75% of *Ornithodoros moubata porcinus* ticks of $10^{0.9}$-10^{4} HAD_{50}, with persistence of virus in the tick for 15 months. In contrast, the ASFV Tengani strain only produced a persistent infection in 5% of these ticks and required 10^{4}-10^{5} HAD_{50}.[36]
- There is no evidence that ASFV can replicate in hard ticks or that hard ticks can transmit ASFV.[85] [45]

There is limited evidence for transmission via non-tick vectors.

- The stable fly (*Stomoxys calcitrans*)[39-40] has been linked to transmission, and has been demonstrated under experimental conditions when pigs eat flies that have fed on infected blood within 24 hours.[86]
- ASFV was not detected in *Culicoides punctatus*, *Obsoletus* biting midges, mosquitoes (*Aedes* spp., *Anopheles* spp., *Culiseta annulata*), or *Haematopota pluvialis* tabanid beetles collected from wild boar habits, suggesting they do not contribute to ASFV transmission.[45]
- ASFV DNA was detected in *Stomoxys calcitrans* and *Culicoides* spp. collected from locations experiencing outbreaks in domestic pigs. It was not confirmed if infectious virus was also present.[87] ASFV DNA was also detected in several families of non-biting flies (Calliphoridae, Sarcophagidae, Fanniidae, Drosophilidae, and Muscidae) in locations experiencing outbreaks in domestic pigs. It was not confirmed if infectious virus was also present.[42]
- Under experimental conditions, an ASFV genotype II strain could survive in leeches and could be transmitted to pigs that consumed the leeches and water contaminated by the infected leeches; however, it remains unclear how leeches may contribute to natural spread of ASFV.[88-89]
- Under experimental conditions, ASFV could survive in nine gastropod (snail) species, and all but two of these species extended the circulation of the virus when compared to the virus in water without gastropods. While this study did acknowledge that gastropods are a part of the diet of wild and free-grazing domesticated pigs, they did not provide supporting evidence that the gastropods could directly cause infection through ingestion.[90]

What do we need to know?

- What are the tissue tropism and infection dynamics in different host and carrier species?
- What is the vector competence of U.S. vector species (e.g., *Otobius megnini*, *Ornithodoros lagophilus*, *Ornithodoros kelleyi*, *O. coriaceus*, *O. turicata*, *O. puertoricensis*)? Has transmission from parent to offspring been demonstrated in these tick species?
- How often do soft-bodied ticks interact with domestic and feral swine in the United States?
- What are the preferred mammalian hosts for the U.S. soft tick species that are competent for

ASFV?

- Do potential vectors such as flies and leeches contribute to natural spread of ASFV?[86, 89, 91]

Incubation Period
How long after infection do symptoms appear? Are animals infectious during this time?

What do we know?

In natural infections, the time from exposure to development of clinical signs ranges from 3-21 days; domestic and wild swine have incubation periods around 15 days.[72, 92]

- Less virulent strains produce mild signs, while more virulent strains have a shorter incubation period (around 3 days) and may result in death before clinical signs are observed.[72, 93]
- Pigs experimentally infected with a highly virulent I ASFV isolate shed infectious virus before the onset of clinical signs, and were generally infectious for 3-7 days overall.[17] The latent infection period (onset of infectiousness) showed a median of 4.5 days.[64, 94]
- For genotype II, the latent period averaged at 3.6 days by blood titration detection, but follows closely with the incubation period, with animals having detectable virus in their blood just prior to or immediately following the onset of fever, and they can remain infectious at a minimum of 1-6 days during acute infection.[6, 64]

In experimental studies, the incubation period depends on exposure dose, exposure route, and virus strain, but clinical signs generally occur within 4 days, with a wide range of 1-28 days.[6-7, 52, 54, 95-99]

- For genotype II strains, clinical illness can last 6.3 days on average (range 0-18), indicating that death can be rapid in experimentally infected individuals.[100] The duration of infectiousness is broad, ranging from 1-40 days, depending on the virulence of the ASFV strain.[6, 48, 100]
- The incubation period for genotype II strains, with swine experimentally inoculated by various routes, is approximately 4 days with a range of 1-28 days.[6-7, 95, 98-101]
- Like domesticated pigs, the incubation period was variable and dependent on exposure location, dose, and animal age. In general, incubation periods in wild boars ranged from 11 days to 4 weeks.[102] An experiment by another team indicated that wild boars exposed to a genotype II strain intranasally had shorter incubation periods (4 days) when compared to infected domestic pigs (7 days).[103]

What do we need to know?

- Are incubation periods genotype-dependent? Are there some strains with longer incubation periods than others, and are animals infected with these strains contagious prior to the presence of clinical signs?
- What are the incubation periods for wild boar and warthogs for genotypes and strains of ASFV that have not yet been tested, and does the onset of infection correlate with that timeline or vary based on the animal?

Clinical Presentation
What are the signs and symptoms of an infected animal?

What do we know?

Clinical signs include fever, loss of appetite, dull/depressed attitude, red/purple skin lesions, respiratory distress, vomiting/diarrhea/bloody diarrhea, high mortality, sudden death, and abortion.[93]

- There are 24 known ASFV genotypes, and clinical presentation may differ for these genotypes.[104]
- Depending on the virulence of the isolate, pigs can develop different clinical forms of disease that are distinctly described as peracute, acute, subacute, and chronic. [72, 93, 104-106]
- Clinical signs also vary depending on age,[107] breed, route of exposure, and whether the virus is endemic to the region.[72, 93] Clinical symptoms in several individuals may need to be observed before clinical diagnosis is pursued.[108]

Peracute Clinical Form

- Clinical signs of peracute infection occur within 3-7 days and include high fever (40.5-42°C), loss of appetite, hemorrhage, vomiting, bloody diarrhea, abortion, and inactivity.[72, 108-110] Swine may die before onset of clinical signs.[72, 74, 93, 109-111]

Acute Clinical Form

- Clinical signs of acute infection tend to appear after 3-7 days and include fever, lack of appetite, increased respiratory rate, and weakness or depression.[103, 109, 111] Other signs include hemorrhages or blue-purple spots on ears,[110] abdomen, and/or hind legs; ocular/nasal discharge; reddening or necrosis of skin; vomiting/constipation/diarrhea; and abortion in pregnant sows.[93]
- Death may occur as early as 6 days (highly virulent strains) or up to 20 days (moderately virulent strains).[93, 103, 110, 112-113]

Subacute Clinical Form

- Clinical signs of subacute infection are similar to acute infection, but are generally less severe and may show initially as prodromal signs of fever, apathy, and reduced feed intake and may also include swollen joints and fluctuating fever.[7]
- Duration of illness can be 5-30 days.[110] Subacute infections may be found in endemic areas, with mortality ranging from 30-70%, with some dependence on age of population, and death occurring within 7-45 days.[74, 93, 110]
- Infection with an attenuated strain can produce a mild or subclinical disease.[114]

Chronic Clinical Form

- Chronic infection is generally caused by low-virulence isolates that do not immediately kill the host.[115]
- Clinical signs of chronic ASF occur 14-21 dpi.[93] Chronic infections have variable, mild clinical signs, complicated diagnoses, and develop over 2-15 months.[109-110] Survivors may carry the virus for life.[110]

Morbidity is high for all forms of disease; mortality rates vary, but can reach 100% in peracute/acute infections.[72, 93]

- Lethality ranges from <20% in chronic forms to, 30-70% in subacute forms, and up to 100% overall.[92-93, 110 103, 111]
- All members of the pig family are thought to be susceptible to infection; however, African wild pigs, warthogs, and bush pigs, are frequently asymptomatic carriers of ASFV.[72, 93, 110] Warthogs exhibit transient viremia, but the mechanisms of tolerance are not well understood.[116] Early historical accounts indicate that peccaries (*Tayassu* spp., also called javelinas), pig-like animals similar to warthogs, may be resistant.[72-73]

What do we need to know?

- Variable presentation of clinical signs complicates diagnosis. What signs can be used for early detection?[35]
- Are warthogs able to clear virus without showing clinical signs, or do they tolerate viremia without clinical signs?

Biosurveillance and Clinical Diagnosis
Are there tools to diagnose infected animals and herds?

What do we know?

The United States has ongoing efforts in place to continuously monitor for ASFV to protect U.S. swine against the virus. A protection zone with increased surveillance, biosecurity, and sanitary policies was established for Puerto Rico and the U.S. Virgin Islands to minimize the risk of ASFV spread to the continental U.S. due to detection in Hispaniola in 2021.[72]

- The USDA Animal and Plant Health Inspection Service (APHIS) Foreign Animal Disease Preparedness & Response Plan (FAD PReP) sets guidelines for surveillance and biosecurity of ASFV.[72]
- Passive surveillance is ongoing in the United States for swine and relies on laboratory personnel, veterinarians, swine producers, or other stakeholders' suspicion of a case of ASF.[72] Suspected cases are reported to local, state, and Federal animal health officials, after which samples are collected by a FAD Diagnostician.[117]
- Active surveillance (the routine collection of whole blood and serum samples from healthy animals) occurs in Florida, Texas, Georgia, Louisiana, New York, New Jersey, Puerto Rico, and the U.S. Virgin Islands as these areas have been identified to have a higher risk of introduction than swine in other areas.[118]

There is currently no global biosurveillance framework for ASFV.

- There are four WOAH reference laboratories for ASFV: CSIRO Australian Centre for Disease Preparedness (Geelong, AU), Onderstepoort Veterinary Institute (Onderstepoort, S. Africa), Centro de Vigilancia Sanitaria Veterinaria (VISVET) (Madrid, Spain), and Pirbright Institute (Surrey, UK).

Symptoms of ASF are similar to other pig diseases such as CSF and necessitate diagnostic tests.[110] ASF should be considered in swine with febrile disease, disseminated hemorrhage, and high mortality.

- Clinical signs of acute disease cannot be used to distinguish ASF from CSF and other pig diseases that have similar clinical presentation and mortality rates; laboratory tests are needed for a definitive diagnosis.[93, 110]
- Differential diagnosis should include CSF and porcine reproductive and respiratory syndrome, porcine dermatitis and nephropathy syndrome, erysipelas, Aujeszky's disease, salmonellosis, other septicemic conditions, and poisoning.[72, 93]
- Clinical evidence can be non-specific, so active surveillance should be based on rapid diagnostic testing.[109, 119] Testing should include a combination of both virus and antibody tests to provide serological and virological differentiation, as animals may be in different stages of disease.[93, 119]
- In the case of acute infection, death may occur before antibodies are produced.[109] Blood sampling, for both PCR and serological analyses, is essential for the accurate diagnosis of

ASF and provides the highest probability of detection of the disease.[120] While not common, a few naturally occurring non-hemadsorbing strains (i.e., strains that do not attract red blood cells) exist that produce acute infection.[121]

Rapid ASFV detection methods are being researched to improve early detection and response.[122-125]

- PCR is the most sensitive technique and can detect ASFV DNA at early stages of infection (3-4 days post-infection).[110] ELISA can detect antibodies 7-14 dpi, with utility for confirmation of clinical cases;[110] antibodies may last for months to years.[70, 119] Commercially available ELISA, lateral flow, and PCR kits are available for use with blood, serum, and oral fluids from pigs, but some may not be available in the U.S.[125-128]
- ASFV genome and antibodies are detectable in meat exudate and provide an alternative method for disease surveillance.[129]

What do we need to know?

- What biosurveillance systems can be implemented in endemic areas?[130]
- Should *Ornithodoros* species-specific surveillance occur throughout the United States to monitor ASFV presence?[131]
- How can diagnostics be improved to overcome current short-comings?[70]

Veterinary Medical Countermeasures
Are there effective vaccines and treatments to limit or prevent infection and/or spread?

What do we know?

There is no approved treatment method for ASF for use in the United States.

- To prevent the spread of disease, the United States' response plan includes containment of infected populations by quarantine and movement controls, tracing and surveillance, and depopulation.[72]

There is not an approved vaccine for use in the U.S. Several vaccine candidates have been tested and some approved for use outside of the U.S., including inactivated vaccines, live attenuated vaccines, and subunit vaccines, but protection and side effects vary.

Inactivated Vaccines

- Inactivated ASFV vaccines have not demonstrated protection.[132-135]

Attenuated Vaccines

- Researchers with USDA Agricultural Research Service have recently identified a promising deletion mutant live attenuated vaccine NAVET-ASFVAC, which is produced using the Georgia 07 isolate (a genotype II ASFV) ASFV-G-ΔI177L strain. It is the first commercially available ASF vaccine in the world and has been approved for domestic commercial use in Vietnam.[135-136] [137] [138] A drawback to this vaccine is that it does not allow differentiation of infected from vaccinated animals (DIVA). This vaccine is the same as virulent ASFV except for the deleted I177L gene.[137-138] This vaccine passed a test in April 2022 confirming that the virus does not undergo a reversion to virulence, which is required for regulatory approval.[139]
- Live, attenuated vaccines (LAVs) have had some success at protecting pigs from subsequent challenge,[95, 140-146] though this protection does not usually extend to other ASFV strains.[147-148] LAVs can spread the virus to other pigs and cause chronic ASF that later regains virulence or limiting fertility.[149-150]

- Real-time PCR assays (qPCR) have been developed that detect the difference between infected and pigs vaccinated with three recombinant live, attenuated vaccine candidates.[151] Research on DIVA through PCR and ELISA-based methods are ongoing.[152-153]

Subunit Vaccines

- Protein subunit vaccines, which help generate antibodies, have shown some protective efficacy against ASFV,[154-156] though the effects are not universal.[157-158]
- Other subunit vaccines, delivered by viral, bacterial, or plasmid vectors have also been developed,[149] and have granted either no,[159-161,162] partial,[160, 163] or robust[164] protection from ASFV. Similarly, vaccines based on DNA constructs also exhibit variable protective efficacy, and efficacy is dependent on the virulence of the ASFV challenge virus.[165-168]
- There has been some evidence of vaccine-induced disease enhancement, where vaccinated pigs have developed more severe disease upon secondary challenge.[166, 168]

WOAH warns against risks associated with using poor quality or non-compliant vaccines[169]

- Poor quality vaccines do not convey protection but could also lead to recombination with field strains to generate novel strains. [169]
- WOAH warns that even with efficacious vaccines, vaccination alone is not a sufficient disease control measure. Rather, vaccines are part of a robust prevention program that includes vaccinations, strict biosecurity measures, and importation and movement controls. [169]

What do we need to know?

- What factors contribute to vaccine or immune-mediated disease enhancement?
- What factors contribute to the lack of protective efficacy among challenge strains?
- Can antibodies limit viral load and transmission potential in treated swine?[170]
- Are there treatments/interventions that could be implemented prior to the onset of clinical signs to prevent culling of herds?
- Can alternatives to ASFV challenge experiments for determining vaccine efficacy be created?[171]
- What are the barriers for designing a test for DIVA?[108]
- Are there treatments that reduce transmission potential in already-infected swine?

Virus Introduction and Spread
What are the risks of the virus entering the United States through agricultural/food vectors?

What do we know?

In 2021, ASFV was detected in the Dominican Republic and has since spread throughout the country and to neighboring Haiti.[99] According to WOAH, the outbreak is ongoing as of 04 December 2024.[172]

- As of 04 December 2024, ASFV has never been detected in the United States, however the presence of ASFV in the Caribbean is concerning.
- In response to the detection of ASFV in the Dominican Republic, USDA APHIS suspended interstate movement of all swine and swine products from U.S. territories, Puerto Rico, and U.S. Virgin Islands due to their proximity to the United States, as well as passenger and

trade movements with the Dominican Republic, until mitigation efforts were instated.[173] A FAD protection zone was established around Puerto Rico and the U.S. Virgin Islands. The USDA also enhanced ASF surveillance in Puerto Rico and the U.S. Virgin Islands, combined sampling and testing for CSF with ASF, and continued efforts to remove feral swine in Puerto Rico. Finally, continued efforts between the USDA and CBP include increased inspections of flights from the Dominican Republic to ensure proper disposal of garbage to reduce the risk of ASFV importation.[173]

Infected animals are the primary source of ASFV, but infection can occur via ingestion of waste food that contains infected pig meat products or unprocessed pig meat. Routes of concern are legal and illegal entry of infected animals and animal products or intentional release of ASFV.[131]

- There is a risk of viral incursion via pathways that may include live pigs, semen, swine products/by-products, wildlife, feed (animal and plant origin; supplements), fomites, and regulated garbage. A qualitative assessment of the likelihood of ASFV entry into the United States determined that illegal entry of products/by-products through air passenger baggage and foreign mail is the largest potential pathway. The risk is considered to be high, with low uncertainty.[72, 174]

Human-mediated import likely contributed to the spread of ASFV in Europe and elsewhere, possibly through illegal importation of infected meat, hunting tourism, or fomites associated with farmers, farming professionals, and importation of bedding.[175]

- It is estimated that limited travel and decreased restaurant food waste due to COVID-19 has reduced ASFV importation in Japan, as the illegal importation of pig products to meet tourism needs is the largest potential for ASFV entry into Japan.[176]
- While there is a decreasing trend of legal imports in swine products and by-products into the United States from ASFV affected countries, there has been no change in the amount of confiscated illegal products from ASFV affected countries at ports of entry. There has been an increase in the monthly number of merchant ships and value of imported goods and percentage of commercial flights from ASFV affected countries.[177] It is estimated that >90% of the risk from ASFV importation via air travel involves five airports from China and eastern European countries.[178]
- Illegal entry of pork, ham, and sausage products is a potential pathway for introducing ASFV.[174] Although fomites and feed ingredients are potential pathways, there are minimal data on transmission.[179-180]
- A recent assessment determined that in 2020, moderate- and high-risk ingredients from ASFV-positive countries represented 3.1% of all ingredients imported into the U.S.[181]

What do we need to know?

- What is the stability of infectious virus on fomites (e.g., on footwear)?[108]
- How much contaminated meat would a pig need to eat to become infected, and would the strain of ASFV (virulence, infectivity) impact the viability of introducing the disease to an endemic area (i.e., would one or two pigs eating the material be enough to introduce the disease to a swine population)?
- How can the risk of transmission via fomites (e.g., food baths, cargo hold fogging) or passenger travel (e.g., public education, signage) be reduced?
- What is the prevalence of ASFV in circulating swine products?

- What is the prevalence of ASFV in seized/illegal/non-permitted pork products at U.S. ports of entry?

Viral Persistence and Environmental Stability
How long does the agent live in the environment?

What do we know?

ASFV has high environmental resistance and can be transmitted via fomites including shoes, clothes, vehicles, glass, metal, rubber, paper, boards, bricks, and equipment.[26, 182-183] Of note, infection of domestic pigs via fomites is not well characterized.[20]

- Dried ASFV persists on non-porous and porous fomites at 42°C, 33°C, and 25°C for 1–2, 6-12, and 11-17 days, respectively.[26]
- In one experiment, ASFV remained at a detectable level as determined by hemadsorption units in water for three weeks at 22 – 24°C, while genomic DNA was detected for up to five weeks.[90]

ASFV is stable in raw and processed pork/meat products, showing viability from 16-155 days in room and chilled temperatures; frozen products have shown ASFV viability from 103-118 days, with estimates as long as 1,000 days.[15, 51, 184]

- ASFV is viable in blood, feces, and tissues, including pork products that are uncooked/undercooked. The virus is highly resistant to temperature and pH fluctuations,[72, 109] and is extremely stable at low temperatures.[184]
- In unprocessed pig meat, the virus is stable for weeks to months;[15] heating to 70°C for 30 minutes destroys the virus.[109] Stability in salted and smoked/cured meats ranges have been reported from 30-399 days.[15, 184] *In vivo* experiments showed detection in Italian salami, pork belly, and loin, up to 18, 60, and 83 days, respectively.[185]

Infectious virus can be shed and detected in urine and feces for approximately 2 weeks, depending on environmental temperature. Excretions should be considered a viable pathway for transmission.[186]

- Of biological samples collected 5 dpi, feces were the least sensitive for ASFV detection in subclinical infections, whereas splenic homogenate was the most sensitive. From highest to lowest, the probability of detecting ASFV in these samples was: spleen > lymph node > tonsil > serum > feces.[187]

Stability of ASFV within carcasses and potential contamination of surrounding area varies by environmental conditions (temperature, time, soil type) and disposal method.[188-190]

- Biosecure carcass disposal remains a high priority due to assumed high viral stability and loads in infected carcasses.[191] In one study in Lithuania, ASFV genome was detectable in previously buried wild boar carcasses up to 440 days post-burial, although infectious ASFV could not be isolated.[192] Using an Estonian 2014 isolate, another study suggests that infected carcasses may remain infectious for nearly 2 years.[193]

ASFV is stable in animal feed (half-life of 2-14 days),[194-195] which is a potential route of transmission.

- Feed trucks have been implicated as a potential mechanical fomites capable of spreading ASFV to production sites, as they were commonly detected as PCR positive for ASFV in one study.[180]
- Although spray-drying of infected porcine plasma can inactivate ASFV (a laboratory spray

drying process reduced infectious ASFV by 2.11 log_{10} $TCID_{50}$/mL),[196] spray-dried porcine plasma dietary supplements experimentally contaminated with ASFV maintained infectivity for 5 weeks at 4°C. A > 5.7 log_{10} loss was observed when the supplements were stored at 21°C for 2 weeks.[197]

- The stability of ASFV varies depending on feed matrices and storage temperature.[195] Infectious ASFV was most stable in soybean meal, and found to retain infectivity for at least 112 days at 40°F, at least 21 days at 68°F, and at least 7 days at 95°F.[21]

What do we need to know?

- What is the stability of ASFV in agricultural or transportation settings and what are suggested hold times for ASFV degradation on commonly used farm equipment?
- What is the stability of ASFV in hydrolyzed proteins, gelatin, collagen, calcium phosphate, and rendered fats?[198]
- What considerations should be given to handling and transportation of feed related products under unknown conditions?
- Does rendering or other manufacturing activities decrease amount of or inactivate ASFV and is this process dependent?
- Is it beneficial in a domestic setting to till the contaminated soil with some type of pH modifying agent?[188]

Environmental Detection
What methods are available for detecting the agent in the environment?

What do we know?

Rapid ASFV detection methods are being researched to improve early detection and response including in environmental samples.[122-124, 199]

- PCR is the gold standard for the detection of ASFV,[200] with both real time PCR (rtPCR)[2, 180, 200-201 202-203] and quantitative PCR (qPCR)[204-205] used for environmental detection. qPCR testing for environmental DNA (eDNA) can be performed to detect viral DNA in water and soil samples;[204] however, qPCR can sometimes lead to false negatives in low viral load or environmental samples. Droplet digital PCR (ddPCR) is a third generation PCR technique that can be used for ASFV and has a detection limit of 1.97 copies/reaction, which is 19 times more sensitive than qPCR.[205] Other near-field-based testing using amplification of nucleic acid such as loop-mediated isothermal amplification or recombinase polymerase amplification (RPA) can also be used.[203206]
- Detecting infectious virus in the environment can be achieved by performing virus isolation followed by hemadsorption tests; however, some ASFV is non-hemadsorbing. Virus isolation must be performed in a Biosafety Level 3 laboratory in non-endemic countries.[2, 150]
- Environmental sampling presents challenges of low concentration as well as microbial or organic contaminants naturally existing in soil or on farm surfaces, which can degrade viral nucleic acid. Ongoing research is focused on environmental detection sampling techniques for increased sensitivity, including pre-moistened sample collection swabs or sponges, inactivation of detection inhibitors, as well as improved eluants.[199, 201-202]
- Rapid diagnostic point-of-care tests for animal disease diagnosis from animal blood or tissue exist, but caution must be taken as most field diagnostic kits are not made for environmental sampling or even testing animal samples from carcasses.[207] Recently commercial rapid detection kits for environmental sampling have become available, including kits from RingBio, Flashtest, and TianLong.[208-210]

- There are multiple field-deployable genetic tests to detect ASFV under study, including MatMaCorp's portable ASFV detection system.[211-213]

What do we need to know?

- Can methods other than virus isolation be used to detect infectious virus?
- At what level would genetic material detection in the environment be relevant to spread/transmission of ASFV?

What do we need to know?

Decontamination
What are effective methods to kill the agent in the environment?

What do we know?

When decontaminating ASFV, the first course of action recommended is use of disinfectants listed by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Section 3 labels claims for use against ASFV, which are registered by the U.S. Environmental Protection Agency (EPA).[214]

- If a suitable commercially available EPA and state-registered disinfectant is unavailable, a disinfectant approved for use against ASFV under Section 18 of FIFRA (i.e., a Section 18 disinfectant) be used according to its Section 18 label. The labels and EPA approval letters from the current Section 18 exemptions are available online from APHIS.[214]

The use of effective disinfectants for cleaning infected premises, trucks, and fomites is an important measure for preventing spread of ASFV to new areas. Care should be taken to use a disinfectant specifically approved for ASFV.

- In an agricultural setting and for the disinfection of transportation vehicles the recommended decontamination options are Virkon S (10 minutes), Neogen Viroxide Super (10 minutes), Clearon Bleach Tablets (30 minutes), Virocid (10 minutes), Klor-Kleen (30 minutes), Klorsept (30 minutes), Klorkleen 2 (30 minutes), and Accel Concentrate Disinfectant Cleaner (5 minutes).[214] This efficacy may be improved by allowing farms to remain empty for 1 to 2 weeks.[215]
- Additional disinfectants listed under FIFRA Section 18 for use when registered disinfectants are not available include: sodium hypochlorite (15 minutes non-porous, 30 minutes porous), citric acid (15 minutes non-porous, 30 minutes porous), and Benefact (15 minutes on non-porous surfaces inside and outside aircraft).[214]

ASFV is stable at low temperatures and survives freezing. However, it can be heat inactivated at 56°C for 70 minutes or 60°C for 20 minutes. ASFV can be inactivated at pH 11.5 in serum-free medium. Serum increases the resistance of the virus (e.g., at pH 13.4, resistance lasts up to 21 hours without serum, and 7 days with serum).[72, 92, 184, 216]

- A detailed decontamination table with benefits and limitations of multiple methods is available from USDA APHIS[217] or from The European Cooperation in Science and Technology.[218]
- Disinfection of feed products can be challenging; additives, heat inactivation, and strict adherence to storage guidelines can reduce ASFV contamination in animal feed. Strict adherence to storage processes is crucial to reduce virus contamination.[198] Heat treatments (30 minutes at 70°C; 5 minutes at 85°C) increase viral decay and reduce the risk of contamination in feed.[219]
- Ultraviolet-C (UV-C) treatment (at 3,000 J/L) and spray-drying can reduce up to 99.99% (4 $\log_{10}$ $TCID_{50}$/mL) ASFV in dried animal feed.[196] UV-C treatment of water-inactivated ASFV

after 30 minutes at 110-120 μW/cm^2 or within 3 seconds at 3,600 μW/cm^2.[220]

Other disinfectants have been evaluated in the literature but may not be registered or approved for use against ASFV.[92, 110, 215, 221-223]

What do we need to know?

- What variables are most important when deciding the best decontamination method for ASFV (i.e., cost or waste generated)?
- What are the risks of recontamination of animal feed post-processing or during transport?
- What are the inactivation kinetics of ASFV under various heat treatment conditions for different feed matrices?
- Current analytical methodologies have significant limitations in sensitivity, repeatability, ability to detect viable virus particles and association with infectivity. How can this be improved?[224]

Personal Protective Equipment (PPE)
What PPE is effective and who should be using it?

What do we know?

The guidance for PPE follows Occupational Safety and Health Administration (OSHA) Level C, which covers most non-zoonotic FAD events and includes NIOSH-approved respirators, chemical resistant clothing, inner and outer gloves, and boots.

- OSHA classifies PPE into four levels of protection. The levels range from D (lowest level of protection) to A (highest level). Most non-zoonotic FAD events, including ASFV, will require Level C protection for biosecurity, which provides standard contact and droplet precautions, which includes protection for the body, hand, foot, eye, face, head, hearing, and for the respiratory system, and should be chosen based on hazards. In most cases, protective equipment that can be cleaned and disinfected will be required. Respiratory protection will depend on the specific situation and environmental risks.[72]
- In addition to the zoonotic and biosecurity risks, other factors influence the selection of PPE that veterinary responders should wear, including:
  - Tasks that individuals must perform and the extent of physical work involved.
  - Physical conditions such as ambient temperature and relative humidity independent of the event.
  - Amount of time PPE must provide the necessary level of protection.
  - Classification of premises involved.[225]
- The Food and Agriculture Organization (FAO) recommends having the following items when working with ASFV on a farm: one pair of quality gumboots that are easy to clean and disinfect, disposable biosecurity suit, waterproof suit (in cold and wet countries), safety glasses, overshoes or boot covers, examination gloves, plastic mat, buckets (three), detergent, disinfectant (approved for ASFV), scrubbing brushes (two), refuse bags, biohazard bags), Ziplock bags, disinfectant wipes, and GPS device to record geocoordinates.[93]

PPE guidelines will differ for various tasks associated with ASFV. For instance, the protection required for surveillance and depopulation could differ from the protection required for chemical decontamination, or for working on an infected or uninfected area.[72, 226-227]

- Additional general (non-ASFV specific) information can be found in FAD PReP Standard Operating Procedures (SOP): Health and Safety/Personal Protective Equipment (PPE).[225]
- Though ASFV is not a threat to public health, responders may be exposed to other health hazards; prevention of adverse human health events related to emergency response efforts is extremely important. For general information, please see the National Animal Health Emergency Management System (NAHEMS) Guidelines: Health and Safety and NAHEMS Guidelines: Personal Protective Equipment. In an incident, refer any health and safety questions or concerns to the Safety Officer or other designated response official.[225]

What do we need to know?

- What types of PPE efficiently reduce viral spread during remediation efforts yet are permissive to the tasks at hand?

Depopulation and Carcass Disposal
What are the most effective methods of depopulation and disposal of infected carcasses?

What do we know?

Guidelines for the depopulation of animals set by the American Veterinary Medical Association must be followed when euthanizing swine. [228] There are several methods for effective depopulation of confirmed or suspected ASFV-infected swine in an agricultural setting, including inhalant gases, penetrating or non-penetrating captive bolt, electrocution head to heart, veterinarian-administered anesthetic overdose, and in certain permitted circumstances, gunshot, ventilation shutdown or sodium nitrate.[217] Gunshot and penetrating bolts are biosecurity risks due to the possibility of blood contamination, but may be the only effective depopulation method in certain locations.[228] [217]

- The method and procedures used for depopulation will depend on available resources and the population dynamics of susceptible animals on the premises, which requires location-specific planning and preparation that is addressed in the FAD PReP/NAHEMS Guidelines and SOP: Mass Depopulation and Euthanasia (2011).[225]
- Farmers are encouraged to comply with reporting suspected ASF cases to minimize losses that could occur if an outbreak occurred, with economic compensation tailored to underlying socioeconomic considerations.[108, 229]

Carcass disposal and environmental regulations differ by setting and state.[217]

- A comprehensive list of approved methods and regulatory considerations is available from the U.S. Veterinary Compliance Assistance Group.[230]
- The authority in charge of carcass disposal regulations may vary by circumstance. If the disposal of carcasses is necessary beyond what is legally allowed, the competent authority must be contacted to approve plans.[228]
- Composting of whole carcasses with a sustained windrow daily temperature ≥55°C inactivated infectious ASFV in samples from swine spleens within five days although viral DNA remains detectable for at least 28 days.[231] Horizontal grinders, typically used for vegetative debris, are being investigated as a way to increase the efficiency of composting carcasses on farms in less time.[232-233]

Tools and SOPs for depopulation exist.[234]

- USDA APHIS maintains a Carcass Management Dashboard, which contains information on disposal options for planning or response purposes.[217, 234]

- NAHEMS guidelines/SOPs includes detailed activities that are essential to prepare for and manage an FAD outbreak.[225]
- To minimize transmission to domestic pigs through infected wild boar carcasses, especially in areas where free-range farming is practiced, wild boar carcasses should be identified and properly disposed of as quickly as possible.[192, 235]

What do we need to know?

- Are there other depopulation and disposal methods that would limit environmental viral transmission?
- Are there options for depopulation, disposal, and decontamination that take into account swine numbers, body weight, housing, handling, local environmental, or soil conditions? Are current techniques effective in killing the virus and preventing spread to the environment?
- Are there alternative methods that could be easily performed in resource-limited countries/regions that would still be effective?

Swine Wastewater Treatment
Is on-farm wastewater a significant risk of transmission?

What do we know?

There is minimal information about ASFV persistence in farm wastewater systems, though transmission via contaminated water (e.g., during seasonal rains) is suspected in several African communities.[236]

- Studies have shown that highly pathogenic avian influenza RNA (another important agricultural pathogen) in wastewater can contaminate the water supply of nearby farms and households,[237] which is a potential route for ASFV, as organic material in wastewater can protect the virus from inactivation.[235] In fact, ASFV sequences were found in samples from a wastewater treatment plant in Spain.[238] ASFV was also detected in farming wastewater samples in China in late 2017 and early 2018, prior to initial ASFV reports.[239]
- Pig slurry can harbor ASFV for 84-112 days depending on temperature[240] and ASFV can survive in pig feces for 11 days at room temperature.[241]
- Contaminated water sources can harbor ASFV for 50-60 days, with colder temperatures facilitating persistence.[242]

Chemical disinfectants and heat are able to inactivate ASFV in pig slurry,[243] though effectiveness in wastewater systems needs to be determined.

- USDA APHIS provides guidance for decontamination in slurry, including heat (50°C for 24 hours or 60°C for 15 minutes) or 1% solutions of NaOH or $Ca(OH)_2$ (for 5 minutes at 4°C).[217]
- In 2023, the U.S. EPA and APHIS published a report on the feasibility of management of swine lagoons (waste lagoons containing solid and liquid manure, rainwater, and wastewater storage) following ASF outbreak. While there are potential options, most are not possible or practical, such as chemical disinfection of the entire lagoon, UV treatment or heat treatment of the entire lagoon, thermophilic anaerobic digestion or AD (only if the farm already has an AD system in place), aeration, or physical structural containment of the lagoon. Overall, there is still minimal information and research on this topic.[244]
- Other studies are investigating the efficacy of treating wastewater with NaOH or $Ca(OH)_2$ in reducing the virus.[245] Heat as a method to inactivate the virus is also being investigated, with temperatures of 56°C for 90 seconds being shown to inactivate the virus.[243] Finally,

decontamination of wastewater with ozonated water is also being investigated.[222]

What do we need to know?

- What are typical concentrations of ASFV in on-farm wastewater plants and can these values be used to conduct qualitative or quantitative risk assessment associated with the threat of wastewater plants in disease transmission and disease recovery?
- Does heat treatment of wastewater provide similar viral inactivation as heat treatment of pig slurry?
- What additional options are needed for effective treatment of wastewater to mitigate risk of viral spread?
- How long does ASFV persist in wastewater specifically, accounting for enhanced protein content compared to water?
- Do procedures such as aerobic thermophilic stabilization (i.e., liquid composting) or anaerobic digestion sufficiently inactivate ASFV in pig slurry?
- Are there ways to treat natural reservoirs near farms that may be a source of accumulating farm wastewater runoff that are not processed through wastewater treatment plants?

Genomics
How do genotypes and strains of the virus compare to each other?

What do we know?

ASFV is a large double-stranded DNA virus with a 170-180 kb genome,[246] and the sole member of its family (*Asfarviridae*).[183]

- ASFV is endemic to Sub-Saharan Africa and was first identified in Kenya in 1921.[70]

There are 24 known genotypes of ASFV, but only two (genotypes I and II) are outside the natural range in Africa.

- There are currently 24 genotypes of ASFV based on the sequences of the p72 gene, with all 24 genotypes being present in Africa over time with genotypes I and II found outside of Africa.[247]
- African outbreaks of ASFV are associated with a highly diverse pool of viruses of multiple genotypes, even within relatively restricted geographies.[248-255]
- Genotypes are regularly identified in regions where they were previously thought to be absent, suggesting that many genotypes may have distributions encompassing most of Sub-Saharan Africa.[248, 250-252, 254-255]
- Up until 2007, genotype I was the only ASFV strain to have spread beyond Africa. In 1957 genotype I was found in Lisbon, Portugal and subsequently spread.[247] A second outbreak in 1960 led to outbreaks into Spain, France, Belgium, The Netherlands, Italy, Malta, the islands of Cuba and Hispaniola, the Dominican Republic, Haiti, and Brazil.[247] In September 2023, a genotype II outbreak was identified in an area in Italy historically affected by genotype I.[256]
- In June of 2007, ASFV was found in the country of Georgia that matched genotype II found in Africa. Since then, genotype II has diversified and spread to other areas including Europe and Asia.[70, 247] In 2021, ASFV was detected in the Dominican Republic and spread throughout the country and to neighboring Haiti.[99]

The ASFV genome is variable in size and can include more than 150 distinct ORFs

encoding multiple proteins via the production of multiple transcripts from the same gene. The functions of some proteins are known, and several are involved in evasion of host defenses.[249, 257]

- ASFV evolves faster than other large DNA viruses; its evolutionary rate is closer to that of some RNA viruses[258] with recombination playing a major role.[249, 259] While the major capsid protein involved in cell entry does not appear to be under selective pressure,[260] at least one immune evasion protein appears to be a hotspot for recombination,[259] suggesting diversification in the proteins responsible for evading the host immune system.
- Genomic analysis of genotype II strains over time show high overall identity between type II strains in different regions.[261] In fact, the globally circulating strain maintains > 99.9% sequence identity with the strain that emerged from Africa around 2007.[250, 262-265] Despite the similarity, *in vivo* studies have shown variability in virulence,[266-269] suggesting more work is needed to understand the genomic markers related to virulence.[270-271]

Multiple genetic evaluations have shown that removal of specific genes reduces the virulence of the virus. However, changes and deletions of other genes allows the virus to still retain virulence. [150, 272]

- Studies with virulence-associated genes of ASFV genotype II strains have shown the ability to attenuate the virus and provide protection to swine from homologous (same genotype) strains, providing an available approach for vaccine development.[273-275]
- Some studies indicated that deletion of target genes resulted in less dangerous ASFV strains. Of note: deletion of EP153R, EP402R and MGF_360-12L-14L genes caused a decrease in pathogenicity, deletion of 11 genes within the MGF300 and MGF360 regions showed reduction in virulence compared to the parent strain, deletion of the H240R gene decreases infectious titer, deletion of the H108R gene resulted in a less virulent ASFV, and deletion of the E184L and DP148R genes resulted in reduced virulence and immune protection compared to non-deletion strains.[276-279.]

What do we need to know?

- What is the true diversity of ASFV in Africa, and are there meaningful biological differences between genotypes that may have operational consequences?
- What is the probability of spread of other ASFV genotypes from Africa and how does evolution affect that probability?
- Are there any differences in sequence between viruses isolated from the main tick vector *Orinthodoros* and a similar genotype isolated from pigs or hogs?
- What are the range of host cell (swine macrophage) receptors?
- What are the functional genomics of ASFV proteins and how do they impact infection and disease presentation?

Commonly Used Acronyms and Abbreviations

Acronym/Term	Definition	Description
APHIS	Animal and Plant Health Inspection Service	N/A
ASF	African Swine Fever	Disease caused by ASFV
ASFV	African Swine Fever Virus	Causative agent of the disease African Swine Fever (ASF)
CBP	Customs and Border Protection	N/A
CSF	Classical Swine Fever	Disease caused by Classical Swine Fever Virus; virus is highly contagious in swine
CSIRO	Commonwealth Scientific and Industrial Research Organisation	N/A
DHS S&T	U.S. Department of Homeland Security Science and Technology Directorate	N/A
DIVA	Differentiation of ASFV-Infected from Vaccinated Animals	N/A
DNA	Deoxyribonucleic Acid	N/A
ELISA	Enzyme-Linked Immunosorbent Assay	Assay used to detect the presence of antibodies to a specific protein
EPA	U.S. Environmental Protection Agency	N/A
FAD	Foreign Animal Disease	N/A
FAD PReP	Foreign Animal Disease Preparedness & Response Plan	N/A
FAO	Food and Agriculture Organization	N/A
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act	N/A
GARA	Global African Swine Fever Research Alliance	N/A
GPS	Global Positioning System	N/A
HAD_{50}	Median Hemadsorbing Dose	Dose necessary to result in hemadsorption in 50% of cultured cells; used as a standard measure of viral particles (e.g., it required 10^3 HAD_{50} to produce clinical signs in exposed pigs)
HAU	Hemadsorbing Unit	Reaction occurs in erythrocytes in the presence of hemagglutinin, which is used to quantify hemagglutinin-producing viruses
ID_{50}	Median Infectious Dose	Dose required to cause an infection in 50% of subjects
IZ	Infection Zone	N/A
LAV	Live Attenuated Vaccine	N/A
LD_{50}	Median Lethal Dose	Dose required to cause a lethal effect in 50% of subjects

Acronym/Term	Definition	Description
MQL	Master Question List	N/A
MVAV	Modified Vaccinia Ankara Virus	Attenuated strain of vaccinia virus that is used as a vaccine against smallpox and monkeypox
NAHEMS	National Animal Health Emergency Management System	N/A
NIOSH	National Institute for Occupational Safety and Health	N/A
ORF	Open Reading Frame	Stretches of DNA between a start and stop codon that may contain genes encoding proteins
OSHA	Occupational Safety and Health Administration	N/A
PCR	Polymerase Chain Reaction	Assay used to amplify RNA or DNA molecules representing a specific sequence target that are present in a sample
PPE	Personal Protective Equipment	Equipment intended to protect individuals against hazardous environments
QDM	Quantum Dot Microsphere	N/A
qPCR	Quantitative Polymerase Chain Reaction	Assay used to determine the number of RNA or DNA molecules representing a specific sequence target that are present in a sample
R_0	Basic Reproductive Number	Average number of new infections that each case is expected to generate in a population where all individuals are susceptible to infection
RNA	Ribonucleic Acid	N/A
SOP	Standard Operating Procedure	N/A
$TCID_{50}$	Median Tissue Culture Infectious Dose	Dose necessary to infect 50% of tissue cells.; used as a standard measure of infectivity (e.g., it required 10^3 $TCID_{50}$ to produce clinical signs in exposed pigs)
USDA	United States Department of Agriculture	N/A
UV	Ultraviolet	Light with wavelength in the 100-400 nm range
VISVET	Centro de Vigilancia Sanitaria Veterinaria	N/A
WOAH or OIE	World Organisation for Animal Health	N/A

References

1. de Leon, P., et al., Laboratory methods to study African swine fever virus. *Virus Res* **2013,** *173* (1), 168-79. https://www.ncbi.nlm.nih.gov/pubmed/23041357

2. WOAH, African Swine Fever (Infection with African Swine Fever Virus). https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.09.01_ASF.pdf (accessed 01 March 2023).

3. Pan, I. C.; Hess, W. R., Virulence in African swine fever: its measurement and implications. *Am J Vet Res* **1984,** *45* (2), 361-6. https://www.ncbi.nlm.nih.gov/pubmed/6711963

4. Niederwerder, M. C., et al., Infectious Dose of African Swine Fever Virus When Consumed Naturally in Liquid or Feed. *Emerg Infect Dis* **2019,** *25* (5), 891-897. https://www.ncbi.nlm.nih.gov/pubmed/30761988

5. Blazquez, E., et al., Commercial feed containing porcine plasma spiked with African swine fever virus is not infective in pigs when administered for 14 consecutive days. *PLoS One* **2020,** *15* (7), e0235895. https://www.ncbi.nlm.nih.gov/pubmed/32697784

6. Guinat, C., et al., Dynamics of African swine fever virus shedding and excretion in domestic pigs infected by intramuscular inoculation and contact transmission. *Vet Res* **2014,** *45* (1), 93. https://www.ncbi.nlm.nih.gov/pubmed/25256695

7. Walczak, M., et al., Analysis of the Clinical Course of Experimental Infection with Highly Pathogenic African Swine Fever Strain, Isolated from an Outbreak in Poland. Aspects Related to the Disease Suspicion at the Farm Level. *Pathogens* **2020,** *9* (3). https://www.ncbi.nlm.nih.gov/pubmed/32235758

8. Walczak, M., et al., Blood Counts, Biochemical Parameters, Inflammatory, and Immune Responses in Pigs Infected Experimentally with the African Swine Fever Virus Isolate Pol18_28298_O111. *Viruses* **2021,** *13* (3). https://www.ncbi.nlm.nih.gov/pubmed/33810057

9. Yamada, M., et al., Experimental infection of pigs with different doses of the African swine fever virus Armenia 07 strain by intramuscular injection and direct contact. *J Vet Med Sci* **2021,** *82* (12), 1835-1845. https://www.ncbi.nlm.nih.gov/pubmed/33162428

10. Lv, C., et al., Infection Characteristics and Transcriptomics of African Swine Fever Virus in Bama Minipigs. *Microbiol Spectr* **2022,** *10* (6), e0383422. https://www.ncbi.nlm.nih.gov/pubmed/36445132

11. Pietschmann, J., et al., Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. *Arch Virol* **2015,** *160* (7), 1657-67. https://www.ncbi.nlm.nih.gov/pubmed/25916610

12. de Carvalho Ferreira, H. C., et al., African swine fever virus excretion patterns in persistently infected animals: a quantitative approach. *Vet Microbiol* **2012,** *160* (3-4), 327-40. https://www.ncbi.nlm.nih.gov/pubmed/22795261

13. Gabriel, C., et al., Characterization of African swine fever virus Caucasus isolate in European wild boars. *Emerg Infect Dis* **2011,** *17* (12), 2342-5. https://www.ncbi.nlm.nih.gov/pubmed/22172247

14. Blome, S., et al., High virulence of African swine fever virus caucasus isolate in European wild boars of all ages. *Emerg Infect Dis* **2012,** *18* (4), 708. https://www.ncbi.nlm.nih.gov/pubmed/22469497

15. EFSA Panel on Animal Health Welfare, Scientific Opinion on African swine fever. *EFSA Journal* **2014,** *12* (4), 3628. https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2014.3628

16. Oh, S. I., et al., Pathobiological analysis of african swine fever virus contact-exposed pigs and estimation of the basic reproduction number of the virus in Vietnam. *Porcine Health Manag* **2023,** *9* (1), 30. https://www.ncbi.nlm.nih.gov/pubmed/37386526

17. Guinat, C., et al., Experimental pig-to-pig transmission dynamics for African swine fever virus, Georgia 2007/1 strain. *Epidemiol Infect* **2016,** *144* (1), 25-34. https://www.ncbi.nlm.nih.gov/pubmed/25989921

18. Gervasi, V.; Guberti, V., African swine fever endemic persistence in wild boar populations: Key mechanisms explored through modelling. *Transbound Emerg Dis* **2021,** *68* (5), 2812-2825. https://www.ncbi.nlm.nih.gov/pubmed/34255414

19. Lange, M., et al., Modelling advanced knowledge of African swine fever, resulting surveillance patterns at the population level and impact on reliable exit strategy definition. *EFSA Supporting Publications* **2021,** *18* (3), 6429E.

20. Guinat, C., et al., Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions. *Vet Rec* **2016,** *178* (11), 262-7. https://www.ncbi.nlm.nih.gov/pubmed/26966305

21. Niederwerder, M. C., et al., Stability of African swine fever virus in feed during environmental storage. *Transbound Emerg Dis* **2022,** *69* (6), 3216-3224. https://www.ncbi.nlm.nih.gov/pubmed/35881701

22. Galvis, J. A.; Machado, G., The role of vehicle movement in swine disease dissemination: Novel method accounting for pathogen stability and vehicle cleaning effectiveness uncertainties. *Prev Vet Med* **2024,** *226*, 106168. https://www.ncbi.nlm.nih.gov/pubmed/38507888

23. Li, X., et al., Evidence of aerosol transmission of African swine fever virus between two piggeries under field conditions: a case study. *Front Vet Sci* **2023,** *10*, 1201503. https://www.ncbi.nlm.nih.gov/pubmed/37323846

24. Yoo, D. S., et al., Transmission Dynamics of African Swine Fever Virus, South Korea, 2019. *Emerg Infect Dis* **2021,** *27* (7), 1909-1918. https://www.ncbi.nlm.nih.gov/pubmed/34152953

25. Elijah, C. G., et al., Evaluating the distribution of African swine fever virus within a feed mill environment following manufacture of inoculated feed. *PLoS One* **2021,** *16* (8), e0256138. https://www.ncbi.nlm.nih.gov/pubmed/34383843

26. Nuanualsuwan, S., et al., Persistence of African swine fever virus on porous and non-porous fomites at environmental temperatures. *Porcine Health Manag* **2022,** *8* (1), 34. https://www.ncbi.nlm.nih.gov/pubmed/35902922

27. Olesen, A. S., et al., Short time window for transmissibility of African swine fever virus from a contaminated environment. *Transbound Emerg Dis* **2018,** *65* (4), 1024-1032. https://www.ncbi.nlm.nih.gov/pubmed/29457702

28. Lohse, L., et al., Experimental Infections of Pigs with African Swine Fever Virus (Genotype II); Studies in Young Animals and Pregnant Sows. *Viruses* **2022,** *14* (7). https://www.ncbi.nlm.nih.gov/pubmed/35891368

29. Friedrichs, V., et al., Artificial Insemination as an Alternative Transmission Route for African Swine Fever Virus. *Pathogens* **2022,** *11* (12). https://www.ncbi.nlm.nih.gov/pubmed/36558873

30. Roszyk, H., et al., The Role of Male Reproductive Organs in the Transmission of African Swine Fever. *Viruses* **2022,** *14* (1), 31. https://www.mdpi.com/1999-4915/14/1/31

31. Pietschmann, J., et al., African swine fever virus transmission cycles in Central Europe: Evaluation of wild boar-soft tick contacts through detection of antibodies against Ornithodoros erraticus saliva antigen. *BMC Vet Res* **2016,** *12*, 1. https://www.ncbi.nlm.nih.gov/pubmed/26728767

32. Penrith, M. L., African swine fever. *Onderstepoort J Vet Res* **2009,** *76* (1), 91-5. https://www.ncbi.nlm.nih.gov/pubmed/19967933

33. Kleiboeker, S. B.; Scoles, G. A., Pathogenesis of African swine fever virus in Ornithodoros ticks. *Anim Health Res Rev* **2001,** *2* (2), 121-8. https://www.ncbi.nlm.nih.gov/pubmed/11831434

34. Saegerman, C., et al., An expert opinion assessment of blood-feeding arthropods based on their capacity to transmit African swine fever virus in Metropolitan France. *Transbound Emerg Dis* **2021,** *68* (3), 1190-1204. https://www.ncbi.nlm.nih.gov/pubmed/32750188

35. Sánchez-Vizcaíno, J. M., Early detection and contingency plans for African swine fever. https://www.woah.org/fileadmin/Home/eng/Publications_&_Documentation/docs/pdf/TT/2010_139-147_SanchezVizcaino_A.pdf.

36. Plowright, W., et al., Experimental infection of the argasid tick, Ornithodoros moubata porcinus, with African swine fever virus. *Arch Gesamte Virusforsch* **1970,** *31* (1), 33-50. https://www.ncbi.nlm.nih.gov/pubmed/5475061

37. Kayaga, E. B., et al., Detection of antibodies against Ornithodoros moubata salivary antigens and their association with detection of African swine fever virus in pigs slaughtered in central Uganda. *Front Vet Sci* **2024,** *11*, 1328040. https://www.ncbi.nlm.nih.gov/pubmed/38605921

38. Obanda, V., et al., Epidemiology and ecology of the sylvatic cycle of African Swine Fever Virus in Kenya. *Virus Res* **2024,** *348*, 199434. https://www.ncbi.nlm.nih.gov/pubmed/39004284

39. Olesen, A. S., et al., Survival and localization of African swine fever virus in stable flies (Stomoxys calcitrans) after feeding on viremic blood using a membrane feeder. *Vet Microbiol* **2018,** *222*, 25-29. https://www.ncbi.nlm.nih.gov/pubmed/30080669

40. Vergne, T., et al., Mechanical transmission of African swine fever virus by Stomoxys calcitrans: Insights from a mechanistic model. *Transbound Emerg Dis* **2021,** *68* (3), 1541-1549. https://www.ncbi.nlm.nih.gov/pubmed/32910533

41. Olesen, A. S., et al., Detection of African Swine Fever Virus and Blood Meals of Porcine Origin in Hematophagous Insects Collected Adjacent to a High-Biosecurity Pig Farm in Lithuania; A Smoking Gun? *Viruses* **2023,** *15* (6). https://www.ncbi.nlm.nih.gov/pubmed/37376554

42. Balmos, O. M., et al., African swine fever virus DNA is present in non-biting flies collected from outbreak farms in Romania. *Parasit Vectors* **2024,** *17* (1), 278. https://www.ncbi.nlm.nih.gov/pubmed/38943218

43. Frant, M., et al., African Swine Fever (ASF) and Ticks. No Risk of Tick-mediated ASF Spread in Poland and Baltic States. *J Vet Res* **2017,** *61* (4), 375-380. https://www.ncbi.nlm.nih.gov/pubmed/29978098

44. Afe, A. E., et al., African Swine Fever Virus Interaction with Host Innate Immune Factors. *Viruses* **2023,** *15* (6). https://www.ncbi.nlm.nih.gov/pubmed/37376520

45. Herm, R., et al., No evidence for African swine fever virus DNA in haematophagous arthropods collected at wild boar baiting sites in Estonia. *Transbound Emerg Dis* **2021,** *68* (5), 2696-2702. https://www.ncbi.nlm.nih.gov/pubmed/33527715

46. Qin, W., et al., Preliminary analysis of whether mosquitoes can carry and transmit African swine fever. *BMC Vet Res* **2021,** *17* (1), 152. https://www.ncbi.nlm.nih.gov/pubmed/33836768

47. Arzt, J., et al., Quantitative impacts of incubation phase transmission of foot-and-mouth disease virus. *Sci Rep* **2019,** *9* (1), 2707. https://www.ncbi.nlm.nih.gov/pubmed/30804426

48. de Carvalho Ferreira, H. C., et al., Transmission rate of African swine fever virus under experimental conditions. *Vet Microbiol* **2013,** *165* (3-4), 296-304. https://www.ncbi.nlm.nih.gov/pubmed/23664069

49. Lim, J. S., et al., Basic reproduction number of African swine fever in wild boars (Sus scrofa) and its spatiotemporal heterogeneity in South Korea. *J Vet Sci* **2021,** *22* (5), e71. https://www.ncbi.nlm.nih.gov/pubmed/34553516

50. Main, A. R., et al., Estimating transmission dynamics of African swine fever virus from experimental studies. *Transbound Emerg Dis* **2022,** *69* (6), 3858-3867. https://www.ncbi.nlm.nih.gov/pubmed/36346271

51. Gao, Y., et al., Estimating the impact of low temperature on African swine fever virus transmission through contaminated environments. *Prev Vet Med* **2023,** *219*, 105991. https://www.ncbi.nlm.nih.gov/pubmed/37678000

52. Olesen, A. S., et al., Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes. *Vet Microbiol* **2017,** *211*, 92-102. https://www.ncbi.nlm.nih.gov/pubmed/29102127

53. O'Neill, X., et al., Modelling the transmission and persistence of African swine fever in wild boar in contrasting European scenarios. *Sci Rep* **2020,** *10* (1), 5895. https://www.ncbi.nlm.nih.gov/pubmed/32246098

54. Gallardo, C., et al., Experimental Transmission of African Swine Fever (ASF) Low Virulent Isolate NH/P68 by Surviving Pigs. *Transbound Emerg Dis* **2015,** *62* (6), 612-22. https://www.ncbi.nlm.nih.gov/pubmed/26432068

55. Martinez Aviles, M., et al., Epidemiological impacts of attenuated African swine fever virus circulating in wild boar populations. *Res Vet Sci* **2023,** *162*, 104964. https://www.ncbi.nlm.nih.gov/pubmed/37531717

56. Dankwa, E. A., et al., Stochastic modelling of African swine fever in wild boar and domestic pigs: Epidemic forecasting and comparison of disease management strategies. *Epidemics* **2022,** *40*, 100622. https://www.ncbi.nlm.nih.gov/pubmed/36041286

57. Pepin, K. M., et al., Risk of African swine fever virus transmission among wild boar and domestic pigs in Poland. *Front Vet Sci* **2023,** *10*, 1295127. https://www.ncbi.nlm.nih.gov/pubmed/38026636

58. Calo, S., et al., Evaluation of the Risk of African Swine Fever Virus Transmission at the Interface between Feral and Domestic Pigs in Lombardy, with a View to Establishing Preventive Measures for Domestic Pigs. *Pathogens* **2023,** *12* (12). https://www.ncbi.nlm.nih.gov/pubmed/38133345

59. Akhmetzhanov, A. R., et al., Reconstruction and analysis of the transmission network of African swine fever in People's Republic of China, August 2018-September 2019. *Epidemiol Infect* **2024,** *152*, e27. https://www.ncbi.nlm.nih.gov/pubmed/38282573

60. Carlos, G. C., et al., Assessing the epidemiological risk at the human-wild boar interface through a one health approach using an agent-based model in Barcelona, Spain. *One Health* **2023,** *17*, 100598. https://www.ncbi.nlm.nih.gov/pubmed/37520846

61. Goicolea, T., et al., Landscape connectivity for predicting the spread of ASF in the European wild boar population. *Sci Rep* **2024,** *14* (1), 3414. https://www.ncbi.nlm.nih.gov/pubmed/38341478

62. Mai, T. N., et al., Dynamic Models of Within-Herd Transmission and Recommendation for Vaccination Coverage Requirement in the Case of African Swine Fever in Vietnam. *Vet Sci* **2022,** *9* (6). https://www.ncbi.nlm.nih.gov/pubmed/35737344

63. Mai, T. N., et al., Estimation of the herd-level basic reproduction number for African swine fever in Vietnam, 2019. *Vet World* **2022,** *15* (12), 2850-2855. https://www.ncbi.nlm.nih.gov/pubmed/36718319

64. Matsumoto, N., et al., Novel estimation of African swine fever transmission parameters within smallholder villages in Lao P.D.R. *Trop Anim Health Prod* **2024,** *56* (5), 166. https://www.ncbi.nlm.nih.gov/pubmed/38758410

65. Lee, H. S., et al., A stochastic simulation model of African swine fever transmission in domestic pig farms in the Red River Delta region in Vietnam. *Transbound Emerg Dis* **2021,** *68* (3), 1384-1391. https://www.ncbi.nlm.nih.gov/pubmed/32810366

66. European Food Safety, A., et al., Research gap analysis on African swine fever. *EFSA J* **2019,** *17* (8), e05811. https://www.ncbi.nlm.nih.gov/pubmed/32626417

67. Yoon, H., et al., Arthropods as potential vectors of African swine fever virus outbreaks in pig farms in the Republic of Korea. *Vet Med Sci* **2021,** *7* (5), 1841-1844. https://www.ncbi.nlm.nih.gov/pubmed/34085400

68. Probst, C., et al., The potential role of scavengers in spreading African swine fever among wild boar. *Sci Rep* **2019,** *9* (1), 11450. https://www.ncbi.nlm.nih.gov/pubmed/31391480

69. Netherton, C. L., et al., The Genetics of Life and Death: Virus-Host Interactions Underpinning Resistance to African Swine Fever, a Viral Hemorrhagic Disease. *Front Genet* **2019,** *10* (402), 402. https://www.ncbi.nlm.nih.gov/pubmed/31130984

70. Gaudreault, N. N., et al., African Swine Fever Virus: An Emerging DNA Arbovirus. *Front Vet Sci* **2020,** *7* (215), 215. https://www.ncbi.nlm.nih.gov/pubmed/32478103

71. Ewers, R. M., et al., African swine fever ravaging Borneo's wild pigs. *Nature* **2021,** *593* (7857), 37. https://www.ncbi.nlm.nih.gov/pubmed/33947991

72. United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS), *African Swine Fever Response Plan: The Red Book*; USDA APHIS VS: July 2023, 2023; pp 1-92. https://www.aphis.usda.gov/animal_health/emergency_management/downloads/asf-responseplan.pdf

73. Dardiri, A. H., et al., Clinical and serologic response of American white-collared peccaries to African swine fever, foot-and-mouth disease, vesicular stomatitis, vesicular exanthema of Swine, hog cholera, and rinderpest viruses. *Proc Annu Meet U S Anim Health Assoc* **1969,** *73*, 437-52. https://www.ncbi.nlm.nih.gov/pubmed/4322827

74. Salguero, F. J., Comparative Pathology and Pathogenesis of African Swine Fever Infection in Swine. *Front Vet Sci* **2020,** *7*, 282. https://www.ncbi.nlm.nih.gov/pubmed/32509811

75. Kim, H. J., et al., Humoral immune response of pigs, Sus scrofa domesticus, upon repeated exposure to blood-feeding by Ornithodoros turicata Duges (Ixodida: Argasidae). *Parasit Vectors* **2020,** *13* (1), 66. https://www.ncbi.nlm.nih.gov/pubmed/32051021

76. Golnar, A. J., et al., Reviewing the Potential Vectors and Hosts of African Swine Fever Virus Transmission in the United States. *Vector Borne Zoonotic Dis* **2019,** *19* (7), 512-524. https://www.ncbi.nlm.nih.gov/pubmed/30785371

77. Tulman, E. R.; Rock, D. L., Novel virulence and host range genes of African swine fever virus. *Curr Opin Microbiol* **2001,** *4* (4), 456-61. https://www.ncbi.nlm.nih.gov/pubmed/11495811

78. Szewczyk, M., et al., Evaluation of the Presence of ASFV in Wolf Feces Collected from Areas in Poland with ASFV Persistence. *Viruses* **2021,** *13* (10). https://www.ncbi.nlm.nih.gov/pubmed/34696492

79. Galindo, I.; Alonso, C., African Swine Fever Virus: A Review. *Viruses* **2017,** *9* (5), 103. https://www.ncbi.nlm.nih.gov/pubmed/28489063

80. Mays Maestas, S. E., et al., Pathogen and Host Associations of Soft Ticks Collected in South Texas. *Vector Borne Zoonotic Dis* **2025,** *25* (1), 34-42. https://www.ncbi.nlm.nih.gov/pubmed/39133644

81. Hess, W. R., et al., Potential arthropod vectors of African swine fever virus in North America and the Caribbean basin. *Vet Parasitol* **1987,** *26* (1-2), 145-55. https://www.ncbi.nlm.nih.gov/pubmed/3326244

82. Sames, W. J.; Teel, P. D., Ornithodoros turicata (Duges) on a Feral Hog in Real County, Texas, USA. *J Wildl Dis* **2022,** *58* (1), 238-240. https://www.ncbi.nlm.nih.gov/pubmed/34695206

83. Gabbatt, A., Incredibly intelligent, highly elusive: US faces new threat from Canadian 'super pig'. https://www.theguardian.com/us-news/2023/feb/20/us-threat-canada-super-pig-boar (accessed 01 March 2023).

84. Rousselle, C., Wild 'super pigs' in Canada poised to wreak environmental havoc, spread disease in US, expert says. https://www.foxnews.com/lifestyle/wild-super-pigs-canada-poised-wreak-environmental-havoc-spread-disease-us-expert (accessed 01 March 2023).

85. de Carvalho Ferreira, H. C., et al., No evidence of African swine fever virus replication in hard ticks. *Ticks Tick Borne Dis* **2014,** *5* (5), 582-9. https://www.ncbi.nlm.nih.gov/pubmed/24980962

86. Olesen, A. S., et al., Infection of pigs with African swine fever virus via ingestion of stable flies (Stomoxys calcitrans). *Transbound Emerg Dis* **2018,** *65* (5), 1152-1157. https://www.ncbi.nlm.nih.gov/pubmed/29877056

87. Balmoș, O. M., et al., African Swine Fever Virus Load in Hematophagous Dipterans Collected in Outbreaks from Romania: Risk Factors and Implications. *Transboundary and Emerging Diseases* **2023,** *2023*, 1-9. https://doi.org/10.1155/2023/3548109

88. Avagyan, H. R., et al., The pattern of stability of African swine fever virus in leeches. *Vet Microbiol* **2023,** *284*, 109835. https://www.ncbi.nlm.nih.gov/pubmed/37515978

89. Karalyan, Z., et al., Presence and survival of African swine fever virus in leeches. *Vet Microbiol* **2019,** *237*, 108421. https://www.ncbi.nlm.nih.gov/pubmed/31585648

90. Poghosyan, A., et al., The role of gastropods in African swine fever virus ecology. *Virol J* **2024,** *21* (1), 180. https://www.ncbi.nlm.nih.gov/pubmed/39113041

91. Mellor, P. S., et al., Mechanical transmission of capripox virus and African swine fever virus by Stomoxys calcitrans. *Res Vet Sci* **1987,** *43* (1), 109-12. https://www.ncbi.nlm.nih.gov/pubmed/2820006

92. Spickler, A. R., African Swine Fever. http://www.cfsph.iastate.edu/Factsheets/pdfs/african_swine_fever.pdf (accessed 02 Nov 2020).

93. Beltran-Alcrudo, D., et al., *African Swine Fever: Detection and Diagnosis - A manual for veterinarians*; 2017; pp 1-88.

94. Malladi, S., et al., Predicting the time to detect moderately virulent African swine fever virus in finisher swine herds using a stochastic disease transmission model. *BMC Vet Res* **2022,** *18* (1), 84. https://www.ncbi.nlm.nih.gov/pubmed/35236347

95. C, G., et al., Evolution in Europe of African swine fever genotype II viruses from highly to moderately virulent. *Vet Microbiol* **2018,** *219*, 70-79. https://www.ncbi.nlm.nih.gov/pubmed/29778207

96. Howey, E. B., et al., Pathogenesis of highly virulent African swine fever virus in domestic pigs exposed via intraoropharyngeal, intranasopharyngeal, and intramuscular inoculation, and by direct contact with infected pigs. *Virus Res* **2013,** *178* (2), 328-39. https://www.ncbi.nlm.nih.gov/pubmed/24076499

97. Oh, S. I., et al., Dynamic Variations in Infrared Skin Temperature of Weaned Pigs Experimentally Inoculated with the African Swine Fever Virus: A Pilot Study. *Vet Sci* **2021,** *8* (10). https://www.ncbi.nlm.nih.gov/pubmed/34679053

98. Goonewardene, K. B., et al., Superficial Inguinal Lymph Nodes for Screening Dead Pigs for African Swine Fever. *Viruses* **2022,** *14* (1), 83. https://www.ncbi.nlm.nih.gov/pubmed/35062287

99. Ramirez-Medina, E., et al., Experimental Infection of Domestic Pigs with an African Swine Fever Virus Field Strain Isolated in 2021 from the Dominican Republic. *Viruses* **2022,** *14* (5). https://www.ncbi.nlm.nih.gov/pubmed/35632831

100. Pershin, A., et al., A Long-Term Study of the Biological Properties of ASF Virus Isolates Originating from Various Regions of the Russian Federation in 2013-2018. *Vet Sci* **2019,** *6* (4). https://www.ncbi.nlm.nih.gov/pubmed/31817809

101. Lee, H. S., et al., Pathogenicity of an African swine fever virus strain isolated in Vietnam and alternative diagnostic specimens for early detection of viral infection. *Porcine Health Manag* **2021,** *7* (1), 36. https://www.ncbi.nlm.nih.gov/pubmed/33934707

102. Sanchez-Cordon, P. J., et al., African Swine Fever: Disease Dynamics in Wild Boar Experimentally Infected with ASFV Isolates Belonging to Genotype I and II. *Viruses* **2019,** *11* (9). https://www.ncbi.nlm.nih.gov/pubmed/31540341

103. Sanchez-Cordon, P. J., et al., Comparative evaluation of disease dynamics in wild boar and domestic pigs experimentally inoculated intranasally with the European highly virulent African swine fever virus genotype II strain "Armenia 2007". *Vet Res* **2024,** *55* (1), 89. https://www.ncbi.nlm.nih.gov/pubmed/39010163

104. Sanchez-Cordon, P. J., et al., Evaluation of Lesions and Viral Antigen Distribution in Domestic Pigs Inoculated Intranasally with African Swine Fever Virus Ken05/Tk1 (Genotype X). *Pathogens* **2021,** *10* (6). https://www.ncbi.nlm.nih.gov/pubmed/34207265

105. Blome, S., et al., African swine fever - A review of current knowledge. *Virus Res* **2020,** *287*, 198099. https://www.ncbi.nlm.nih.gov/pubmed/32755631

106. Li, Z., et al., African Swine Fever Virus: A Review. *Life (Basel)* **2022,** *12* (8). https://www.ncbi.nlm.nih.gov/pubmed/36013434

107. Oh, T., et al., Age-related viral load and severity of systemic pathological lesions in acute naturally occurring African swine fever virus genotype II infections. *Comp Immunol Microbiol Infect Dis* **2021,** *79*, 101709. https://www.ncbi.nlm.nih.gov/pubmed/34543808

108. Dixon, L. K., et al., African Swine Fever Epidemiology and Control. *Annu Rev Anim Biosci* **2020,** *8*, 221-246. https://www.ncbi.nlm.nih.gov/pubmed/31743062

109. Oura, C., African Swine Fever. https://www.merckvetmanual.com/generalized-conditions/african-swine-fever/african-swine-fever (accessed 01 March 2023).

110. World Organisation for Animal Health (OIE), African Swine Fever Technical Disease Card. https://www.oie.int/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/Disease_cards/AFRICAN_SWINE_FEVER.pdf (accessed 01 March 2023).

111. Pegu, S. R., et al., Clinicopathological and ultrastructural study of African swine fever outbreak in North-East India. *Microb Pathog* **2023,** *185*, 106452. https://www.ncbi.nlm.nih.gov/pubmed/37972743

112. Hershey, B. J., et al., Clinical Indicators of Moribundity in Swine Experimentally Inoculated with African Swine Fever Virus. *J Am Assoc Lab Anim Sci* **2021,** *60* (1), 96-102. https://www.ncbi.nlm.nih.gov/pubmed/33198851

113. Ardelean, F., et al., The course of African swine fever in Romanian backyard holdings - A case report. *Vet Med Sci* **2021,** *7* (6), 2273-2279. https://www.ncbi.nlm.nih.gov/pubmed/34378334

114. Gallardo, C., et al., Dynamics of African swine fever virus (ASFV) infection in domestic pigs infected with virulent, moderate virulent and attenuated genotype II ASFV European isolates. *Transbound Emerg Dis* **2021,** *68* (5), 2826-2841. https://www.ncbi.nlm.nih.gov/pubmed/34273247

115. Sun, E., et al., Genotype I African swine fever viruses emerged in domestic pigs in China and caused chronic infection. *Emerg Microbes Infect* **2021,** *10* (1), 2183-2193. https://www.ncbi.nlm.nih.gov/pubmed/34709128

116. Zhang, J., et al., Fecal microbiota transplantation from warthog to pig confirms the influence of the gut microbiota on African swine fever susceptibility. *Sci Rep* **2020,** *10* (1), 17605. https://www.ncbi.nlm.nih.gov/pubmed/33077775

117. United States Department of Agriculture (USDA), *Swine Hemorrhagic Fevers: African and Classical Swine Fever Integrated Surveillance Plan*; 2022; pp 1-20. https://www.aphis.usda.gov/animal_health/downloads/animal_diseases/swine/hemorrhagic-fevers-integrated-surveillance-plan.pdf

118. United States Department of Agriculture (USDA), *Swine Hemorrhagic Fevers: African and Classical Swine Fever Integrated Surveillance Plan*; 2019; pp 1-20. https://www.aphis.usda.gov/animal_health/downloads/animal_diseases/swine/hemorrhagic-fevers-integrated-surveillance-plan.pdf

119. Global African Swine Fever Research Alliance (GARA), *Global African Swine Fever Research Alliance (GARA) Gap Analysis Report*; 2018; pp 1-110. https://go.usa.gov/xPfWr

120. Walczak, M., et al., Non-Invasive Sampling in the Aspect of African Swine Fever Detection-A Risk to Accurate Diagnosis. *Viruses* **2022,** *14* (8). https://www.ncbi.nlm.nih.gov/pubmed/36016380

121. Borca, M. V., et al., Deletion of CD2-like gene from the genome of African swine fever virus strain Georgia does not attenuate virulence in swine. *Sci Rep* **2020,** *10* (1), 494. https://www.ncbi.nlm.nih.gov/pubmed/31949276

122. Daigle, J., et al., Rapid and highly sensitive portable detection of African swine fever virus. *Transbound Emerg Dis* **2021,** *68* (2), 952-959. https://www.ncbi.nlm.nih.gov/pubmed/32762007

123. Yang, Y., et al., Development of a chemiluminescence immunoassay to accurately detect African swine fever virus antibodies in serum. *J Virol Methods* **2021,** *298*, 114269. https://www.ncbi.nlm.nih.gov/pubmed/34454001

124. Li, J., et al., Rapid and ultra-sensitive detection of African swine fever virus antibody on site using QDM based-ASFV immunosensor (QAIS). *Anal Chim Acta* **2022,** *1189*, 339187. https://www.ncbi.nlm.nih.gov/pubmed/34815032

125. Inui, K., et al., *The OIE ASF Reference Laboratory Network's overview of African swine fever diagnostic tests for field application*. 2022.

126. Auer, A., et al., Comparison of the sensitivity, specificity, correlation and inter-assay agreement of eight diagnostic in vitro assays for the detection of African swine fever virus. *Transbound Emerg Dis* **2022,** *69* (5), e3231-e3238. https://www.ncbi.nlm.nih.gov/pubmed/35189029

127. Havas, K. A., et al., An Assessment of Diagnostic Assays and Sample Types in the Detection of an Attenuated Genotype 5 African Swine Fever Virus in European Pigs over a 3-Month Period. *Pathogens* **2022,** *11* (4). https://www.ncbi.nlm.nih.gov/pubmed/35456079

128. Schambow, R., et al., Modeling the accuracy of a novel PCR and antibody ELISA for African swine fever virus detection using Bayesian latent class analysis. *Front Vet Sci* **2023,** *10*, 1079918. https://www.ncbi.nlm.nih.gov/pubmed/36908521

129. Onyilagha, C., et al., Meat Exudate for Detection of African Swine Fever Virus Genomic Material and Anti-ASFV Antibodies. *Viruses* **2021,** *13* (9). https://www.ncbi.nlm.nih.gov/pubmed/34578325

130. Mulumba-Mfumu, L. K., et al., African swine fever: Update on Eastern, Central and Southern Africa. *Transbound Emerg Dis* **2019,** *66* (4), 1462-1480. https://www.ncbi.nlm.nih.gov/pubmed/30920725

131. Brown, V. R.; Bevins, S. N., A Review of African Swine Fever and the Potential for Introduction into the United States and the Possibility of Subsequent Establishment in Feral Swine and Native Ticks. *Front Vet Sci* **2018,** *5*, 11. https://www.ncbi.nlm.nih.gov/pubmed/29468165

132. Forman, A. J., et al., The immunological response of pigs and guinea pigs to antigens of African swine fever virus. *Arch Virol* **1982,** *74* (2-3), 91-100. https://www.ncbi.nlm.nih.gov/pubmed/7165511

133. Cadenas-Fernandez, E., et al., High Doses of Inactivated African Swine Fever Virus Are Safe, but Do Not Confer Protection against a Virulent Challenge. *Vaccines (Basel)* **2021,** *9* (3). https://www.ncbi.nlm.nih.gov/pubmed/33802021

134. Pikalo, J., et al., Vaccination With a Gamma Irradiation-Inactivated African Swine Fever Virus Is Safe But Does Not Protect Against a Challenge. *Front Immunol* **2022,** *13*, 832264. https://www.ncbi.nlm.nih.gov/pubmed/35558083

135. Zhang, H., et al., Vaccines for African swine fever: an update. *Front Microbiol* **2023,** *14*, 1139494. https://www.ncbi.nlm.nih.gov/pubmed/37180260

136. Reuters, Vietnam approves commercial use of first African swine fever vaccines. https://www.reuters.com/business/healthcare-pharmaceuticals/vietnam-approves-commercial-use-first-african-swine-fever-vaccines-2023-07-24/ (accessed 04 September 2023).

137. Borca, M. V., et al., Development of a Highly Effective African Swine Fever Virus Vaccine by Deletion of the I177L Gene Results in Sterile Immunity against the Current Epidemic Eurasia Strain. *J Virol* **2020,** *94* (7), e02017-19. https://www.ncbi.nlm.nih.gov/pubmed/31969432

138. Tran, X. H., et al., African swine fever virus vaccine candidate ASFV-G-DeltaI177L efficiently protects European and native pig breeds against circulating Vietnamese field strain. *Transbound Emerg Dis* **2022,** *69* (4), e497-e504. https://www.ncbi.nlm.nih.gov/pubmed/34582622

139. USDA, African Swine Fever Virus Vaccine Passes Tests Required for Regulatory Approval. https://www.ars.usda.gov/news-events/news/research-news/2022/african-swine-fever-virus-vaccine-passes-tests-required-for-regulatory-approval/ (accessed 01 March 2023).

140. Leitao, A., et al., The non-haemadsorbing African swine fever virus isolate ASFV/NH/P68 provides a model for defining the protective anti-virus immune response. *J Gen Virol* **2001,** *82* (Pt 3), 513-523. https://www.ncbi.nlm.nih.gov/pubmed/11172092

141. Ramirez-Medina, E., et al., Recombinant Vaccine Strain ASFV-G-Delta9GL/DeltaUK Produced in the IPKM Cell Line Is Genetically Stable and Efficacious in Inducing Protection in Pigs Challenged with the Virulent African Swine Fever Virus Field Isolate Georgia 2010. *Pathogens* **2024,** *13* (4). https://www.ncbi.nlm.nih.gov/pubmed/38668274

142. Truong, Q. L., et al., A Cell-Adapted Live-Attenuated Vaccine Candidate Protects Pigs against the Homologous Strain VNUA-ASFV-05L1, a Representative Strain of the Contemporary Pandemic African Swine Fever Virus. *Viruses* **2023,** *15* (10). https://www.ncbi.nlm.nih.gov/pubmed/37896866

143. Abrams, C. C., et al., Deletion of virulence associated genes from attenuated African swine fever virus isolate OUR T88/3 decreases its ability to protect against challenge with virulent virus. *Virology* **2013,** *443* (1), 99-105. https://www.ncbi.nlm.nih.gov/pubmed/23725691

144. Borca, M. V., et al., The L83L ORF of African swine fever virus strain Georgia encodes for a non-essential gene that interacts with the host protein IL-1β. *Virus research* **2018,** *249*, 116-123.

145. Chen, W., et al., A seven-gene-deleted African swine fever virus is safe and effective as a live attenuated vaccine in pigs. *Sci China Life Sci* **2020,** *63* (5), 623-634. https://www.ncbi.nlm.nih.gov/pubmed/32124180

146. Sang, H., et al., Progress Toward Development of Effective and Safe African Swine Fever Virus Vaccines. *Front Vet Sci* **2020,** *7* (84), 84. https://www.ncbi.nlm.nih.gov/pubmed/32154279

147. Lacasta, A., et al., Live attenuated African swine fever viruses as ideal tools to dissect the mechanisms involved in viral pathogenesis and immune protection. *Vet Res* **2015,** *46*, 135. https://www.ncbi.nlm.nih.gov/pubmed/26589145

148. Sanchez-Cordon, P. J., et al., Absence of Long-Term Protection in Domestic Pigs Immunized with Attenuated African Swine Fever Virus Isolate OURT88/3 or BeninDeltaMGF Correlates with Increased Levels of Regulatory T Cells and Interleukin-10. *J Virol* **2020,** *94* (14). https://www.ncbi.nlm.nih.gov/pubmed/32376618

149. Gaudreault, N. N.; Richt, J. A., Subunit Vaccine Approaches for African Swine Fever Virus. *Vaccines (Basel)* **2019,** *7* (2), 56. https://www.ncbi.nlm.nih.gov/pubmed/31242632

150. Ambagala, A., et al., Characterization of an African Swine Fever Virus Field Isolate from Vietnam with Deletions in the Left Variable Multigene Family Region. *Viruses* **2024,** *16* (4). https://www.ncbi.nlm.nih.gov/pubmed/38675912

151. Velazquez-Salinas, L., et al., Development Real-Time PCR Assays to Genetically Differentiate Vaccinated Pigs From Infected Pigs With the Eurasian Strain of African Swine Fever Virus. *Front Vet Sci* **2021,** *8*, 768869. https://www.ncbi.nlm.nih.gov/pubmed/34778441

152. Wang, T., et al., Current efforts towards safe and effective live attenuated vaccines against African swine fever: challenges and prospects. *Infect Dis Poverty* **2021,** *10* (1), 137. https://www.ncbi.nlm.nih.gov/pubmed/34949228

153. Ramirez-Medina, E., et al., Evaluation of the Deletion of MGF110-5L-6L on Swine Virulence from the Pandemic Strain of African Swine Fever Virus and Use as a DIVA Marker in Vaccine Candidate ASFV-G-ΔI177L. *Journal of Virology* **2022,** *96* (14), e00597-22.

154. Burmakina, G., et al., African swine fever virus serotype-specific proteins are significant protective antigens for African swine fever. *J Gen Virol* **2016,** *97* (7), 1670-1675. https://www.ncbi.nlm.nih.gov/pubmed/27114233

155. Ruiz-Gonzalvo, F., et al., Functional and immunological properties of the baculovirus-expressed hemagglutinin of African swine fever virus. *Virology* **1996,** *218* (1), 285-9. https://www.ncbi.nlm.nih.gov/pubmed/8615037

156. Gómez-Puertas, P., et al., The African swine fever virus proteins p54 and p30 are involved in two distinct steps of virus attachment and both contribute to the antibody-mediated protective immune response. *Virology* **1998,** *243* (2), 461-471.

157. Angulo, A., et al., Inhibition of African swine fever virus binding and infectivity by purified recombinant virus attachment protein p12. *J Virol* **1993,** *67* (9), 5463-71. https://www.ncbi.nlm.nih.gov/pubmed/8350406

158. Neilan, J. G., et al., Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection. *Virology* **2004,** *319* (2), 337-42. https://www.ncbi.nlm.nih.gov/pubmed/14980493

159. Murgia, M. V., et al., Evaluation of an African swine fever (ASF) vaccine strategy incorporating priming with an alphavirus-expressed antigen followed by boosting with attenuated ASF virus. *Arch Virol* **2019,** *164* (2), 359-370. https://www.ncbi.nlm.nih.gov/pubmed/30367292

160. Lokhandwala, S., et al., Adenovirus-vectored African Swine Fever Virus antigen cocktails are immunogenic but not protective against intranasal challenge with Georgia 2007/1 isolate. *Vet Microbiol* **2019,** *235*, 10-20. https://www.ncbi.nlm.nih.gov/pubmed/31282366

161. Cadenas-Fernandez, E., et al., Adenovirus-vectored African Swine Fever Virus Antigens Cocktail Is Not Protective against Virulent Arm07 Isolate in Eurasian Wild Boar. *Pathogens* **2020,** *9* (3). https://www.ncbi.nlm.nih.gov/pubmed/32121082

162. Gladue, D. P., et al., Deletion of CD2-Like (CD2v) and C-Type Lectin-Like (EP153R) Genes from African Swine Fever Virus Georgia-Δ9GL Abrogates Its Effectiveness as an Experimental Vaccine. *Viruses* **2020,** *12* (10). https://www.ncbi.nlm.nih.gov/pubmed/33092057

163. Argilaguet, J. M., et al., BacMam immunization partially protects pigs against sublethal challenge with African swine fever virus. *Antiviral Res* **2013,** *98* (1), 61-5. https://www.ncbi.nlm.nih.gov/pubmed/23428670

164. Goatley, L. C., et al., A Pool of Eight Virally Vectored African Swine Fever Antigens Protect Pigs Against Fatal Disease. *Vaccines (Basel)* **2020,** *8* (2). https://www.ncbi.nlm.nih.gov/pubmed/32443536

165. Argilaguet, J. M., et al., DNA vaccination partially protects against African swine fever virus lethal challenge in the absence of antibodies. *PLoS One* **2012,** *7* (9), e40942. https://www.ncbi.nlm.nih.gov/pubmed/23049728

166. Jancovich, J. K., et al., Immunization of Pigs by DNA Prime and Recombinant Vaccinia Virus Boost To Identify and Rank African Swine Fever Virus Immunogenic and Protective Proteins. *J Virol* **2018,** *92* (8). https://www.ncbi.nlm.nih.gov/pubmed/29386289

167. Lacasta, A., et al., Expression library immunization can confer protection against lethal challenge with African swine fever virus. *Journal of virology* **2014,** *88* (22), 13322-13332.

168. Sunwoo, S.-Y., et al., DNA-protein vaccination strategy does not protect from challenge with African swine fever virus Armenia 2007 strain. *Vaccines* **2019,** *7* (1), 12.

169. World Organisation for Animal Health (WOAH), African swine fever: WOAH warns Veterinary Authorities and pig industry of risk from use of sub-standard vaccines. https://www.woah.org/en/african-swine-fever-woah-warns-veterinary-authorities-and-pig-industry-of-risk-from-use-of-sub-standard-vaccines/ (accessed 01 December 2024).

170. Escribano, J. M., et al., Antibody-mediated neutralization of African swine fever virus: myths and facts. *Virus Res* **2013,** *173* (1), 101-9. https://www.ncbi.nlm.nih.gov/pubmed/23159730

171. Lopez, E., et al., Deletion Mutants of the Attenuated Recombinant ASF Virus, BA71ΔCD2, Show Decreased Vaccine Efficacy. *Viruses* **2021,** *13* (9), 1678. https://www.mdpi.com/1999-4915/13/9/1678

172. WOAH, Animal Disease Events. https://wahis.woah.org/#/event-management (accessed 01 March 2023).

173. USDA, APHIS Suspends the Interstate Movement of Live Swine, Swine Germplasm, Swine Products, and Swine Byproducts from Puerto Rico and the U.S. Virgin Islands. APHIS, U., Ed. 2021. https://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/vs-federal-order-signed-rbs.pdf

174. United States Department of Agriculture (USDA), *Qualitative Assessment of the likelihood of African swine fever virus entry to the United States: Entry Assessment*; 2019. https://www.aphis.usda.gov/animal_health/downloads/animal_diseases/swine/asf-entry.pdf

175. Mauroy, A., et al., Semi-quantitative risk assessment by expert elicitation of potential introduction routes of African swine fever from wild reservoir to domestic pig industry and subsequent spread during the Belgian outbreak (2018-2019). *Transbound Emerg Dis* **2021,** *68* (5), 2761-2773. https://www.ncbi.nlm.nih.gov/pubmed/33713549

176. Sugiura, K., et al., Change in the ASF entry risk into Japan as a result of the COVID-19 pandemic. *Transbound Emerg Dis* **2021,** *68* (3), 1700-1703. https://www.ncbi.nlm.nih.gov/pubmed/32969591

177. Fanelli, A., et al., Is the COVID-19 pandemic impacting on the risk of African Swine Fever virus (ASFV) introduction into the United States? A short-term assessment of the risk factors. *Transbound Emerg Dis* **2022,** *69* (4), e505-e516. https://www.ncbi.nlm.nih.gov/pubmed/34549530

178. Jurado, C., et al., Risk of African swine fever virus introduction into the United States through smuggling of pork in air passenger luggage. *Sci Rep* **2019,** *9* (1), 14423. https://www.ncbi.nlm.nih.gov/pubmed/31594957

179. USDA, African Swine Fever. https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/swine-disease-information/african-swine-fever/seminar (accessed Aug 23 2022).

180. Gebhardt, J. T., et al., Sampling and detection of African swine fever virus within a feed manufacturing and swine production system. *Transbound Emerg Dis* **2022,** *69* (1), 103-114. https://www.ncbi.nlm.nih.gov/pubmed/34554652

181. Harrison, O. L., et al., Developing a gateway program for importing non-animal origin ingredients from regions with African swine fever virus. *Transbound Emerg Dis* **2022,** *69* (5), e1407-e1416. https://www.ncbi.nlm.nih.gov/pubmed/35156315

182. Wales, A. D.; Davies, R. H., Disinfection to control African swine fever virus: a UK perspective. *J Med Microbiol* **2021,** *70* (9). https://www.ncbi.nlm.nih.gov/pubmed/34477547

183. WOAH, African Swine Fever. https://www.woah.org/app/uploads/2021/03/a-african-swine-fever-v2-0.pdf (accessed 01 March 2023).

184. Mazur-Panasiuk, N., et al., African Swine Fever Virus - Persistence in Different Environmental Conditions and the Possibility of its Indirect Transmission. *J Vet Res* **2019,** *63* (3), 303-310. https://www.ncbi.nlm.nih.gov/pubmed/31572808

185. Petrini, S., et al., Survival of African swine fever virus (ASFV) in various traditional Italian dry-cured meat products. *Prev Vet Med* **2019,** *162*, 126-130. https://www.ncbi.nlm.nih.gov/pubmed/30621891

186. Davies, K., et al., Survival of African Swine Fever Virus in Excretions from Pigs Experimentally Infected with the Georgia 2007/1 Isolate. *Transbound Emerg Dis* **2017,** *64* (2), 425-431. https://www.ncbi.nlm.nih.gov/pubmed/26104842

187. Niederwerder, M. C.; Hefley, T. J., Diagnostic sensitivity of porcine biological samples for detecting African swine fever virus infection after natural consumption in feed and liquid. *Transbound Emerg Dis* **2022,** *69* (5), 2727-2734. https://www.ncbi.nlm.nih.gov/pubmed/34914859

188. Carlson, J., et al., Stability of African Swine Fever Virus in Soil and Options to Mitigate the Potential Transmission Risk. *Pathogens* **2020,** *9* (11). https://www.ncbi.nlm.nih.gov/pubmed/33238521

189. Prodelalova, J., et al., Experimental Evidence of the Long-Term Survival of Infective African Swine Fever Virus Strain Ba71V in Soil under Different Conditions. *Pathogens* **2022,** *11* (6). https://www.ncbi.nlm.nih.gov/pubmed/35745502

190. Tummeleht, L., et al., Wild boar (Sus scrofa) carcasses as an attraction for scavengers and a potential source for soil contamination with the African swine fever virus. *Front Vet Sci* **2024,** *11*, 1305643. https://www.ncbi.nlm.nih.gov/pubmed/38545558

191. European Food Safety, A., et al., Epidemiological analyses of African swine fever in the European Union (November 2017 until November 2018). *EFSA J* **2018,** *16* (11), e05494. https://www.ncbi.nlm.nih.gov/pubmed/32625771

192. Zani, L., et al., African swine fever virus survival in buried wild boar carcasses. *Transbound Emerg Dis* **2020,** *67* (5), 2086-2092. https://www.ncbi.nlm.nih.gov/pubmed/32216049

193. Fischer, M., et al., Stability of African Swine Fever Virus in Carcasses of Domestic Pigs and Wild Boar Experimentally Infected with the ASFV "Estonia 2014" Isolate. *Viruses* **2020,** *12* (10). https://www.ncbi.nlm.nih.gov/pubmed/33019736

194. Dee, S. A., et al., Survival of viral pathogens in animal feed ingredients under transboundary shipping models. *PLoS One* **2018,** *13* (3), e0194509. https://www.ncbi.nlm.nih.gov/pubmed/29558524

195. Stoian, A. M. M., et al., Half-Life of African Swine Fever Virus in Shipped Feed. *Emerg Infect Dis* **2019,** *25* (12), 2261-2263. https://www.ncbi.nlm.nih.gov/pubmed/31524583

196. Blazquez, E., et al., Effect of spray-drying and ultraviolet C radiation as biosafety steps for CSFV and ASFV inactivation in porcine plasma. *PLoS One* **2021,** *16* (4), e0249935. https://www.ncbi.nlm.nih.gov/pubmed/33909651

197. Fischer, M., et al., Stability of African swine fever virus on spiked spray-dried porcine plasma. *Transbound Emerg Dis* **2021,** *68* (5), 2806-2811. https://www.ncbi.nlm.nih.gov/pubmed/34171166

198. European Food Safety, A., et al., Research objectives to fill knowledge gaps in African swine fever virus survival in the environment and carcasses, which could improve the control of African swine fever virus in wild boar populations. *EFSA J* **2021,** *19* (6), e06675. https://www.ncbi.nlm.nih.gov/pubmed/34188717

199. Kwon, T., et al., Development and optimization of sampling techniques for environmental samples from African swine fever virus-contaminated surfaces with no organic contaminants. *Front Vet Sci* **2024,** *11*, 1425928. https://www.ncbi.nlm.nih.gov/pubmed/39091398

200. World Organisation for Animal Health (WOAH), Addressing African Swine Fever: Protocols and Guidelines for Laboratory Diagnosis. https://www.woah.org/app/uploads/2024/06/woah-asf-lab-guidelines-170524-en.pdf (accessed 04 December 2024).

201. Kosowska, A., et al., A new method for sampling African swine fever virus genome and its inactivation in environmental samples. *Sci Rep* **2021,** *11* (1), 21560. https://www.ncbi.nlm.nih.gov/pubmed/34732758

202. Zhang, L., et al., A novel easy-to-desorb eluant contributes to address environmental contamination of African swine fever virus. *AMB Express* **2024,** *14* (1), 55. https://www.ncbi.nlm.nih.gov/pubmed/38730054

203. Food and Agricultural Organization (FAO), African swine fever prevention, detection and control in resource-limited settings. https://openknowledge.fao.org/server/api/core/bitstreams/773b174a-a962-4242-9dde-97cb1c86b9fb/content (accessed 04 December 2024).

204. Varzandi, A. R., et al., Detection of African swine fever virus and wild boar eDNA in soil and turbid water samples: towards environmental surveillance. *European Journal of Wildlife Research* **2023,** *70* (1), 4. https://doi.org/10.1007/s10344-023-01758-z

205. Yang, R., et al., Enhanced detection of African swine fever virus in samples with low viral load using digital PCR technology. *Heliyon* **2024,** *10* (7), e28426. https://www.ncbi.nlm.nih.gov/pubmed/38689956

206. Phillips, D. E., et al., Use of Field Based Loop Mediated Isothermal Amplification (LAMP) Technology for a Prevalence Survey and Proof of Freedom Survey for African Swine Fever in Timor-Leste in 2019. *Front Vet Sci* **2021,** *8*, 672048. https://www.ncbi.nlm.nih.gov/pubmed/34235203

207. Deutschmann, P., et al., Lateral flow assays for the detection of African swine fever virus antigen are not fit for field diagnosis of wild boar carcasses. *Transbound Emerg Dis* **2022,** *69* (4), 2344-2348. https://www.ncbi.nlm.nih.gov/pubmed/34312995

208. Ringbio, African Swine Fever Antigen ASFV Real-time PCR Kit. https://www.ringbio.com/solutions/swine/asfv-african-swine-fever-asfv-rt-pcr-kit (accessed 04 December 2024).

209. TianLong, African Swine Fever Virus (ASFV) Nucleic Acid Detection Kit (Fluorescence PCR Method). https://www.medtl.net/products/african-swine-fever-virus-asfv-nucleic-acid-detection-kit-fluorescence-pcr-method.html (accessed 04 December 2024).

210. FlashTest, African swine fever (ASFV) (P72 gene, environmental samples) Nucleic Acid Test Kit (Lyophilized). https://www.flashtestbio.com/products/asfv-environmental-asfv-vp72-nucleic-acid-test-kit-lyophilized.html#:~:text=This%20is%20a%20PCR%20nucleic%20acid%20detection%20reagent,of%20fast%2C%20sensitive%2C%20highly%20specific%20and%20high%20accuracy.

211. Department of Homeland Security (DHS), News Release: DHS S&T Completes Successful Test of DNA Tool to Detect African Swine Fever in Pigs. https://www.dhs.gov/science-and-technology/news/2020/03/24/news-release-st-completes-test-dna-tool-detect-african-swine-fever (accessed 02 Nov 2020).

212. Aira, C., et al., Simultaneous Detection of Antigen and Antibodies of African Swine Fever in a Novel Combo Lateral Flow Assay. *Vaccines (Basel)* **2024,** *12* (3). https://www.ncbi.nlm.nih.gov/pubmed/38543941

213. Luan, H., et al., KP177R-based visual assay integrating RPA and CRISPR/Cas12a for the detection of African swine fever virus. *Front Immunol* **2024,** *15*, 1358960. https://www.ncbi.nlm.nih.gov/pubmed/38655256

214. USDA, Disinfectants Approved for Use Against African Swine Fever Virus in Farm Settings. https://www.aphis.usda.gov/animal_health/emergency_management/downloads/asf-virus-disinfectants.pdf (accessed 02 Nov 2020).

215. Tian, L., et al., A quadruple protection procedure for resuming pig production in small-scale ASFV-positive farms in China. *Curr Res Microb Sci* **2021,** *2*, 100014. https://www.ncbi.nlm.nih.gov/pubmed/34841307

216. Oura, C. A., et al., Virological diagnosis of African swine fever--comparative study of available tests. *Virus Res* **2013,** *173* (1), 150-8. https://www.ncbi.nlm.nih.gov/pubmed/23131492

217. USDA, *ASF Response - Depopulation, Disposal, and Decontamination Guidance - Option Matrices and Considerations*; United States Department of Agriculture: 2019. https://www.aphis.usda.gov/animal_health/carcass/docs/asf-3d-summary-guidance.pdf

218. Štukelj, M., et al., Cleaning and disinfection in the domestic pig sector. In *Understanding and combatting African Swine Fever: A European perspective*, Wageningen Academic Publishers: 2021; pp 133-152.

219. Niederwerder, M. C., Risk and Mitigation of African Swine Fever Virus in Feed. *Animals (Basel)* **2021,** *11* (3). https://www.ncbi.nlm.nih.gov/pubmed/33803495

220. Xu, R., et al., Disinfection Effect of Short-wave Ultraviolet Radiation(UV-C) on ASFV in Water. *J Infect* **2020,** *80* (6), 671-693. https://www.ncbi.nlm.nih.gov/pubmed/32135172

221. Taesuji, M., et al., In vitro primary porcine alveolar macrophage cell toxicity and African swine fever virus inactivation using five commercially supply compound disinfectants under various condition. *Journal of Veterinary Medical Science* **2021,** *83* (11), 1800-1804.

222. Zhang, L., et al., Efficient inactivation of African swine fever virus by ozonized water. *Vet Microbiol* **2020,** *247*, 108796. https://www.ncbi.nlm.nih.gov/pubmed/32768237

223. Gabbert, L. R., et al., Recovery and chemical disinfection of foot-and-mouth disease and African swine fever viruses from porous concrete surfaces. *J Appl Microbiol* **2020,** *129* (5), 1092-1101. https://www.ncbi.nlm.nih.gov/pubmed/32379950

224. Shurson, G. C., et al., New perspectives for evaluating relative risks of African swine fever virus contamination in global feed ingredient supply chains. *Transbound Emerg Dis* **2022,** *69* (1), 31-56. https://www.ncbi.nlm.nih.gov/pubmed/34076354

225. USDA, NAHEMS Guidelines: Personal Protective Equipment (PPE). https://www.aphis.usda.gov/animal_health/emergency_management/downloads/nahems_guidelines/fadprep_nahems_guidelines_ppe_final_april2011.pdf (accessed 02 Nov 2020).

226. Martin, C., African Swine Fever: Secure Zoo Strategy Plan. https://zahp.org/wp-content/uploads/2021/12/ASF-in-Zoo-Response.pdf (accessed 01 March 2022).

227. Galli, F., et al., How do small farms contribute to food and nutrition security? Linking European small farms, strategies and outcomes in territorial food systems. *Global Food Security* **2020,** *26*, 100427.

228. AVMA, AVMA Guidelines for the Depopulation of Animals: 2019 Edition. https://www.avma.org/sites/default/files/resources/AVMA-Guidelines-for-the-Depopulation-of-Animals.pdf (accessed 02 Nov 2020).

229. Barnes, A. P., et al., The effect of animal health compensation on 'positive' behaviours towards exotic disease reporting and implementing biosecurity: A review, a synthesis and a research agenda. *Prev Vet Med* **2015,** *122* (1-2), 42-52. https://www.ncbi.nlm.nih.gov/pubmed/26422364

230. VetCA, Carcass Disposal State Resource Locator. https://www.vetca.org/lacd/index.php (accessed 02 Nov 2020).

231. Gabbert, L., et al., Evaluation of Whole Carcass Composting as a Mortality Disposal Option for African Swine Fever Virus-Infected Swine. *Transboundary and Emerging Diseases* **2023,** *2023*, 1-9. https://doi.org/10.1155/2023/9926250

232. Environmental Protection Agency (EPA), Assessment of the Biosecurity of Animal Mortality Size Reduction Using Horizontal Grinders Prior to On-Farm Composting. https://www.epa.gov/sites/default/files/2020-02/documents/lemieux-a.pdf (accessed 30 January 2025).

233. Environmental Protection Agency (EPA), Assessment of the Biosecurity of Animal Mortality Size Reduction Using Horizontal Grinders Prior to On-Farm Composting- Presentation. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=CESER&dirEntryId=347492 (accessed 30 January 2025).

234. USDA, Carcass Management Dashboard. https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/emergency-management/carcass-management (accessed 02 Nov 2020).

235. Bellini, S., et al., A review of risk factors of African swine fever incursion in pig farming within the European Union Scenario. *Pathogens* **2021,** *10* (1), 84.

236. Fasina, F. O., et al., Drivers, Risk Factors and Dynamics of African Swine Fever Outbreaks, Southern Highlands, Tanzania. *Pathogens* **2020,** *9* (3), 155. https://www.ncbi.nlm.nih.gov/pubmed/32106538

237. Borchardt, M. A., et al., Avian Influenza Virus RNA in Groundwater Wells Supplying Poultry Farms Affected by the 2015 Influenza Outbreak. *Environmental Science & Technology Letters* **2017,** *4* (7), 268-272. <Go to ISI>://WOS:000405543600003

238. Loh, J., et al., Detection of novel sequences related to african Swine Fever virus in human serum and sewage. *J Virol* **2009,** *83* (24), 13019-25. https://www.ncbi.nlm.nih.gov/pubmed/19812170

239. Fu, S., et al., Metagenomics combined with comprehensive validation as a public health risk assessment tool for urban and agricultural run-off. *Water Res* **2022,** *209*, 117941. https://www.ncbi.nlm.nih.gov/pubmed/34920315

240. Haas, B., et al., Inactivation of viruses in liquid manure. *Rev Sci Tech* **1995,** *14* (2), 435-45. https://www.ncbi.nlm.nih.gov/pubmed/7579641

241. Liu, Y., et al., Prevention and Control Strategies of African Swine Fever and Progress on Pig Farm Repopulation in China. *Viruses* **2021,** *13* (12). https://www.ncbi.nlm.nih.gov/pubmed/34960821

242. Sindryakova, I., et al., The influence of temperature on the Russian isolate of African swine fever virus in pork products and feed with extrapolation to natural conditions. *Sel'skokhozyaistvennaya Biol* **2016,** *51*, 467-474.

243. Turner, C., et al., Laboratory scale inactivation of pig viruses in pig slurry and design of a pilot plant for thermal inactivation. *Water Science and Technology* **1998,** *38* (4-5), 79-86. http://www.sciencedirect.com/science/article/pii/S0273122398005009

244. Cleland, J., *Feasibility Study Report: Management of Swine Lagoons Following African Swine Fever Outbreak*; U.S. Environmental Protection Agency,: 2023. https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=357912&Lab=CESER

245. Juszkiewicz, M., et al., Characteristics of selected active substances used in disinfectants and their virucidal activity against ASFV. *Journal of veterinary research* **2019,** *63* (1), 17.

246. Cackett, G., et al., Transcriptome view of a killer: African swine fever virus. *Biochem Soc Trans* **2020,** *48* (4), 1569-1581. https://www.ncbi.nlm.nih.gov/pubmed/32725217

247. Penrith, M. L., et al., Review of the Pig-Adapted African Swine Fever Viruses in and Outside Africa. *Pathogens* **2022,** *11* (10). https://www.ncbi.nlm.nih.gov/pubmed/36297247

248. Janse van Rensburg, L., et al., Understanding African swine fever outbreaks in domestic pigs in a sylvatic endemic area: The case of the South African controlled area between 1977-2017. *Transbound Emerg Dis* **2020,** *67* (6), 2753-2769. https://www.ncbi.nlm.nih.gov/pubmed/32438525

249. Dixon, L. K., et al., African swine fever virus replication and genomics. *Virus Res* **2013,** *173* (1), 3-14. https://www.ncbi.nlm.nih.gov/pubmed/23142553

250. Njau, E. P., et al., The first genotype II African swine fever virus isolated in Africa provides insight into the current Eurasian pandemic. *Sci Rep* **2021,** *11* (1), 13081. https://www.ncbi.nlm.nih.gov/pubmed/34158551

251. Peter, E., et al., Detection of African swine fever virus genotype XV in a sylvatic cycle in Saadani National Park, Tanzania. *Transbound Emerg Dis* **2021,** *68* (2), 813-823. https://www.ncbi.nlm.nih.gov/pubmed/32696552

252. Bisimwa, P. N., et al., First detection of African swine fever (ASF) virus genotype X and serogroup 7 in symptomatic pigs in the Democratic Republic of Congo. *Virol J* **2020,** *17* (1), 135. https://www.ncbi.nlm.nih.gov/pubmed/32883295

253. Yona, C. M., et al., Persistent domestic circulation of African swine fever virus in Tanzania, 2015-2017. *BMC Vet Res* **2020,** *16* (1), 369. https://www.ncbi.nlm.nih.gov/pubmed/33004025

254. Minoungou, G. L., et al., Molecular characterization of African Swine fever viruses in Burkina Faso, Mali, and Senegal 1989-2016: Genetic diversity of ASFV in West Africa. *Transbound Emerg Dis* **2021,** *68* (5), 2842-2852. https://www.ncbi.nlm.nih.gov/pubmed/34323385

255. Adedeji, A. J., et al., First-Time Presence of African Swine Fever Virus Genotype II in Nigeria. *Microbiol Resour Announc* **2021,** *10* (26), e0035021. https://www.ncbi.nlm.nih.gov/pubmed/34197187

256. Dei Giudici, S., et al., The Long-Jumping of African Swine Fever: First Genotype II Notified in Sardinia, Italy. *Viruses* **2023,** *16* (1). https://www.ncbi.nlm.nih.gov/pubmed/38257733

257. Zhu, Z., et al., Classification and characterization of multigene family proteins of African swine fever viruses. *Brief Bioinform* **2021,** *22* (4). https://www.ncbi.nlm.nih.gov/pubmed/33333556

258. Michaud, V., et al., Comprehensive phylogenetic reconstructions of African swine fever virus: proposal for a new classification and molecular dating of the virus. *PLoS One* **2013,** *8* (7), e69662. https://www.ncbi.nlm.nih.gov/pubmed/23936068

259. Nefedeva, M., et al., Recombination shapes African swine fever virus serotype-specific locus evolution. *Sci Rep* **2020,** *10* (1), 18474. https://www.ncbi.nlm.nih.gov/pubmed/33116230

260. de Villiers, E. P., et al., Phylogenomic analysis of 11 complete African swine fever virus genome sequences. *Virology* **2010,** *400* (1), 128-36. https://www.ncbi.nlm.nih.gov/pubmed/20171711

261. Forth, J. H., et al., Comparative Analysis of Whole-Genome Sequence of African Swine Fever Virus Belgium 2018/1. *Emerg Infect Dis* **2019,** *25* (6), 1249-1252. https://www.ncbi.nlm.nih.gov/pubmed/30907724

262. Mazloum, A., et al., Comparative Analysis of Full Genome Sequences of African Swine Fever Virus Isolates Taken from Wild Boars in Russia in 2019. *Pathogens* **2021,** *10* (5). https://www.ncbi.nlm.nih.gov/pubmed/33925986

263. Mazur-Panasiuk, N., et al., The first complete genomic sequences of African swine fever virus isolated in Poland. *Sci Rep* **2019,** *9* (1), 4556. https://www.ncbi.nlm.nih.gov/pubmed/30872594

264. Mai, N. T. A., et al., Molecular profile of African swine fever virus (ASFV) circulating in Vietnam during 2019-2020 outbreaks. *Arch Virol* **2021,** *166* (3), 885-890. https://www.ncbi.nlm.nih.gov/pubmed/33454861

265. Rajukumar, K., et al., Genetic characterization of African swine fever virus from domestic pigs in India. *Transbound Emerg Dis* **2021,** *68* (5), 2687-2692. https://www.ncbi.nlm.nih.gov/pubmed/33415828

266. Nurmoja, I., et al., Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. *Transbound Emerg Dis* **2017,** *64* (6), 2034-2041. https://www.ncbi.nlm.nih.gov/pubmed/28116841

267. Gallardo, C., et al., African swine fever virus (ASFV) protection mediated by NH/P68 and NH/P68 recombinant live-attenuated viruses. *Vaccine* **2018,** *36* (19), 2694-2704. https://www.ncbi.nlm.nih.gov/pubmed/29609966

268. Zani, L., et al., Deletion at the 5'-end of Estonian ASFV strains associated with an attenuated phenotype. *Sci Rep* **2018,** *8* (1), 6510. https://www.ncbi.nlm.nih.gov/pubmed/29695831

269. Sargsyan, M. A., et al., Third wave of African swine fever infection in Armenia: Virus demonstrates the reduction of pathogenicity. *Vet World* **2018,** *11* (1), 5-9. https://www.ncbi.nlm.nih.gov/pubmed/29479149

270. Arias, M., et al., Gaps in African swine fever: Analysis and priorities. *Transbound Emerg Dis* **2018,** *65 Suppl 1*, 235-247. https://www.ncbi.nlm.nih.gov/pubmed/28941208

271. Gallardo, C., et al., African swine fever (ASF) diagnosis, an essential tool in the epidemiological investigation. *Virus Res* **2019,** *271*, 197676. https://www.ncbi.nlm.nih.gov/pubmed/31362027

272. Vuono, E. A., et al., Deletion of the ASFV dUTPase Gene E165R from the Genome of Highly Virulent African Swine Fever Virus Georgia 2010 Does Not Affect Virus Replication or Virulence in Domestic Pigs. *Viruses* **2022,** *14* (7), 1409.

273. Ramirez-Medina, E., et al., Differential Effect of the Deletion of African Swine Fever Virus Virulence-Associated Genes in the Induction of Attenuation of the Highly Virulent Georgia Strain. *Viruses* **2019,** *11* (7). https://www.ncbi.nlm.nih.gov/pubmed/31269702

274. O'Donnell, V., et al., African Swine Fever Virus Georgia 2007 with a Deletion of Virulence-Associated Gene 9GL (B119L), when Administered at Low Doses, Leads to Virus Attenuation in Swine and Induces an Effective Protection against Homologous Challenge. *J Virol* **2015,** *89* (16), 8556-66. https://www.ncbi.nlm.nih.gov/pubmed/26063424

275. O'Donnell, V., et al., African Swine Fever Virus Georgia Isolate Harboring Deletions of MGF360 and MGF505 Genes Is Attenuated in Swine and Confers Protection against Challenge with Virulent Parental Virus. *J Virol* **2015,** *89* (11), 6048-56. https://www.ncbi.nlm.nih.gov/pubmed/25810553

276. Vuono, E., et al., Deletion of the H108r Gene Reduces Virulence of the Pandemic Eurasia Strain of African Swine Fever Virus with Surviving Animals Being Protected against Virulent Challenge. *Journal of Virology* **2022,** *96* (14), e00545-22.

277. Ramirez-Medina, E., et al., Deletion of E184L, a Putative DIVA Target from the Pandemic Strain of African Swine Fever Virus, Produces a Reduction in Virulence and Protection against Virulent Challenge. *J Virol* **2022,** *96* (1), e0141921. https://www.ncbi.nlm.nih.gov/pubmed/34668772

278. Zhou, P., et al., Deletion of the H240R Gene of African Swine Fever Virus decreases infectious progeny virus production due to aberrant virion morphogenesis and enhances inflammatory cytokine expression in porcine macrophages. *Journal of Virology* **2022,** *96* (3), e01667-21.

279. Xie, Z., et al., Protection Evaluation of a Five-Gene-Deleted African Swine Fever Virus Vaccine Candidate Against Homologous Challenge. *Front Microbiol* **2022,** *13*, 902932. https://www.ncbi.nlm.nih.gov/pubmed/35966648