DHS SCIENCE AND TECHNOLOGY

Master Question List for African Swine Fever Virus (ASFV)

09 March 2023

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Science and Technology

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

African Swine Fever Virus (ASFV) – Master Question List

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Foreword

The following Master Question List (MQL) was developed by the Department of Homeland Security Science and Technology Directorate (DHS S&T) to provide government decision makers with up-to-date information which will enable them to appropriately respond to the 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 is native to Africa, where native hosts include warthogs and bush pigs, neither of which exhibit clinical disease signs. Domestic pigs are non-native hosts that acquire fatal illness. Of 24 known ASFV genotypes, only genotypes I and II have spread outside of Africa and caused devastating outbreaks in Europe, Asia, and some Caribbean islands. ASFV is not a human pathogen; however, a domestic outbreak would result in disruption of the U.S. swine industry with the potential for billions of dollars in economic impact. In 2021, ASFV was detected in the Dominican Republic and spread throughout the country and to neighboring Haiti. According to the World Organisation for Animal Health (WOAH or OIE), the outbreak is on-going as of 01 March 2023. Although ASFV has not been detected in the United States, the United States Department of Agriculture (USDA) and United States Customs and Border Protections (CBP) have increased precautions to prevent importation of the virus and further spread of the virus in the region.

The proximity of this outbreak has caused concern for further spread and motivates evaluation of the threat of ASFV to the United States.

The cutoff date for information gathering related to this document was 01 March 2023.

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Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
BACKGROUND	• ASFV is a large, double-stranded DNA virus with a 170-180 kb genome, and the sole member of its family (*Asfarviridae*). The ASFV genome can include more than 180 distinct open reading frames (ORFs) encoding multiple proteins via the production of multiple transcripts from the same gene. • The median infectious dose (ID_{50}) of ASFV in domestic swine ranges from 10-10,000 viral particles (median hemadsorbing dose; HAD_{50}), but depends on age, body weight, inoculation route, and virus strain. • ASFV has high resistance to degradation and remains infectious for long periods on domestic surfaces (e.g., shoes, clothes, vehicles, boards, bricks, glass, metal, rubber, paper, and equipment), food, body fluids, and carcasses. • From 01 September 2022 to 01 March 2023, there were a total of 107 reports of ASFV in over 25 countries.
INFECTIOUS DOSE	• The ID_{50} of ASFV in domestic swine ranges from 10-10,000 viral particles (HAD_{50}), but depends on age, body weight, inoculation route, and virus strain. • Some domestic pigs can acquire ASFV infection after oronasal exposure to only a few viral particles.
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). • Ticks are crucial for natural ASFV infection in warthogs (reservoir species), though stable flies may also play a role in transmission to swine.
HOST RANGE	• ASFV is native to Africa, where hosts include warthogs (*Phacochoerus africanus*), bush pigs (*Potamochoerus larvatus*), and giant forest hogs (*Hylochoerus meinertzhageni*). 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.
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. • 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.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
CLINICAL PRESENTATION	• Clinical signs of ASF include fever, loss of appetite, dull/depressed attitude, red/purple skin lesions, respiratory distress, vomiting/diarrhea/bloody diarrhea, high mortality, sudden death, and abortion. • Morbidity is high for all forms of disease; mortality rates vary, but can reach 100% in peracute/acute infections. • Symptoms of ASF are similar to other pig diseases such as classical swine fever (CSF) and necessitate diagnostic tests.
BIOSURVEILLANCE AND CLINICAL DIAGNOSIS	• There is currently no global biosurveillance framework specifically for ASFV. There are four WOAH reference laboratories for ASFV: Commonwealth Scientific and Industrial Research Organisation (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). • ASF is difficult to distinguish from CSF and other diseases via clinical signs and post-mortem examination. ASF should be considered in swine with febrile disease, disseminated hemorrhage, and high mortality.
VETERINARY MEDICAL COUNTERMEASURES	• To date, there is no uniformly effective medical treatment or a licensed vaccine for ASF. Several vaccine candidates have been tested, but efficacy varies and generally only applies to closely related ASFV strains.
VIRUS IMPORTATION	• Infected animals are the primary source of the virus, but infection can occur via ingestion of waste food that contains pig meat products or unprocessed pig meat. Routes of concern are legal and illegal entry of animals and animal products infected with the virus. • Human-mediated import likely contributed to the spread of ASFV in Europe, possibly through illegal importation of infected meat, hunting tourism, or fomites associated with farmers, farming professionals, and importation of bedding.
VIRAL PERSISTENCE / ENVIRONMENTAL STABILITY	• ASFV has high environmental resistance and can be transmitted via fomites including shoes, clothes, vehicles, knives, and equipment. Of note, infection of domestic pigs via fomites is not well characterized. • Infectious virus can be shed in urine and feces for approximately 2 weeks, depending on environmental temperature. • ASFV stability in soil, which could potentially be contaminated by an infected carcass, varies by soil type and temperature. • ASFV is also stable in animal feed (half-life of 4-14 days), which is a potential route of transmission.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
DECONTAMINATION	• When decontaminating ASFV, the first course of action recommended is a U.S. Environmental Protection Agency (EPA)-registered disinfectant. • The use of effective disinfectants for cleaning infected premises, trucks, and fomites is an important measure for preventing introductions of ASFV to new areas. Care should be taken to use a disinfectant specifically approved for ASFV. • ASFV is highly resistant to low temperatures and persists despite refrigeration or freezing. However, it can be heat inactivated by treating 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).
PERSONAL PROTECTIVE EQUIPMENT (PPE)	• The guidance for PPE follows Occupational Safety and Health Administration (OSHA) Level C, which covers most non-zoonotic foreign animal disease (FAD) events and includes National Institute for Occupational Safety and Health (NIOSH)-approved respirators, chemical resistant clothing, inner and outer gloves, and boots. • PPE guidelines will differ for various tasks associated with ASFV. For instance, protection required for surveillance and depopulation could differ from the protection required for chemical decontamination, or for working on an infected or uninfected area. • In most cases, protective hooded coveralls, gloves, boots, goggles or a face shield, and waterproof boots that can be cleaned and disinfected will be required.
DEPOPULATION AND CARCASS DISPOSAL	• There are several methods for effective depopulation of confirmed or suspected ASFV-infected swine in an agricultural setting, including inhalant (CO_2 gas), gunshot, penetrating or non-penetrating captive bolt, electrocution head to heart, veterinarian-administered anesthetic overdose, and in certain permitted circumstances, ventilation shutdown or sodium nitrate. • 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. • Carcass disposal and environmental regulations differ by setting and state.
SWINE WASTEWATER TREATMENT	• There is minimal knowledge 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 its effectiveness in wastewater systems needs to be determined.

Major Findings by Topic Area	
Topic	**Overview of Current Knowledge**
GENOMICS	• ASFV is a DNA virus, and the sole member of its family (*Asfarviridae*). • The ASFV genome can include more than 180 distinct ORFs encoding multiple proteins via the production of multiple transcripts from the same gene. • There are 24 known genotypes of ASFV, but only two (genotypes I and II) occur outside the natural range in Africa. • The strain of current potential pandemic concern is classified as genotype II and emerged in the country of Georgia in 2007. • The Georgia 2007 strain has not been found in the United States.

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

What do we know?

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

- The HAD_{50}, which is the preferred method for quantifying ASFV infectivity, is the dose (titer) of virus measured as hemadsorbing units (HAU) that it would take to infect 50% of inoculated pigs. Other more common virological methods like median tissue culture infectious dose ($TCID_{50}$) or plaque forming unit assays are not used for wild-type ASFV strain characterization due to the cell line adaptation that would be required to obtain the titer and the variability in results between cell lines and strains.[1-3]

Some domestic pigs can acquire ASFV infection after exposure to only a few viral particles.

- The infectious dose in approximately 8-week-old, cross-bred pigs for a genotype II strain through ingestion of liquid contaminated with ASFV was extremely low, about 7 HAD_{50}.[4] For contaminated dry feed, the infectious dose was considerably higher at 4.4 x 10^6 HAD_{50},[4] This result may be caused by the stimulation of salivary proteases produced while feeding that may degrade the integrity of the virus or the dried virus may have limited exposure to lymphoid or epithelial tissues before entering the gastrointestinal tract, requiring a higher dose to infect.[4]
- Additional studies in which 4- to 5-week-old pigs were fed dry feed spiked with genotype II Georgia '07 ASFV-contaminated plasma for 14 days did not result in infection at doses of 10^5, confirming a high dose is required for transmission through feed.[5]
- The infectious dose for a genotype II strain in 7-week-old domestic swine inoculated via intramuscular injection is ≤ 10^2 HAD_{50}.[6] Other studies have shown the dose can be as low as 5 HAD_{50} for normal susceptible animals (5-6 weeks old)[7-8] and 0.1 HAD_{50} in healthy 8 - week old pigs (Landrace breeds).[9] Doses for infection via oronasal inoculation were ≤10 HAU for runt (smallest or weakest of the litter at birth) animals (approximately 8-week-old domestic swine).[10] More recent studies on a virulent 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}, as this was the lowest dose administered that was lethal by 14 days.[11]
- For genotype I strains, Malta'78, and Netherlands'86, the ID_{50} was estimated to be ≤ 3.5 $\log_{10}$ $TCID_{50}$ by intraoropharyngeal inoculation, while the median lethal dose (LD_{50}) was estimated to be ≥ 3.5 $\log_{10}$ $TCID_{50}$.[12] Another genotype I strain, Brazil'78, has a reported ID_{50} of -0.25 $\log_{10}$ $TCID_{50}$.[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 $TCID_{50}$ (< 7 x 10^5 HAD_{50}).[13-14] Lower ranges still need to be defined.
- Doses for infection via oronasal inoculation were ≤10 HAU for runt (smallest or weakest of the litter at birth) animals (approximately 4-month-old wild boar).[10]
- Male and female wild boar appear equally susceptible to ASFV.[14]

What do we need to know?

- What are the infectious doses for different breeds of pigs, specifically domestic U.S. breeds (Berkshire, Chester White, Duroc, Hampshire, Landrace, Poland China, Spotted, and Yorkshire) 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?

- What factors are responsible for the differences in susceptibility to ASFV between warthogs in Africa and wild boar in Europe and Asia? Is it strain-dependent, genomics, the immune system, or the level of virus to which they are exposed?

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;[10, 13-15] ingestion of contaminated pig products;[4, 16-17] direct contact between infected and susceptible domestic pigs;[15, 18-19] indirect contact through people, vehicles, fomites (e.g., virus transported on shoes, tools, or PPE) and contaminated pork products;[15, 20-22] contact between infected wild pigs and domestic pigs including infected carcasses;[23-24] limited transplacental transmission;[25] vector transmission by ticks in the genus *Ornithodoros*;[26-29] and through ingestion of infectious stable flies (*Stomoxys calcitrans*).[30-32]

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). ASFV is less transmissible than foot and mouth disease in swine.[33]

- The R_0 of the genotype II Georgia 2007/1 isolate was calculated as 2.8 (1.3-4.8) within pens and 1.4 (0.6-2.4) between animals in adjacent pens.[18] The results were similar with another genotype II strain (POL/2015/Podlaskie), with an R_0 range of 0.68-1.94 within pens and 0.82-2.13 between animals in adjacent pens with variations due to different latent periods and models.[34] The estimated R_0 for a single genotype I virus (Malta'78 isolate) was 18 (6.90-46.9).[35] R_0 analysis in South Korea of transmission clusters determined a mean R_0 of 1.54.[36] Not all genotypes are characterized.
- 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.[37] 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.[38] To effectively inhibit ASF outbreaks, pig movements between farms need to be reduced by 50-75%.[39]
- A spatially explicit disease transmission model utilizing wild pig densities and contact ecology in the Southwest United States suggests that ecology may play a bigger role than the initial R_0 of an outbreak and may necessitate a larger radius of control with higher culling rates, but population density and detection delays should be considered.[40]
- Development of risk mapping on the ability of wild boar in Asia to maintain and spread ASFV utilizes knowledge of wild boar habitat, movement patterns, and small farm notification systems to estimate areas of risk.[41] Studies are continuing in this area with additional models being evaluated to aid in surveillance and include both pigs and wild boar.[42]
- The role of live and dead wild boar in the persistence of ASFV in Europe (e.g., through direct contact with infected animals or indirect contact with contaminated carcasses) is unclear, with research both supporting[19, 43-44] and refuting its importance.[24] Direct transmission by wild boar is thought to be limited to short distances (<20 km) given limited boar dispersal.[45-47]
- Evaluation of virus levels in animal carcasses under various experimental conditions identified infectious ASFV specifically on bones, muscles, and skin at cold temperatures (-20°C), which can provide a source of infection for several months.[44]
- Aerosol transmission of a genotype II virus between an infectious pig and a nearby healthy

pig with no direct contact is possible.[48] Animals in direct contact were infectious by Day 12 and animals that were air exposed were infectious by Day 15.[48]

- Animals that recover from initial infection remain persistent carriers. Viral titers were highest during acute clinical infection and in oropharyngeal swabs with two peaks up to 70 days; lower levels of virus were detected in feces, with the lowest titers in nasal, vaginal, and ocular samples.[12] Persistence can occur with any ASFV strain and reversion back to an infectious state can restart local outbreaks.[49] Pigs chronically infected with low-virulence strains were able to transmit virus through direct contact with co-housed pigs two months post-initial infection.[50]
- Persistence of genotype II strains in mature male boar reproductive organs suggests viral transmission through semen.[51] Experimental studies in wild boar infected with ASFV genotype II through intramuscular infection have shown that ASFV viral genome was detected in semen as early as 2 days post-infection, and that semen collected 4 and 5 days post infection and artificially inseminated into female boars led to transmission of ASFV to both females and implanted embryos.[52]
- ASFV infections in wild boar have shown that genetic relatedness is more important in transmission between live animals, but for carcass-based transmission, nearby infected animals play a larger role than relatedness.[53]

Ticks are crucial for natural ASFV infection in warthogs, though stable flies may also play a role in transmission to swine.[54] Vectors are not needed for transmission among domestic or between domestic and feral swine.

- There is no evidence that mosquitoes play a role in ASFV transmission.[55-56]
- Surface fomite transmission among farm pigs reveals a small window of transmission potential, as uninfected swine were only infected 1 day after introduction into uncleaned pens, but not after 3, 5, or 7 days.[57]

What do we need to know?

- Is there a threshold for virus or time necessary to transmit between domestic pigs?
- Can asymptomatic hosts transmit disease?
- 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?[55, 58-59]
- 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?[19, 24, 60]

Host Range
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.).[61] Neither warthogs nor bush pigs exhibit clinical disease signs.[61] Outside of Africa, domestic pigs (*Sus scrofa domesticus*),[62] wild bearded pigs (*Sus barbatus*) in Borneo,[63] Sulawesi warty pig (*Sus celebensis*) in Asia,[63] and wild boar/feral hogs (*Sus scrofa*)[62] are non-native hosts that can acquire fatal illness.

- Peccaries are thought to be relatively resistant to ASFV,[64-65] 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.[66]

There is currently no evidence that non-suid mammals act as a reservoir for ASFV.[11, 67]

- ASFV utilizes macrophages to infect host cells, and a large portion of host range determination is governed by large clusters of viral genes that confer increased susceptibility

and aid in viral internalization.[68]

- Studies on wolves who have previously fed on ASFV-positive wild boar carcasses did not show evidence of ASFV DNA in their feces, which suggests that though wolves may not be a viable source of transmission of ASF in the wild, they do act as a source to remove ASFV-infected carcasses from the environment, possibly helping prevent the transmission to other animals.[69]

Soft-bodied ticks (genus *Ornithodoros*) are a natural vector in Eastern Africa and Europe.[70] Ticks are required for transmission between warthogs, but not between domestic and/or feral pigs.[67]

- In the United States, there are at least three species of potentially competent tick vectors (*Ornithodoros coriaceus*, *Ornithodoros turicata*, and *Ornithodoros puertoicensis*).[67]
- Interestingly, different tick species within *Ornithodoros* appear to have different capacities to transmit individual strains of ASFV, as demonstrated by experimental trials in which tick viral load was dependent on both tick species and ASFV isolate,[28] which suggests that host-vector dynamics are complex, and that vector competence depends on circulating viral strain.
- 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 *Ornithodoros moubata porcinus* ticks and required 10^{4}-10^{5} HAD_{50}.[32]
- ASFV did not replicate when inoculated into two species of hard ticks from Europe, suggesting those species have no role in transmission.[71] ASFV DNA could be detected in hard ticks such as *Ixodes ricinus* and *Dermacentor reticulatus* for 6 and 8 weeks after feeding on infected blood, respectively; however, there is no evidence that ASFV can replicate in hard ticks or can transmit ASFV.[71] Additionally, no ASFV was detected in *Ixodes ricinus* ticks collected from wild boar habitats, further suggesting they do not facilitate transmission.[55]

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

- The stable fly (*Stomoxys calcitrans*) has been linked to transmission,[30-31] and has been demonstrated under experimental conditions when pigs eat flies that have fed on infected blood within 24 hours.[72]
- 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.[55]
- 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.[73]
- Under experimental conditions, ASFV genotype II Arm07 strain could survive in leeches and could be transmitted to pigs that consumed the leeches and water contaminated by the infected leeches.[74]

What do we need to know?

- What are the cellular entry receptor(s) for ASFV infection and which suid species express them?
- What is the vector competence of U.S. vector species (e.g., *Otobius megnini*, *Ornithodoros lagophilus*, *Ornithodoros kelleyi*, *O. coriaceus*, *O. turicata*, *O. puertoicensis*)? 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?
- Are peccaries/javelinas potential reservoirs of ASFV in the United States?
- Are White Collared Peccaries that inhabit the Southwestern United States susceptible to infection by ASFV genotype II?

- 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?[72, 74-75]

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.[64, 76]

- 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.[64, 77]
- Pigs experimentally infected with the highly virulent Georgia/2007 I ASFV isolate shed infectious virus before the onset of clinical signs, and were generally infectious for 3-7 days overall.[18]
- For genotype I, the latent infection period (onset of infectiousness) showed a median of 4.5 days determined by quantitative polymerase chain reaction (qPCR) detection of titer ≥1.92 log $TCID_{50}$ in oropharyngeal swab.[78] 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]

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, 48, 50, 79-83]

- In swine experimentally inoculated intramuscularly, the ASFV genome was first detected in the blood (2.2 ± 0.8 days post-infection) and then in rectal (3.1 ± 0.7 dpi), nasal (3.2 ± 0.4 dpi), and oral (3.6 ± 0.7 dpi) swab samples. Genomic DNA from ASFV was also detected in oral fluid samples collected using a chewed rope starting at 3 dpi.[84]
- The incubation period for genotype II strains, with swine experimentally inoculated by various routes, is approximately 4 days with a range of 1-13 days.[6, 79, 82-85] For intranasal infection, the incubation period was 5-20 days and was dose-dependent.[7]
- For genotype II strains, directly inoculated animals can have a shorter incubation period (around 4 days) than for direct contact (pig to pig or pig to contaminated secretions, around 9 days) or those indirectly exposed to contaminated air (around 12 days), and can vary by dose.[6, 48, 82]
- Clinical illness can last 6.3 days on average (range 0-18) for different genotype II strains, indicating that death can be rapid in experimentally infected individuals.[85] The duration of infectiousness is broad, ranging from 1-40 days, depending on the virulence of the ASFV strain.[6, 35, 85] For instance, the infectious period ranges from 1-6 days for a genotype II strain[6] and up to 20-40 days (depending on exposure dose) for genotype I strains.[35]

What do we need to know?

- What is the incubation period of the various genotypes of ASFV? Are there some strains with longer periods than others and are they infectious prior to the presence of clinical signs? Is this dose- and route-specific?
- What are the incubation periods for wild boar and warthogs and does the onset of infection coordinate 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.[77]

- The typical clinical signs of ASF vary depending on the virulency of the viral strain. Clinical courses of ASF have been characterized as acute – lethal, prolonged – lethal, chronic-like (transient or lethal), and acute transient (early or late virus clearance), all with distinctly associated clinical signs and pathomorphological lesions.[86]
- Thus, depending on the viral isolate, pigs can develop different clinical forms of disease that range from and are distinctly described as peracute, acute, subacute, and chronic.[64, 77, 87-88] Chronic infection is generally caused by low-virulence isolates that do not immediately kill the host.
- 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.[64, 89-91] Swine may die before onset of clinical signs.[64, 66, 77, 89-90]
- 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.[89] Other signs include hemorrhages or blue-purple spots on ears,[90] abdomen, and/or hind legs; ocular/nasal discharge; reddening or necrosis of skin; vomiting/constipation/diarrhea; leukopenia and thrombocytopenia; and abortion in pregnant sows.[77] Death may occur as early as 6 days (highly virulent strains) or up to 20 days (moderately virulent strains).[77, 90, 92-93] While not common, a few naturally occurring non-hemadsorbing strains exist that produce acute infection,[64] and deletion of a gene rendering the Georgia 2010 isolate non-hemadsorbing was not shown to affect virulence.[89, 94]
- 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.[90] 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.[66, 77, 90]
- Infection with an attenuated strain can produce a mild or subclinical disease.[95]
- Clinical signs of chronic ASF occur 14-21 days after infection.[77] Chronic infections have variable, mild clinical signs, complicated diagnoses, and develop over 2-15 months.[89-90] Survivors may carry the virus for life.[90]
- Clinical signs also vary depending on age,[96] breed, route of exposure, and whether the virus is endemic to the region .[64, 77] Clinical symptoms in several individuals may need to be observed before clinical diagnosis is pursued.[91]
- Post-mortem findings include enlarged red to black and friable spleen; hemorrhages on kidneys, lymph nodes, and mucous membranes; enlarged lymph nodes; excess fluid in body cavity and around heart; and pneumonia.[77]

Morbidity is high for all forms of disease; mortality rates vary, but can reach 100% in peracute/acute infections.[64, 77]

- Lethality ranges from <20% in chronic forms to, 30-70% in subacute forms, to 100% in peracute/acute forms.[76-77, 90]

Symptoms of ASF are similar to other pig diseases such as CSF and necessitate diagnostic tests.

- 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.[77, 90]

- 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.[64, 77]
- All members of the Suidae (pig) family are thought to be susceptible to infection; however, African wild pigs, warthogs, and bush pigs, are frequently asymptomatic carriers of ASFV.[64, 77, 90] Warthogs exhibit transient viremia, but the mechanisms of tolerance are not well understood and is thought to be a combination of genetic and environmental adaptive responses.[97] In Mozambique, a population of domestic pigs was found to have increased resistance (circulating antibodies), but the exact mechanism of resistance is unknown and was not heritable.[98] Early historical accounts indicate that peccaries (*Tayassu* spp.), pig-like animals similar to wart hogs (also called javelinas), may be resistant.[64-65]
- There are 24 known ASFV genotypes, and clinical presentation may differ for these genotypes.[99]

What do we need to know?

- Variable presentation of clinical signs complicates diagnosis. What signs can be used for early detection?[100]
- 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?

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).

- Biosurveillance measures employed in infected regions and adjacent areas should include early recognition and rapid laboratory testing.[89] It is unclear whether sero-surveillance is ongoing in ASFV-positive countries neighboring the European Union.[15]
- The U.S. Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS) Foreign Animal Disease Preparedness & Response Plan (FAD PReP) sets guidelines for surveillance and biosecurity of ASFV.[64]
- 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.[64] Suspected cases are reported to local, state, and federal animal health officials, after which samples are collected by a Foreign Animal Disease Diagnostician.[101]
- Active surveillance involves testing the samples collected from passive surveillance, gathering and testing samples from areas of slaughter or dense swine population, and testing feed. USDA APHIS has integrated testing and data for both ASF and CSF.[102]
- Modeling suggests that passive surveillance of wild boar carcasses results in higher detection rates than active surveillance,[103] but these surveillance strategies require extensive effort.[104] Transmission from wild boar to livestock is suspected, but not well documented, and a lack of biosurveillance and lack of implementation of control measures may have contributed to ASFV endemicity in European wild boar.[46] Modeling can be used to identify high-risk locations with regard to spread to or from wild populations.[105]
- The USDA ASF response plan includes the establishment of quarantine zones upon suspected ASFV infection or positive ASFV result of either pig or premises. The average minimum diameter of infection zone (IZ) containment is 1.86 miles (3 km) per positive case

or premises, with a buffer zone an additional 1.24 miles (2 km) from the IZ; however, the actual zone diameter is dependent upon many factors.[64]

- ASFV-positive samples were reported in Haiti and the Dominican Republic in 2021. On September 24, 2021, the United States established its first protection zones for Puerto Rico and the U.S. Virgin Islands. Measures implemented included increased laboratory capabilities within the United States; enhanced surveillance in Puerto Rico, U.S. Virgin Islands, and the United States; and awareness and training programs for professionals and the public.[106]

ASF is difficult to distinguish from CSF and other diseases via clinical signs and post-mortem examination.[90] ASF should be considered in swine with febrile disease, disseminated hemorrhage, and high mortality.

- Clinical evidence can be non-specific, so active surveillance should be based on rapid diagnostic testing.[89, 107] 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.[77, 107] In the case of acute infection, death may occur before antibodies are produced.[89] 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.[108]
- PCR is the most sensitive technique and can detect ASFV DNA at early stages of infection (within 3-4 days).[90] Enzyme-linked immunosorbent assay (ELISA) can detect antibodies 7-14 days post-infection, with utility for confirmation of clinical cases;[90] antibodies may last for months to years.[62, 107] There is a field-deployable genetic test to detect ASFV under study.[109]
- The blood, spleen, kidney, lymph node, lung, bone marrow, and tonsil can be used for virus isolation or detection of antigen.[89-90] Serum for serological tests can be collected 8-21 days after infection.[90]
- Oral fluids have proven compatible with PCR methods and could prove useful for early detection of ASF.[84, 110]
- ASFV genome and antibodies are detectable in meat exudate and provide an alternative method for disease surveillance.[111]

Rapid ASFV detection methods are being researched to improve early detection and response.

- A portable real-time recombinase-aided amplification assay has a 20- to 30-minute detection time.[112-113] A recombinase polymerase amplification-CRISPR assay has also been tested.[114] A handheld portable reverse transcription-polymerase chain reaction instrument has been successful in the field.[115]
- A chemiluminescence assay has been developed based on anti-p54 monoclonal antibody that has high sensitivity, specificity, and reliability for ASFV detection.[116]
- A novel ASFV test method using dual quantum dot microsphere (QDM) probes of recombinant ASF virus protein 30 and 54 were used as a QDM based-ASFV immunosensor. This test allows for a 25-minute ASFV field detection test, with test results more sensitive than either ELISA or colloidal gold immunochromatographic strip methods.[117]

What do we need to know?

- What biosurveillance systems can be implemented in endemic areas?[118]
- How can diagnostics be improved to overcome current short-comings?[62]
- Should *Ornithodoros* species-specific surveillance occur throughout the United States to monitor ASFV presence?[119]

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 vaccine or treatment for ASF.

- 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.[64]

A number of vaccine candidates have been tested, including inactivated vaccines, live attenuated vaccines, and subunit vaccines, but protection and side effects vary.

Inactivated Vaccines

- As of 01 March 2023, inactivated ASFV vaccines have not demonstrated protection.[120-122]

Attenuated Vaccines

- Live, attenuated vaccines (LAVs) have had some success at protecting pigs from subsequent challenge,[79, 123] though this protection does not usually extend to other ASFV strains.[124] Live vaccines can cause their own side effects, including chronic ASF that later regains virulence or limiting fertility.[125] To improve safety of live attenuated vaccines, a number of deletion mutants have been created,[126] though these show variable efficacy[127-129] and protective effects may not last long.[130]
- Deletion mutation LAVs (e.g., Lv17/WB/Rie1) are being investigated for use in wild boar populations to reduce likelihood of transmission to domestic swine, with some evidence they are safe and do not shed virus into the environment.[131-132]
- Researchers with USDA Agricultural Research Service have recently identified a promising deletion mutant vaccine providing protection from the Georgia 07 isolate (a genotype II ASFV).[133] A dose of 10^2 HAD_{50} of SFV-G-Δ177L was able to protect 100% of both European (Yorkshire and Landrace) and Asian (Vietnamese Mong Cai) pig breeds from an intranasal challenge.[134] This is the first evidence of a vaccine derived from the Georgia 2007 ASFV isolate showing protection in Asian pig breeds and from an Asian ASFV strain. 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.[135]
- The deletion of genes, A137R of the Georgia 07 isolate and I226R from another genotype II ASFV (SY18), have been shown to attenuate the virus and offer protection from virulent challenge and are experimental vaccine candidates.[136-137]
- Real-time PCR assays (qPCR) have been developed that detect the difference in blood samples between infected and pigs vaccinated with three recombinant live, attenuated vaccine candidates.[138] Research on differentiation of ASFV-infected from vaccinated animals (DIVA) through PCR and ELISA-based methods are ongoing in China.[139] Deletion of the non-essential MGF110-5L-6L gene in the ASFV-G- ΔI177L vaccine platform was considered as a DIVA marker; however, deletion of MGF110-5L-6L reduced the protective efficacy of the vaccine, highlighting the complexity of identifying DIVA markers.[140]

Subunit Vaccines

- Protein subunit vaccines, which help generate antibodies, have shown some protective efficacy against ASFV,[141-143] though the effects are not universal.[144-145]
- Other subunit vaccines, delivered viral, bacterial, or plasmid vectors have also been developed,[125] and have granted either no,[146-148,149] partial,[147, 150] or robust[151] 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.[152-155]
- A cell-penetrating peptide (called Z12) was used to deliver a fusion protein (p30/p54) subunit vaccine that elicited a robust immune response in inoculated mice.[156] *In vivo* trials with swine are needed to estimate protective efficacy.
- There has been some evidence of vaccine-induced disease enhancement, where

vaccinated pigs have developed more severe disease upon secondary challenge.[153, 155]

Severity of clinical signs in domestic swine may be influenced by the gut microbiota.

- The gut microbiome has been shown to play a role in susceptibility to low-virulence ASFV strains, as pigs inoculated with fecal microbes from warthogs showed reduced clinical signs when infected with a low-pathogenicity ASFV isolate.[97]

What do we need to know?

- What are the roles of pro-inflammatory responses and specific cytokines in pathogenesis?
- 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?[157]
- 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?[158]
- Are mRNA vaccines being explored in addition to LAV and DNA subunit?
- What are the barriers for designing a test for DIVA?[91]
- Are there treatments that reduce transmission potential in already-infected swine?

Virus Importation
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 spread throughout the country and to neighboring Haiti.[83] According to WOAH, the outbreak is on-going as of 01 March 2023 with the most recent report on 20 December 2022.[159]

- As of 01 March 2023, 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.[160] A FAD protection zone was established around Puerto Rico and the U.S. Virgin Islands. The USDA has 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.[160]

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.[119]

- 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.[64, 161]
- An experimental study duplicating feed mill contamination demonstrated that an ASFV-contaminated ingredient introduced to a feed mill results in detectable ASFV spread throughout the facility,[21] which demonstrates how imported ASFV-contaminated grain could

cause ASFV spread in the United States.

- A known vector of transmission for ASFV, is the *Ornithodoros* soft ticks,[28, 119, 162-165] and in particular the *Ornithodoros turicata* (Duges) has been collected from a feral pig in Texas, suggesting that if introduced, it could be a possible source of transmission of ASFV through the sylvatic cycle.[166]
- A recent expansion of the feral cross-bred (*Sus scrofa* x *Sus scrofa domesticus*) swine population in Canada has caused concern about the spread of this species into Northern U.S. states as it is a potential source of transmission of ASFV.[167-168]

Human-mediated import likely contributed to the spread of ASFV in Europe, possibly through illegal importation of infected meat, hunting tourism, or fomites associated with farmers, farming professionals, and importation of bedding.[169]

- ASFV moved from Western Poland to Germany in 9 months.[170] 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.[171]
- 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 ASFV detection in confiscated illegal products at ports of entry. There has been and an increase in the monthly number of merchant ships and value of imported goods and percentage of commercial flights from ASFV affected countries, primarily from China. [172]
- Illegal entry of pork, ham, and sausage products is a potential pathway for introducing ASFV,[161] for instance by international travelers who do not legally declare items. Although fomites and feed ingredients are potential pathways, there are minimal data on transmission and lack of quantification of infectious dose that remains, for example, on footwear.
- 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 United States.[173]
- There are no high-risk means of legal importation, but animal feed, regulated garbage, and swine products and by-products have non-negligible importation risk.[161, 174-176] Pig movement networks should be monitored to ensure earlier detection and response.[177-178] In addition, five U.S. seaports receive 91% of the imported soybean oilcake feed from India and surveillance at these locations could be extremely effective.[174] Similarly, it is estimated that >90% of the risk from ASFV importation via air travel involves five airports from China, and eastern European countries.[179] For illegal entry pathways, swine products and by-products are considered high-risk, while meat and hunting trophies, animal feed, fomites, and swine semen are associated with non-negligible importation risk.[161]
- The time required to reduce ASFV by 99.99% (3-log reduction) was 113 to 135 days in conventional soybean meal, 150 to 186 days in organic soybean meal, and 142 to 168 days in choline chloride.[180]
- One study determined that the mean annual probability of ASFV importation into the United States via contaminated pork products prior to 2016 in air passengers' luggage is 0.061%; meaning ASFV could be expected to enter the United States illegally once every 17 years. This value has been revised to a mean annual probability risk of 0.21 prior to customs inspection, and 0.11 after customs inspection. ASFV-contaminated agricultural products have been seized at airports in South Korea, Japan, Taiwan, Thailand, Australia, the Philippines, and Northern Ireland.[179]
- Clothing that has been in contact with infected pigs can be a source of spreading ASFV to other farms or countries.[181-182]

What do we need to know?

- What is the stability of infectious virus on fomites (e.g., on footwear)?[91]
- What is the potential underreporting of visits to foreign farms and would this affect the risk of ASFV entering the United States?
- How stable is ASFV through processing and shipping steps of potentially contaminated animal products and swine feed?
- How much virus may be present in various raw, dry-cured, or heat-processed pork products that were produced from an infected carcass?
- 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 are potential wildlife reservoirs for ASFV in North America?[67]

Viral Persistence / 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. [22, 183-184] Of note, infection of domestic pigs via fomites is not well characterized.[16]

- 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.[22]

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, 185]

- 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,[64, 89] and is extremely stable at low temperatures.[185]
- In unprocessed pig meat, the virus is stable for weeks to months;[15] heating to 70°C for 30 minutes destroys the virus.[89]
- Pigs with less virulent strains can transmit the virus up to one month after infection; blood remains infectious for up to 6 weeks. In acute infections, all body fluids and tissues are infectious from onset of clinical disease until death.[89]
- Stability in salted and smoked/cured meats ranges have been reported from 30-399 days.[15, 185] *In vivo* experiments showed detection in Italian salami, pork belly, and loin, up to 18, 60, and 83 days, respectively.[186]
- Stability in blood is dependent on temperature; at 4°C, early studies reported persistence in lyophilized blood in ampules up to 2,900 days, while more recent studies report up to 540 days[15] or 105 days in putrefied blood.[183]
- Virus in body fluids and serum is inactivated at 60°C for 20 minutes.[76] It is stable and infectious for months at room temperature and at least 61 weeks when stored at 4°C.[89, 185]

Infectious virus can be shed in urine and feces for approximately 2 weeks, depending on environmental temperature. Excretions should be considered a viable pathway for transmission.[187]

- Of biological samples collected 5 days post-infection, 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.[188]

- Stability studies of the Georgia 2007/1 strain suggest that feces will retain infectious virus for up to 5 days at 4-12°C, 3 days at 21°C, and 1 day at 37°C.[187] Georgia 2007/1 strain has been detected in urine for up to 5 days at 4-12°C and 1 day at 37°C.[187]

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

- It is assumed that due to viral stability and high titers, carcass disposal remains a high priority.[189] However, the actual rate of transmission is unclear; in one study, excavated carcasses in Lithuania demonstrated presence of ASFV genome although infectious ASFV could not be isolated.[190] Using an Estonian 2014 isolate, another study suggests that infected carcasses may remain infectious for nearly 2 years.[44]
- Infectious virus could be found in yard soil for 1 week and sandy soil for 3 weeks, though none could be isolated from acidic forest soils.[191] Lower temperatures are conducive to persistent infectious virus stability in soil.[191-192]
- ASFV was inoculated into soil and stability was tested in the laboratory at 22°C and 4°C. Infectious ASFV was detected after 112 days of incubation at 4°C both with and without the sheep erythrocytes. Infectious ASFV was detected through 22 days incubation (soil only) at 22°C and 42 days (soil + erythrocytes).[192]
- Composting wild boar carcasses in straw and sawdust during winter months has been demonstrated to inactivate ASFV in a time- and temperature-dependent manner.[193]

ASFV is stable in animal feed (half-life of 2-14 days),[180, 194] which is a potential route of transmission.

- The use of uncooked swill has proven as a significant source of contamination and transmission among pigs.[195] A modeling study ranked compound feeds and cereals as the highest potential to introduce ASFV in pig farming.[196]
- Transmission via contaminated feed is possible and is dependent upon biosecurity conditions during processing, packing, and transport.[197] Studies show viable virus pathogens in feed and water contaminated by blood can last 30 to at least 60 days (extent of study) when stored at 4°C, but only 1 day in feed and up to 50 days in water when stored at room temperature.[198]
- Animal feed trucks have been commonly identified as a common site of ASFV source contamination.[182]
- Although spray-drying can inactivate ASFV,[199] dietary supplements contaminated after spray-drying maintained infectivity for 5 weeks at 4°C and had a > 5.7 $\log_{10}$ loss when stored at 21°C for 2 weeks.[200]
- Infectious ASFV was found in feed dust in processing equipment after 30 minutes, but not found 480 minutes post-processing.[175]
- The stability of Georgia 2007/1 was tested in three feed matrices (complete feed, soybean meal, ground corncobs) at three storage temperatures (40°F, 68°F, 95°F) for up to 365 days. ASFV DNA was highly stable and detectable in almost all feed matrices through the end of each study. Infectious ASFV was most stable in soybean meal, maintaining infectivity for at least 112 days at 40°F, at least 21 days at 68°F, and at least 7 days at 95°F.[17]

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?[176]
- 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?[191]

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 an EPA-registered disinfectant.[201]

- If a suitable commercially available EPA and state-registered disinfectant is not available, only then may a disinfectant approved for use against ASFV under Section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (i.e., a Section 18 disinfectant) be used according to its Section 18 label. There are currently three Section 18 exemptions approved for use against ASFV; the labels and EPA approval letters are available online from APHIS.[201]

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

- **In an agricultural setting,** recommended decontamination options are Virkon S (10 minutes), Clearon Bleach Tablets (30 minutes), Klor-Kleen (30 minutes), Klorsept (30 minutes), Klorkleen 2 (30 minutes), and Accel Concentrate Disinfectant Cleaner (5 minutes).[201] This efficacy may be improved by allowing farms to remain empty for 1 to 2 weeks.[202]
- **In pork packing plants,** decontamination should be done with acid-based disinfectants (such as CD631, acid quaternary ammonium-based) following the manufacturer's instructions, specifically regarding pre-washing procedures. Hypochlorite-based disinfectants such as XY12 and bleach should be avoided when organic load (e.g., blood, feces) is high. Also, concrete surfaces should be sealed to render them non-porous in order to allow appropriate disinfection.[203]
- **In transportation vehicles,** decontamination options are Virkon S (10 minutes, animal transport and regular transport vehicles), Klorsept (30 minutes, animal transport vehicles), and Klorkleen 2 (30 minutes, animal transport vehicles). If none of the above are available, FIFRA Section 18 options are sodium hypochlorite (15 minutes non-porous, 30 minutes porous) and Benefact (15 minutes on non-porous surfaces inside and outside aircraft).[201, 204]
- Disinfectants used in an enhanced ASFV control program on pig farms in China include strong alkalis, surface active agents, or strong oxidants (3% and 5% sodium hydroxide), calcium hydroxide, glutaraldehyde, formalin and potassium permanganate, potassium persulfate, chlorine dioxide, povidone iodine, glutaraldehyde decyl ammonium bromide solution, and sodium tricholoroisocyanurate.[202]
- Either 2% citric acid or a high concentration of sodium hypochlorite (2,000 ppm) can disinfect the virus on wood.[76]
- ASFV can be inactivated by sodium hydroxide (0.8% 30 minutes, 2-5%),[90, 202] calcium hydroxide (10-20%),[202] quaternary ammonium compounds,[205] hypochlorites (between 0.03% and 0.5% chlorine for 30 minutes), 3/1000 formalin (30 minutes), 3% ortho-phenylphenol (30 minutes), iodine compounds,[90] flame guns, glutaraldehyde, potassium persulfate (0.1%),[202] and 5 to 20 mg/L of ozone in water.[206] Virkon S also inactivates ASFV on stainless steel or concrete.[207]

ASFV is highly resistant at low temperatures and endures 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).[64, 76, 185, 208]

- A detailed decontamination table with benefits and limitations of multiple methods is available from USDA APHIS[209] or from The European Cooperation in Science and Technology.[210]
- 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.[176] Heat treatments (30 minutes at 70°C; 5 minutes at 85°C) increase viral decay and reduce the risk of contamination in feed.[211]
- 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.[199] 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.[212]
- A quantitative risk assessment model suggests minimal risk of ASFV contamination from soybean feed, assuming that thermal processing has inactivated any ASFV present in the feed prior to processing and no recontamination occurs.[213]
- Physical similarities (e.g., DNA genome and double membranes) suggests the Modified Vaccinia Ankara Virus (MVAV) may be a suitable surrogate for ASFV disinfection studies.[214]
- Disinfection study of both MVAV and ASFV in soil showed 0.1% peracetic acid solution was able to reduce viral load by 4 logs.[214]
- Slaked lime, milk of lime, and quick lime in forest soils showed at least a 4-log reduction of MVAV and ASFV virus titer.[215]
- Povidone Iodine (PVP-I, common name Betadine) and proprietary iodine complexes were compared for inactivation of ASFV,[216] and was able to inactivate ASFV at lower concentrations and contact times, while also being less cytotoxic.

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?
- What are the most effective storage parameters to reduce ASFV survival in animal feed?
- Current analytical methodologies have significant limitations in sensitivity, repeatability, ability to detect viable virus particles and association with infectivity. How can this be improved?[217]

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

What do we know?

The guidance for PPE follows 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 hooded coveralls, gloves, boots, goggles or a face shield, and waterproof boots that can be cleaned and disinfected will be required. Respiratory protection will be based on the specific situation and any environmental risks.[64]
- 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 such as surveillance, depopulation, disposal, and cleaning, and disinfection.
  - Exertion levels and the extent of physical work at premises.
  - Ambient temperature, relative humidity, and other conditions independent of the event.
  - Length of time PPE must provide a specific level of protection.
  - Classification of premises.[218]
- The Food and Agriculture Organization (FAO) recommends when working with ASFV to have these items to ensure good biosecurity when entering a farm: one pair of good-quality gumboots that are easy to clean and disinfect, disposable biosecurity suit, waterproof suit if required (in cold and wet countries), safety glasses for eye protection, overshoes or boot covers, examination gloves (ensure correct fit), plastic mat, buckets (three ideally), detergent, disinfectant (approved for ASFV), scrubbing brushes (two), refuse bags (including biohazard bags), Ziplock bags (for transporting phones or other equipment), disinfectant wipes for face, sealing tape, scissors, and GPS device to record geocoordinates.[77]

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.

- Recommendations for PPE may vary based on the classification of the premise due to the differences in risk of exposure to a hazardous agent. For example, PPE may be different when working at an infected premise than when working on surveillance at a premise in an infection-free area.[64]
- Commercially available PPE for prevention of the spread of infectious agents as well as protection from decontamination chemicals are available and marketed toward ASFV work, such as those offered by Dupont.[219]
- Additional general (non-ASFV specific) information can be found in FAD PReP Standard Operating Procedures (SOP): Health and Safety/Personal Protective Equipment (PPE).[218]
- Zoo staff should wear protective gear such as gloves and boot covers when handling or entering a new animal's enclosure.[220]
- Recommendations include providing external visitors with farm-specific protective clothing and footwear, and changing clothing and thoroughly washing hands after carcass disposal.[221]

In most cases, protective hooded coveralls, gloves, boots, goggles or a face shield, and waterproof boots that can be cleaned and disinfected will be required.

- 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.[218]

What do we need to know?

- Are there specific types/brands of PPE more effective for ASFV than for general use in animal disease responses?
- What are the effects of decontamination methods on PPE/personnel?
- Can more effective PPE be developed?

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

What do we know?

There are several methods for effective depopulation of confirmed or suspected ASFV-infected swine in an agricultural setting, including inhalant (CO_2 gas), gunshot, penetrating or non-penetrating captive bolt, electrocution head to heart, veterinarian-administered anesthetic overdose, and in certain permitted circumstances, ventilation shutdown or sodium nitrate.[209] 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.

- 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).[218]
- The size of the pigs to be depopulated can help determine the method that best fits the urgency of the situation. Swine can vary in size from 1 lb (0.5 kg) for neonates to over 600 lb (272.7 kg) for mature breeding stock.[222]
- Gunshot and veterinarian-administered anesthetic overdose can be used for pigs of any size, as can ventilation shutdown or sodium nitrate in approved constrained circumstances. Penetrating captive bolt and electrocution (head to heart) are additional options specifically for pigs over 100 lbs, and CO_2 inhalant or non-penetrating captive bolt are additional options available for pigs under 100 lbs.[209]
- Other factors to take into consideration when choosing which depopulation method is most appropriate are the skill level of the person/facility performing the procedure, the risk to human health involved in each technique, and if there are carcass disposal restrictions (as with veterinarian-administered overdose) or biosecurity risks of blood contamination (as with gunshot or penetrating bolt electrocution).[209]
- 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.[91, 223]

Carcass disposal and environmental regulations differ by setting and state.[209]

***Disposal in Agricultural Settings*[209]**

- Animals should be tested for ASFV to determine proper depopulation and disposal methods.
- Composting is likely to inactivate ASFV by multiple mechanisms, though infectious surrogate viruses porcine reproductive and respiratory syndrome virus and porcine epidemic diarrhea virus was found for up to 2 weeks during carcass composting.[224] When testing specifically for ASFV, compost samples that were positive at Day 0 with titers of $10^{5.5}$ to $10^{6.3}$ HAD_{50} were found to have no infectious ASFV by Day 3, even though ASFV DNA was found in compost samples up to 21 days.[225]
- Composting effectiveness can be enhanced when accompanied by grinding of carcasses; however, aerosol production is a concern.[226]
- Open burning is likely to inactivate ASFV but is also a potential to cause adverse human health impacts.
- Mobile incineration is safer than open burning but has limited capacity.
- Landfill is a negligible risk if leachate passes to a wastewater treatment step; ASFV-negative herds should be prioritized for landfill.
- ASFV-negative herds should be prioritized for rendering if renderers will accept non-infected material from infected premises or control area.
- Incineration/energy-from-waste is highly likely to inactivate ASFV but requires packaging

carcasses.

Processing Plant

- Carcass disposal and environmental regulations differ by state. A comprehensive list of approved methods and regulatory considerations is available from the U.S. Veterinary Compliance Assistance Group.[227]
- The competent authority in charge of carcass disposal regulations may vary by jurisdiction and by situation. In situations where the disposal of carcasses is necessary beyond what is legally allowed, it is imperative that the competent authority be contacted to approve carcass disposal plans.[222]

Tools and SOPs for depopulation exist.[228]

- USDA APHIS maintains a Carcass Management Dashboard, which contains information on disposal options for planning or response purposes.[209, 228]
- NAHEMS guidelines/SOPs includes detailed guidelines for 23 critical activities such as disposal, depopulation, cleaning and disinfection, and biosecurity that are essential to effective preparedness and response to an FAD outbreak.[218]
- 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.[190, 229]
- Research is being done on composting techniques as a means of safe disposal of carcasses, which requires less land space, and destroys tissues faster, thereby more quickly reducing the viable virus.[224] Recent studies during cold winter temperatures in Europe show open-air composting inactivates ASFV in wild boar in a time- and temperature-dependent manner, with infectious virus recovered up to 35 days later; however the viral genome was still detectable through the 112-day study.[193]

What do we need to know?

- Are there other depopulation methods that would limit environmental viral transmission?
- Are there other carcass disposal methods that would limit environmental viral transmission?
- 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.[230]

- When infected swine tissue (e.g., muscle) is soaked in water, ASFV DNA can be detected in the solution for up to 24 months (by PCR), and infectious virus can be detected for 0-8 months.[44] Results, though, depend on temperature and tissue type,[44] and the study did not test wastewater *per se*.
- 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,[231] which is a potential route for ASFV, as organic material in wastewater can protect the virus from inactivation[229] and the infectious dose via ingestion is thought to be extremely low (only a few particles, or 1 $TCID_{50}$).[4] In fact, ASFV sequences were found in samples from a wastewater treatment plant in Spain[232] and proximity to water was identified as a possible risk factor for an ASFV outbreak in Romania centered around the Danube Delta Biosphere Reservation.[189] ASFV was also detected in farming wastewater samples in

China in late 2017 and early 2018, prior to the epidemic, which began in Aug 2018.[233]

- Pig slurry can harbor ASFV for 84-112 days depending on temperature[234] and ASFV can survive in pig feces for 11 days at room temperature.[235]
- Contaminated water sources can harbor ASFV for 50-60 days, with colder temperatures facilitating persistence.[198]

Chemical disinfectants and heat are able to inactivate ASFV in pig slurry,[236] though effectiveness in wastewater systems needs to be determined.

- In concentrated pig slurry (i.e., liquid manure), a 1% solution of NaOH or $Ca(OH)_2$ at 4°C or 22°C for 5 minutes was sufficient for inactivating ASFV.[236] Treatment of wastewater with NaOH or $Ca(OH)_2$ reduces virus in wastewater as well.[237] Alternatively, heating the slurry to 56°C for 90 seconds inactivated the virus;[236] it is unknown whether these methods would also apply to wastewater *per se*.
- 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).[209]
- Germany recommends several methods of slurry disinfection, including $Ca(OH)_2$ (40% solution at rate of 40-60 l/m^3 for >4 days), NaOH (50% solution at 16-30 l/m^3 for >4 days), formalin (35-37% solution at rate of 24-40 l/m^3 for 4 days), and peracetic acid (24-40 l/m^3 for >1 hour), though these values have not been tested explicitly with ASFV.[234]
- Decontamination of wastewater with ozonated water is also being investigated.[206]
- The FAO does not have recommended decontamination procedures for water, but recommends burying, burning, alkalis, or acids for effluent and manure disposal.[238]

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 170-180kb genome,[239] and the sole member of its family (*Asfarviridae*).[184]

- ASFV is endemic to Sub-Saharan Africa and was first identified in Kenya in 1921.[62]

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.[240]
- African outbreaks of ASFV are associated with a highly diverse pool of viruses of multiple genotypes, even within relatively restricted geographies.[241-248]

- 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.[241, 243-245, 247-248]
- 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.[240] 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.[240]
- 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.[62, 240] In 2021, ASFV was detected in the Dominican Republic and spread throughout the country and to neighboring Haiti.[83]

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.[242, 249]

- ASFV evolves faster than other large DNA viruses; its evolutionary rate is closer to that of some RNA viruses[250] with recombination playing a major role.[242, 251] While the major capsid protein involved in cell entry does not appear to be under selective pressure,[252] at least one immune evasion protein appears to be a hotspot for recombination,[251] 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.[253] In fact, the globally circulating strain maintains > 99.9% sequence identity with the strain that emerged from Africa around 2007.[243, 254-257] Despite the similarity, *in vivo* studies have shown variability in virulence,[258-261] suggesting more work is needed to understand the genomic markers related to virulence.[262-263]

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.

- 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. More work is needed on additional genetic factors that play a role in virulence, but this provides a framework for vaccine development to prevent future pandemics.[264-266]
- A study showed that the deletion of EP153R, EP402R and MGF_360-12L-14L genes caused the virus to decrease in pathogenicity, indicating that these genes are essential for viral infection and propagation.[267]
- Deletion of 11 genes within the MGF300 and MGF360 regions reduces virulence compared to the parent strain.[268]
- The ASFV strain, SF/VN/CanTho-OM/2021, which caused an outbreak in the Mekong Delta, was found to have a unique sequence in the EP402R gene that contains an 18 base pair (bp) deletion.[269]
- The H240R gene has been associated with regulating the structure of the ASFV capsid and deletion of this gene decreases infectious titer.[270]
- ASFV with the H108R gene removed were shown to grow slower in cultures and to be less virulent in animal studies.[271]
- Deletion of the dUTPase gene, E165R, from the ASFV Georgia 2010 stain has been shown to not have a significant effect on the replication of the virus.[272]
- Removing the EP296R gene from ASFV Georgia 2010 caused the virial infection to develop slower, but it maintained lethality and viral reproduction.[273]
- The E184L gene has been highlighted as a novel virulence factor. Infection with an E184L

deletion mutant in animals showed reduced virulence and immune protection against non-deletion strains.[274]

- The DP148R gene has been highlighted as a virulence factor. Infection with an DP148R deletion mutant in animals showed reduced virulence and immune protection against non-deletion strains.[264]

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?

Commonly Used Acronyms and Abbreviations

Acronym/Term	Definition	Description
APHIS	Animal and Plant Health Inspection Service	N/A
ASF	African Swine Fever	The disease caused by ASFV
ASFV	African Swine Fever Virus	Causative agent of the disease African Swine Fever (ASF)
CSF	Classical Swine Fever	The disease caused by Classical Swine Fever Virus. This 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
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	A reaction occurs in erythrocytes in the presence of hemagglutinin, and this 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
MQL	Master Question List	N/A

Acronym/Term	Definition	Description
MVAV	Modified Vaccinia Ankara Virus	An 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 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 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	World Organisation for Animal Health	N/A

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