DHS SCIENCE AND TECHNOLOGY

Master Question List for African Swine Fever (ASF) Virus

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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.

Homeland Security

Science and Technology

FOREWORD

The Department of Homeland Security (DHS) is paying close attention to the worldwide spread and impacts of the African Swine Fever (ASF) Virus, to best prepare for the possibility of it reaching domestic swine populations in the United States. In accordance with Homeland Security Presidential Directive (HSPD) 9, as well as the Securing our Agriculture and Food Act of 2017 (Public Law 115-43), DHS, specifically the DHS Countering Weapons of Mass Destruction (CWMD) Office, has the responsibility to coordinate interagency activities to defend U.S. agriculture and food sectors against terrorist attacks, major disasters, and other emergencies. DHS, especially DHS Science and Technology Directorate (S&T)'s facility at the Plum Island Animal Disease Center (PIADC), is working closely with the U.S. Department of Agriculture (USDA), other federal agencies, and domestic and international partners, to conduct research on the ASF virus. These efforts are undertaken to understand prevention, transmission, impacts, and potential countermeasures and other mitigation efforts for this agricultural pathogen, whose worldwide spread has resulted in the loss of hundreds of millions of domesticated swine and poses a catastrophic risk to U.S. pork production if it were to be introduced into the United States.

The purpose of this document is to enhance scientific collaboration on ASF research by creating a central, regularly updated clearinghouse to communicate the current state of ASF research, allow information sharing and input from ASF researchers and generally promote domestic and worldwide cooperation on this global threat. Based on the response to similar products generated for the recent SARS-CoV-2 outbreak and the 2014 Ebolavirus outbreak in West Africa, DHS S&T developed the following "master question list" for the ASF virus that quickly summarizes what is known, what additional information is needed, and who may be working to address such fundamental questions as, "What is the infectious dose?" and "How long does the virus persist in the environment?" The Master Question List (MQL) is intended to quickly present the current state of available information to government decision makers in the response to a potential future outbreak of ASF in the United States, to allow 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.

The information contained in the following table has been assembled from publicly available sources to include reports and articles found in scientific and technical journals, selected sources on the internet, and various media reports. It is intended to serve as a "quick reference" tool and should not be regarded as a comprehensive source of information, nor as necessarily representing the official policies, either expressed or implied, of DHS or the U.S. government. DHS does not endorse any products or commercial services mentioned in this document. All sources of the information provided are cited so that individual users of this document may independently evaluate the source of that information and its suitability for any particular use. This document is a "living document" that will be updated as needed when new information becomes available.

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Table of Contents

Infectious Dose – How much virus will make a healthy pig ill?

What do we know about ASFV?

The median infectious dose 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 median hemadsorbing dose (HAD_{50}), which is the preferred method for quantifying ASFV infectivity, is the dose (titer) of virus that it would take to infect 50% of inoculated pigs. Other more common virological methods like $TCID_{50}$ or PFU 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.
- The method to determine the virus titer is based on the adsorption of swine red blood cells to ASFV-infected monocytes. Initially, monocytes from uninfected swine are exposed to an aliquot of ASFV virus. Then, uninfected swine red blood cells are incubated with the monocyte and ASFV sample mixture. Following an incubation, the ASFV will infect the monocytes and when infected monocytes are in the presence of swine red blood cells, the red blood cells form a rosette pattern around the infected cell, which can be readily visualized and counted.[34, 37]

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

- The infectious dose in approximately 8-week-old, crossbred pigs for a genotype II strain through ingestion of liquid contaminated with ASFV was very low, ~7 HAD_{50}.[82] For contaminated dry feed, the infectious dose was considerably higher at $4.4x10^6$ HAD_{50}.[82] This 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.[82]
- The infectious dose for a genotype II strain in 7-week-old domestic swine, inoculated by intramuscular injection is $\leq 10^2$ median hemadsorbing doses (HAD_{50}).[63] Other studies have shown the dose can be as low as 5 HAD_{50} for normal susceptible animals (5-6 weeks old)[131] and 3 HAD_{50} for runt (smallest or weakest of the litter at birth) animals (~8-week-old domestic swine and ~4-month-old wild boar) following oronasal inoculation.[97]

By some exposure routes, ASFV has a lower infectious dose than foot and mouth disease (FMD) in domestic swine.

- ASFV administered via the ingestion, oronasal, or intramuscular routes may have a lower infectious dose than foot and mouth disease (FMD),[112] which causes infection in swine after minimum doses of >1,500 infectious units by the inhalation route.[3] It should be noted, however, that the methods used to quantify infectious doses differ substantially across these diseases (for FMD, infectivity is quantified by tissue culture infectious doses, or $TCID_{50}$), and comparisons should be made with care. Additionally, variation in host and/or virus characteristics (e.g., strain, age, weight) make direct comparisons difficult.
- Studies in wild boars of various ages (9 weeks-10 years) with a pathogenic genotype II strain was equally lethal at high doses by oral and intramuscular inoculation, suggesting an infectious dose $<10^6$ $TCID_{50}$ ($<7x10^5$ HAD_{50}).[16, 43] Lower ranges still need to be defined.
- For genotype I strains, the infectious dose was $\leq 10^{3.5}$ $TCID_{50}$ by intraoropharyngeal inoculation.[33] The infectious dose for a genotype VIII strain (Malawi Lil-20/1, 1983 isolate exclusive to Africa) in ~3-month-old pigs was $\leq 10^2$ (HAD_{50}) by intramuscular injection and $\leq 10^4$ (HAD_{50}) by intraoropharyngeal or intranasopharyngeal inoculation.[67]
- Male and female wild boar appear equally susceptible to ASFV.[16]

What are the gaps?

- 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 is 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?
- Are White Collared Peccaries that inhabit the Southwestern United States susceptible to infection by ASFV genotype II?

Who is doing experiments in this area?

- **USA**: Department of Homeland Security (DHS) Science and Technology Directorate (S&T) Plum Island Disease Center (PIADC),[33] Kansas State University[82]
- **International**: Germany (Friedrich Loeffler Institute),[97] Kenya (East African Veterinary Research Organization),[60] Poland (National Veterinary Research Institute),[131] Russia (National Research Institute for Veterinary Virology and Microbiology),[23] Spain (Universidad Complutense of Madrid[109]; Centre de Recerca en Sanitat Animal [CReSA][8]), United Kingdom (Pirbright Institute)[63]

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

What do we know about ASFV?

ASFV can spread between domestic or feral 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;[16, 43, 66, 97] ingestion of contaminated pig products;[61, 82] direct contact between infected and susceptible domestic pigs;[61, 66] indirect contact through people, vehicles, fomites and contaminated pork products;[66] contact between infected wild pigs and domestic pigs;[109] vector transmission by ticks in the genus *Ornithodoros*;[70, 92, 98] and through ingestion of infectious stable flies (*Stomoxys calcitrans*).[87, 89, 99, 128]
- Direct contact transmission is most common from infected to susceptible pigs. Viral blood titers are generally higher in directly inoculated pigs, most likely due to systemic infection following intramuscular inoculation, and can be detected by day three post inoculation vs. contact-exposed animals, which can be identified with blood detection by day 10 post contact, with minimum infectious periods from 1-6 days for a genotype II strain[63] and maximum infectious period of 20-40 days depending on the dose for genotype I strains.[32]
- Aerosol transmission of a genotype II virus, between a directly inoculated pig and a nearby native pig with no direct contact, is possible with a delay in clinical signs for air contact pigs compared to directly inoculated pigs with infectiousness coinciding with clinical signs. Direct contact animals were infectious by day 12 and air exposed animals were infectious by day 15.[88]

ASFV is highly transmissible, with infected domestic swine each infecting an average of 2.8 other individuals sharing a pen.

- The basic reproduction number (R_0) of the genotype II, Georgia 2007/1 ASFV isolate was calculated as 2.8 (1.3-4.8) within pens and 1.4 (0.6-2.4) between animals in adjacent pens.[62] The estimated R_0 for a single genotype I virus (Malta'78 isolate) was 18 (6.90-46.9),[32] demonstrating high transmission potential in certain genotypes and strains. Not all are characterized.

ASFV is less transmissible than FMD in swine, in terms of the average number of infections arising from a single infected individual in an entirely susceptible population (R_0).[10]

- For genotype II strains, viral titers in blood samples can reach 10^6-10^8 HAD_{50}/ml, with titers of 10^4-10^5 HAD_{50}/ml in pharyngeal swabs and lower levels of virus in nasal and rectal swabs of 10^2-10^4 HAD_{50}/ml.[48, 63]
- Stability studies of ASFV in urine and feces suggest that feces will retain infectious virus at 4°C and 37°C for 8.48 and 3.71 days respectively, with infectious urine viable for 15.33 and 2.88 days.[31]
- Chronically infected pigs, those infected with low virulence strains that develop minimal to no clinical signs, can transmit virus to contact animals two months post infection with recoverable virus present in tissues at three months.[49]
- In persistently infected animals, those pigs that recover from initial infection but 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.[33] Persistence can occur with any ASFV strain.

Ticks are crucial for natural ASFV infection in warthogs (reservoir species), though stable flies may also play a role in transmission to swine.[17]

Vectors are not needed for transmission among domestic swine, or between domestic and feral swine.

- Studies in *Ornithodoros* species ticks showed that ticks ingest blood from infected pigs, can retain the infectious virus for months and further transmit the virus to pigs after three months.[99]

What are the gaps?

- What is the extent of aerosol transmission between domestic pigs in direct contact and those not in direct contact? Is there a threshold for the amount of virus or time necessary to transmit between domestic pigs?
- The ability of wild boars/feral pigs to transmit ASFV via aerosol is currently unknown.
- In the wild boar and feral swine population, how long can the transmission chain extend? Do viral titers in each successive transmission/infection event ever reduce below the level of transmission to another animal?
- Based on population dynamics, densities, and behavior, can wild boar or warthogs travel large distances and interact with other susceptible animals while they are infectious and before succumbing to disease if highly pathogenic? What are their behaviors that place them in contact with other wild animals or domestic pig facilities or farms?
- Are there effective methods to decrease the populations of wild boar in affected areas?[4] If so, what are these methods?
- How much is known about the tick vectors and their presence in areas with high populations of wild boar?[4]
- What is known about the presence of ASFV-competent tick vectors in the United States (*Ornithodoros coriaceus, O. parkeri* and *O. turicata*)?
- How do scavengers play a role in transmission and how long is the virus viable in an animal carcass at levels that could transmit?[101] What are the viral titers in the tissues of pig carcasses following death from a genotype II strain?
- Need to understand rate of transmission of strains with different virulence,[54] and the role of feral swine as reservoirs.

Who is doing experiments in this area?

- **USA**: Kansas State University[82]
- **International**: Germany (Friedrich Loeffler Institute),[16, 98] Netherlands (Central Veterinary Institute, University Utrecht),[33] South Africa (University of Pretoria),[92] Spain (Universidad Complutense de Madrid, INI-CISA [Centro de Investigación en Sanidad Animal], Spain),[49, 109] United Kingdom (Pirbright Institute)[30, 63]

Clinical presentation – What are the clinical signs of an infected animal?

What do we know about ASFV?

Clinical signs include fever, loss of appetite, dull/depressed, red/purple skin lesions, respiratory distress, vomiting/diarrhea/bloody diarrhea, high mortality, sudden death, and abortion.[15]

- The clinical signs of ASFV vary depending if the viral strain is classified as a high, moderate, or low virulence isolate. Depending on the viral isolate, pigs can develop different clinical forms of disease ranging from peracute, acute, subacute, and chronic.[15, 122] Chronic infection is generally caused by low-virulence isolates that do not immediately kill the host.
- Clinical signs also vary depending on the breed of pig infected, the route by which a pig is exposed, and whether or not the virus is endemic to the region.[15, 122]
- Postmortem 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.[15]

Morbidity is high for all forms of disease; mortality rates vary but can reach 100% in peracute/acute infections.[15, 122]

- Lethality ranges from <20% in chronic forms to approximately 30-70% in subacute forms, to 100% in peracute/acute forms.[15, 115, 133]
- Clinical signs of peracute infection occur within 3-7 days and include high fever (40.5-42°C), loss of appetite, hemorrhage, and inactivity.[90, 122, 133] Swine may die suddenly before onset of clinical signs.[15, 90, 122, 133]
- 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.[90] Other signs include hemorrhages or blue-purple spots on ears (appearance of lesions depends on isolate),[133] abdomen, and/or hind legs; ocular/nasal discharge; reddening of skin; vomiting/constipation/diarrhea; leukopenia and thrombocytopenia; and abortion in pregnant sows.[15] Death may occur as early as 6 days (highly virulent strains) or up to 20 days (moderately virulent strains) after infection.[15, 133]
- Clinical signs of subacute infection are similar to acute infection but are generally less severe and may also include swollen joints and fluctuating fever. Duration of illness can be 5-30 days.[133] 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.[15, 133]
- Clinical signs of chronic ASFV occur 14-21 days after infection and include fever with mild respiratory distress and joint swelling. Areas of the skin may be red and become necrotic. Lethality rates are generally less than 30%.[15] Chronically infected swine have variable clinical signs, complicating diagnosis, and disease develops over 2-15 months.[90, 133] It is thought some survivors carry the virus for life[133] (i.e., viral persistence).

Symptoms of ASFV are similar to other pig diseases such as classical swine fever (CSF) and necessitate laboratory tests for diagnosis.

- Clinical signs of acute disease cannot be used to distinguish ASFV from classical swine fever (CSF) and other pig diseases that have similar clinical presentation and mortality rates; laboratory tests are needed for a definitive diagnosis.[15, 134]
- Differential diagnosis should include classical swine fever and diseases such as porcine reproductive and respiratory syndrome, porcine dermatitis and nephropathy syndrome, erysipelas, Aujeszky's disease, salmonellosis, other septicemic conditions, and poisoning.[15, 122]
- All members of Suidae (pig) family are thought to be susceptible to infection; however, African wild pigs, warthogs, bush pigs, and giant forest hogs exhibit few to no clinical signs of infection, making them asymptomatic carriers of ASFV. [15, 122, 134] Warthogs exhibit transient viremia but the mechanism of tolerability/resistance is not well understood and is thought to be a combination of genetic and environmental adaptive responses.[138] Fecal microbiota transplantation from warthogs to domestic pigs was shown to confer partial protection again attenuated strains.[138] In Mozambique, a population of domestic pigs was found to have increased resistance (based on circulating antibodies), but the exact mechanism of resistance is unknown and was not shown to be heritable.[93] Early historical accounts indicate that Peccaries (T*ayassu* spp.), pig-like animals similar to wart hogs (also called javelinas), may be resistant.[29, 122]

What are the gaps?

- Variable presentation of clinical signs complicates diagnosis.
- Much work is needed using the ASFV-Georgia/genotype II strain, the highest global pandemic threat strain, with respect to clinical presentation in U.S. domestic swine breeds of different ages using different infection routes.
- Are warthogs able to clear virus without showing clinical signs, or do they tolerate viremia without clinical signs?
- Is the collared peccary (javelina) susceptible to infection by ASFV genotype II?

Who is doing experiments in this area?

- **USA**: United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services, Center for Food Security and Public Health (Iowa State University)[115]
- **International**: France (World Organisation for Animal Health [OIE]), Italy (Food and Agriculture Organization of the United Nations [FAO])[15], Spain (FAO Reference Centre, INIA-CISA),[15, 47] United Kingdom (Institute for Animal Health, Pirbright),[91] South Africa (University of Pretoria)[94]

Incubation period – How long after infection do clinical signs appear? Are animals infectious during this time?

What do we know about ASFV?

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.[115, 122]

- Less virulent strains produce mild signs, while more virulent strains have a shorter incubation period (~3 days) and may result in death before clinical signs are observed.[15, 122]

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.[47, 49, 63, 67, 88, 131]

- The incubation period for genotype II strains, with swine experimentally inoculated by various routes, is ~4 days with a range of 1-13 days.[46, 63, 95] For intranasal infection, the incubation period was 5-20 days and was dose dependent.[131]
- For genotype II strains, directly inoculated animals can have a shorter incubation period (~4 days) than for direct contact (pig to pig or pig to contaminated secretions,~9 days) or those indirectly exposed to contaminated air (~12 days), and can vary by dose.[63, 88]
- For genotype II, the latent period (onset of infectiousness) 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.[63]
- 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.[95] The duration of infectiousness is broad, ranging from 1-40 days, depending on the virulence of the ASFV strain.[32, 63, 95]

What are the gaps?

- 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 infectiousness coordinate with that timeline or vary based on the animal?

Who is doing experiments in this area?

- **USA**: DHS S&T PIADC[32, 67]
- **International**: Denmark (National Veterinary Institute, Technical University of Denmark),[88] Russia (Federal Center for Animal Health, Russia),[95] Spain (Centro de Investigacion en Sanidad Animal [CISA, INIA][9, 46]; Universidad Complutense de Madrid[109]), United Kingdom (Pirbright Institute)[63]

Biosurveillance and clinical diagnosis – What are the most effective Biosurveillance measures for ASFV and methods of confirming infection in individual animals or a small herd?

What do we know about ASFV?

There is currently no global biosurveillance framework for ASFV. There are four OIE 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.[90] It is unclear whether sero-surveillance is ongoing in ASFV-positive countries neighboring the EU.[66]
- Passive surveillance (in the U.S.) is ongoing for all swine and relies on laboratory personnel, veterinarian, producer, or other stakeholders' suspicion of a case of ASFV.[122] USDA APHIS has developed an integrated surveillance plan.[121]
- It has been suggested that in the EU, efficient passive surveillance of wild boar carcasses would need to be equal to 1% of the estimated live post-reproductive wild boar population.[55]

ASFV is hard to distinguish from classical swine fever (CSF) and other diseases via clinical signs and post-mortem examination.[133] ASFV should be considered in swine with febrile disease, disseminated hemorrhage, and high mortality.

- Clinical evidence can be nonspecific, so active surveillance should be based on rapid diagnostic testing.[54, 90] 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.[15, 54] In the case of acute infection, death may occur before antibodies are produced.[90]
- Current methods for diagnosis do have limitations such as cost, time to result, need for specialized equipment/laboratories, less than optimal analytical and clinical sensitivity/specificity, and inability to detect early acute and chronic stages.[50]
- Detection can be accomplished via commercially available tests: PCR is the most sensitive technique and can detect ASFV DNA early stages of infection (within 3-4 days).[134] Enzyme-linked immunosorbent assay (ELISA) can detect antibodies within 7-14 days post infection and is used to detect an immune response, with limited utility for confirmation of clinical cases[86]; antibodies may last for months to years.[50, 54] There is a field-deployable genetic test to detect ASFV under study.[35]
- ASFV was detectable in tonsil scrapings 2-4 days before onset of clinical signs using a fluorogenic probe hydrolysis assay.[139]
- The blood, spleen, kidney, lymph node, lung, bone marrow, and tonsil can be used for virus isolation, detection of antigen, or polymerase chain reaction (PCR) testing.[90, 133] Serum for serological tests can be collected 8-21 days after infection.[133]
- Oral fluids samples have been reported to be compatible with PCR methods.[59]
- Common viral detection and identification techniques include hemadsorption (HAD) cultures, fluorescent antibody tests, and molecular detection via PCR.[54, 133] PCR can detect a range of ASFV isolates across known virus genotypes.[54] While not common, a few naturally occurring non-hemadsorbing strains do exist that produce acute infection. Deletion of 8DR was not shown to affect virulence in the Georgia 2010 isolate.[19, 90] A fluorescent antibody test can be used to detect ASFV antigen in HAD-negative samples.[54, 91, 133] Direct immunofluorescence has high sensitivity to acute ASFV, less so for subacute or chronic forms. Antigen detection is not recommended for chronic or individual diagnosis.[54]
- Common antibody detection techniques include ELISA, immunoblotting assay (IB), indirect immunofluorescence antibody test (IFA), and some pen-side tests (lateral flow device). In areas free from ASV, IFA can be used as a confirmatory test for sera if ELISA is positive. In endemic areas, IFA can be used if results from ELISA are inconclusive.[133]
- Monitoring European and African isolates can aid understanding of viral evolution and genetic markers of virulence.[54]
- A commercially available cell line, MA104, has been identified as a substrate for ASFV virus isolation using archived clinical samples, though further work is required to determine if MA104 cells can detect ASFV virus in contemporary field samples.[103]

What are the gaps?

- Need to identify mammalian continuous (immortalized) cell lines that can be used in ASFV diagnostic laboratories for ASFV isolation directly from recent clinical samples without the need for virus passage/virus adaptation diagnostic procedures.
- Diagnostics should be field-deployable, accurate, rapid, and less costly than existing methods; research is needed for novel DNA amplification and detection strategies, and serological diagnosis should include multiple recombinant ASFV antigens.[50]
- There is a lack of commercial tests and/or lack of molecular diagnostic tools in countries where ASFV is circulating.[91]
- Need pen-side detection to enable on-farm diagnosis[91] and syndromic tests to differentiate ASFV from other pathogens.
- Need to develop novel tests for antigen and antibody detection, to include interlaboratory comparison and validation.
- Need to model epidemiology on level of individual pig, herd, and regional demographics.[54]
- Performance of risk assessments for control/spread of ASFV is needed.[54]
- Need to understand pig and pork value chains related to socioeconomic landscape.[54]

Who is doing experiments in this area?

- **USA:** DHS S&T PIADC, USDA Agricultural Research Service (ARS), USDA Animal and Plant Health Inspection Service (APHIS), Kansas State University,[51] Iowa State University, University of Minnesota
- **International:** Australia (Australian Centre for Disease Preparedness), Canada (National Centre for Foreign Animal Disease, Canadian Food Inspection Agency),[74] France (World Organisation for Animal Health [OIE]), Italy (Food and Agriculture Organization of the United Nations [FAO]), Spain (VISAVET), South Africa (Ondestepoort Veterinary Institute Agricultural Research Council), United Kingdom (Royal Veterinary College[63]; Pirbright Institute[30])

Viral persistence/Environmental stability – How long does the virus persist in the environment?

What do we know about ASFV?

ASFV has high environmental resistance and can be transmitted via fomites, to include shoes, clothes, vehicles, knives, and equipment.[132] Of note, infection of domestic pigs via fomites is not well characterized.[61]

- 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,[90, 122] and is very stable at low temperatures.[75]
- Virus in body fluids and serum is inactivated at 60°C for 20 minutes.[115] It is stable and infectious for months at room temperature and at least 61 weeks when stored at 4°C.[75, 90]
- In unprocessed pig meat, the virus is stable for weeks to months; heating to 70°C for 30 minutes destroys the virus.[90]
- Pigs with less virulent strains can transmit the virus up to one month after infection; blood remains infectious for up to six weeks. In acute infections, all body fluids and tissues are infectious from onset of clinical disease until death.[90]
- Research has been conducted over decades on the stability of isolates in numerous matrices, to include meat, meat tissues/products at different temperatures and preparations, and fluids (e.g., serum, blood).[66]
- Transmission via contaminated feed is possible and is dependent upon biosecurity conditions during processing, packing, and transport.[118] Studies show viable virus pathogens in feed and water contaminated by blood can last 30 to 60 days when stored at 4°C, but 1 day in feed and up to 50 days in water when stored at room temperature.[75]

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.[66, 75]

- Stability in salted and smoked/cured meats ranges have been reported from 30-399 days.[66, 75] In vivo experiments showed detection of viable ASFV in Italian salami, pork belly, and loin, up to 18, 60, and 83 days, respectively, but no virus infectivity was detected beyond these ranges.[96]
- 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.[66]
- Stability in feces has been reported at 11 days at room temperature and up to 160 days at 4–6°C.[66] Excretions should be considered a viable pathway for virus transmission; survivability is dependent on temperature.[30] In experimentally inoculated pigs, feces were infectious for 8 days at 4° C, 3–4 days at 37°C.
- Urine has been shown to contain viable virus (Georgia 2007/1 strain) for 15 days at 4°C, 5 days at 21°C, and 2–3 days at 37°C.[75] A recent study demonstrated that infectious ASFV from the Georgia 2007/1 strain could be detected in feces for up to 5 days at 4°C and 12°C, 3 days at 21°C, and 1 day at 37°C, and can be detected in urine for up to 5 days at 4°C and 1 day at 37° C.[30]
- It is assumed that due to viral stability and high titers of ASFV upon death in the acute phase, carcasses would contain viable virus and carcass removal remains a high priority due to the suspected risk of infection.[11] However, the actual rate of transmission is unclear; in one field study, researchers examining buried wild boar carcasses in Lithuania were not able to demonstrate presence of infectious ASFV across various decomposition stages.[137] Using an Estonian 2014 isolate, other findings suggest that under favorable environmental conditions, wild boar or infected pig carcass may remain infectious for nearly two years.[40]

What are the gaps?

- The stability of ASFV in agricultural or transportation settings and suggested hold times for ASFV degradation is not well characterized. This includes survival on commonly used farm hand equipment such as shovels, rakes, etc.
- Need to determine effect of surface type on stability.
- Need to examine role of fomites and variability of viral shedding in samples (e.g., urine, feces).[120]
- Temperatures required for heat inactivation may not be feasible in the field.[122]
- Consideration should be given to handling and transportation of feedstuff under unknown conditions.
- Underreporting of foreign farm visits may affect approach rate.
- There is a lack of persistence data in soil contaminated with excreta and on fomites and in feed supplements.[120]
- Does rendering or other manufacturing activities decrease amount of or inactivate ASFV and is this process dependent?
- Need to develop standardized sampling methods for environmental materials.[61]
- Are different climates (e.g., temperature, humidity) more or less suitable for ASFV persistence?

Who is doing experiments in this area?

- **USA:** DHS S&T PIADC
- **International:** France (World Organisation for Animal Health [OIE]), Germany (Friedrich-Loeffler Institute),[137] Italy (Food and Agriculture Organization of the United Nations [FAO]; European Food Safety Authority), Lithuania (State Food and Veterinary Services, Lithuanian University of Health Sciences), Poland (National Veterinary Research Institute),[75] United Kingdom (Royal Veterinary College[63]; Pirbright Institute[30])

Virus importation – What are the risks of the virus entering the United States through agricultural/food vectors?

What do we know about ASFV?

Infected animals are the primary source of 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 or intentional release.[22]

- 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/byproducts through air passenger baggage and foreign mail is the largest potential pathway. The risk is considered to be high, with low uncertainty.[120,122]
- Illegal entry of pork, ham, and sausage products is a potential pathway for introducing ASFV,[120] for instance by international travelers who do not legally declare items. Although fomites and feed ingredients are potential pathways, there is little data on transmission and lack of quantification of infectious dose that remains, for example, on footwear.
- 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.[120] For illegal entry pathways, swine products and by-products are considered high-risk, while meat and trophies, animal feed, fomites, and swine semen are associated with non-negligible importation risk.[120]

What are the gaps?

- Need quantification of virus that would remain on fomites (e.g., on footwear) and remain infectious.
- There is potential underreporting of visits to foreign farms which may affect approach rates.
- There is uncertainty in virus survival through processing and shipping steps.
- 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)?

Who is doing experiments in this area?

- **USA**: USDA APHIS Wildlife Services[22]
- **International**: France (World Organisation for Animal Health [OIE]), Italy (Food and Agriculture Organization of the United Nations [FAO])

Host range – How many species does it infect? Can it transfer from species to species? What is the vector range (ticks)?

What do we know about ASFV?

ASFV is native to Africa, where hosts include warthogs (*Phacochoerus africanus*), bush pigs (*Potamochoerus larvatus*), and giant forest hogs (*Hylochoerus meinertzhageni*).[57, 81] Neither warthogs nor bush pigs exhibit clinical disease signs.[81] Outside of Africa, domestic pigs (*Sus scrofa domesticus*) and wild boar/feral hogs (*Sus scrofa*) are non-native hosts that acquire fatal illness.

- Peccaries are thought to be relatively resistant to ASFV,[29, 122] but additional work is required to characterize them as a potential reservoir in the United States.
- There is currently no evidence that non-suid mammals act as reservoir hosts for ASFV.[57]

Soft-bodied ticks (genus *Ornithodoros*) are a natural vector in Eastern Africa and Europe.[44] Ticks are required for transmission between warthogs, but not between domestic and/or feral pigs.[57]

- In the United States, there are at least three species of potentially competent tick vectors (*O. coriaceus*, *O. turicata*, and *O. puertoicensis*).[57]
- The stable fly (*Stomoxys calcitrans*) has also been linked to mechanical transmission,[87, 89, 128] primarily when pigs eat flies that have fed on infected blood within the prior 24 hours.
- ASFV Uganda strain has a very 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}.[99]

What are the gaps?

- What is the vector competence of other U.S. vector species (e.g., *Otobius megnini*, *Ornithodoros lagophilus*, *Ornithodoros kelleyi*, *Ornithodoros coriaceus*, *O. turicata*, *O. puertoicensis*)? Has transovarial transmission been demonstrated?
- How often do soft-bodied ticks interact with domestic and feral swine in the United States?
- Are peccaries potential reservoirs of ASFV in the United States?
- What are the preferred mammalian hosts for the U.S. soft tick species that are competent for ASFV?
- What is the relative risk of introduction from U.S. feral hogs to domestic pigs based on current distribution, movement, and behavior?
- How likely is transmission from feral to domestic swine, particularly in the United States?[61]
- Can asymptomatic hosts transmit disease?
- How long can infected tick vectors maintain disease?[50]
- Which U.S. regions are at highest risk of ASFV establishment through persistently infected (transovarial transmission) tick species?[135]
- How important are other potential vectors like flies?[77, 89]
- Are leeches natural reservoirs of ASFV?[69]

Who is doing experiments in this area?

- **USA**: Kansas State University[50, 73], Texas A&M University[57, 135]
- **International**: Denmark (National Veterinary Institute),[89] Germany (Institute of Diagnostic Virology, Germany),[98] Netherlands (Wageningen Bioveterinary Research),[32, 100] Portugal (Centre for Interdisciplinary Research in Animal Health [CIISA]),[14] United Kingdom (Pirbright Institute[61]; Royal Veterinary College[62])

Medical veterinary countermeasures – Are there effective vaccines and treatments to limit or prevent infection and/or spread?

What do we know about ASFV?

To date, there is no uniformly effective medical treatment or licensed vaccine for ASFV.[110] A number of vaccine candidates have been tested, but efficacy varies and generally only applies to closely related ASFV strains.

- Live, attenuated vaccines have had some success at protecting pigs from subsequent challenge,[47, 72] though this protection does not usually extend to other ASFV strains.[65] Live vaccines can cause their own side effects, including chronic ASFV that later regains virulence.[51] To improve safety of live attenuated vaccines, a number of deletion mutants have been created,[110] though these show variable efficacy[1, 18, 27] and protective effects may not last for long.[108]
- Researchers with USDA Agricultural Research Service (ARS) have recently identified a promising deletion mutant vaccine providing protection from the pandemic Georgia isolate (a genotype II ASFV).[20]
- To date, inactivated ASFV vaccines have not demonstrated protection.[41, 76, 116]
- Protein subunit vaccines, which help generate antibodies, have also shown some protective efficacy against ASFV,[23, 58, 107] though the effects are not universal.[5, 80]
- Other subunit vaccines, delivered viral, bacterial, or plasmid vectors have also been developed,[51] and have granted either no,[24, 73, 79 53] partial,[7, 73] or robust[56] 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.[6, 68, 71, 117]
- There has been some evidence of vaccine-induced disease enhancement, where vaccinated pigs have developed more severe disease upon secondary challenge.[68, 117]
- Currently, the only effective treatment for outbreaks in swine is depopulation.

What are the gaps?

- Understanding of the mechanistic basis for the lack of vaccine cross-genotype protection is needed.
- Identification of correlates of host protective immune mechanism(s), including specific cell-mediated immune mechanism is needed.
- Identification of ASFV proteins associates with the induction of host immune protection is needed.
- We need to understand the role of pro-inflammatory responses and specific cytokines that contribute to disease pathogenesis.
- Identification of reproducible challenge models that better mimic natural infection to better assess vaccine efficacy is needed.
- Research is needed to explain inconsistencies in challenge study results.
- A better understanding of immunological determinants of protection is needed for consistent vaccine efficacy, as it is unclear what the ideal vaccine target is.[51]
- What factors contribute to vaccine or immune-mediated disease enhancement?
- Are there treatments that reduce transmission potential in already-infected swine?
- How should quarantine/depopulation be undertaken to minimize spread?
- What factors contribute to the lack of protective efficacy among challenge strains?
- Currently, there is no validated cell line for use in vaccine production, inhibiting future vaccine commercialization.
- There is a need to differentiate between infected and vaccinated animals (DIVA).
- Can antibodies limit viral load and transmission potential in treated swine?[38]
- Are there any treatments or interventions that could be implemented prior to the onset of clinical signs to prevent culling of entire herds?
- Identification of lead vaccine candidate that can be used to produce 5-10 million emergency vaccine doses for stockpiling in the National Animal Vaccine and Veterinary Countermeasures Bank (NAVVCB) is needed.

Who is doing experiments in this area?

- **USA**: Arizona State University Biodesign Institute[68], Kansas State University[117], USDA ARS, DHS S&T PIADC[18, 51, 73], USDA APHIS[85]
- **International**: China (National High Containment Laboratory for Animal Diseases Control and Prevention),[27] Spain (Centro de Investigación en Sanidad Animal[107]; European Union Reference Laboratory for African Swine Fever[47]; Centre de Recerca en Sanitat Animal [CReSA][6]), United Kingdom (Pirbright Institute)[56, 108]

Decontamination – What are effective methods to kill the virus?

What do we know about ASFV?

When decontaminating ASFV, the first course of action recommended is an EPA-registered disinfectant.[126]

- 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.[126]

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 min), Clearon Bleach Tablets (30 min), Klor-Kleen (30 min), Klorsept (30 min), Klorkleen 2 (30 min), and Accel Concentrate Disinfectant Cleaner (5 min).[126]
  - **In pork packing plants,** decontamination should be done with acid-based disinfectants (such as CD631, acid quaternary ammonium-based) following the manufacturer's instructions, particularly 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 nonporous in order to allow appropriate disinfection.[105]
  - **In transportation vehicles,** decontamination options are Virkon S (10 min, animal transport and regular transport vehicles), Klorsept (30 min, animal transport vehicles), and Klorkleen 2 (30 min, animal transport vehicles). If none of the above are available, FIFRA Section 18 options are Sodium Hypochlorite (15 min nonporous, 30 min porous) and Benefact (15 min on nonporous surfaces inside and outside aircraft).[126]

- For cooking or curing pork products, unprocessed meat must be heated to at least 70°C (158°F) for 30 minutes to inactivate ASFV; 30 minutes at 60°C (140°F) is sufficient for serum and body fluids. Virus in serum-free medium can also be inactivated by pH < 3.9 or > 11.5.[114]
- Sodium hypochlorite, citric acid, and some iodine and quaternary ammonium compounds are reported to destroy ASFV on some nonporous surfaces. Either 2% citric acid or higher concentrations of sodium hypochlorite (e.g., 2000 ppm) can disinfect the virus on wood, though citric acid is more effective.[114]

ASFV is highly resistant to low temperatures and persists despite refrigeration or freezing. However, it can be heat inactivated by 56ºC for 70 minutes or 60ºC for 20 minutes.

ASFV can be inactivated by 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).

- ASFV is susceptible to ether and chloroform: inactivated by 8/1000 sodium hydroxide (30 minutes), hypochlorites – between 0.03% and 0.5% chlorine (30 minutes), 3/1000 formalin (30 minutes), 3% ortho-phenylphenol (30 minutes) and iodine compounds. Disinfectant activity may vary depending on the pH, time of storage, and organic content.[86]
- Washing with water alone reduces contamination by up to 60%, but using a heavy-duty detergent decreases the original organic burden by 99%.[130]
- A detailed decontamination table with benefits and limitations of multiple methods is available from USDA APHIS.[78]

What are the gaps?

- Are there methods clearly more beneficial when considering completeness of decontamination, cost, and environmental waste generated?
- There is a lack of standardized laboratory testing methods for assessing chemical disinfectants, as stringency varies by requirement statute (e.g., OECD, ASTM 1053, and EN 14675).
- Who is responsible for waste management issues?
- What magnitude reduction in viral load is necessary to reduce or eliminate transmission risk?
- Are three rounds of disinfection with three different disinfectants necessary?
- What are the optimal parameters for powerwashing an ASFV-infected barn (e.g., temperature, presoaking, time)?
- Is spot treatment of organic residues with a flame effective or necessary?
- Are there heat treatment options for barns (similar to those used for high-pathogenicity avian influenza) that would be effective without causing substantial damage?
- Is heat treatment a viable option for hoop structures?
- What steps are needed for virus elimination outside of the barn?
- Validating environmental sampling methods is a large gap in our current understanding.

Who is doing experiments in this area?

- **USA**: USDA, Environmental Protection Agency, DHS S&T PIADC[13]
- **International**: Poland (Department of Swine Diseases, National Veterinary Research Institute[131]), Spain (VISAVET Center and Animal Health Department, Complutense University of Madrid),[109] United Kingdom (Pirbright Institute)

Depopulation and carcass disposal – What are the most effective methods of depopulation and disposal of infected carcasses?

What do we know about ASFV?

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 nonpenetrating captive bolt, electrocution head to heart, veterinarian-administered anesthetic overdose, and in certain permitted circumstances ventilation shutdown (VSD) or Sodium Nitrate.[78] 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. This requires location-specific planning and preparation which is addressed in the FAD PReP/NAHEMS Guidelines and SOP: Mass Depopulation and Euthanasia (2011).[127]
- 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.[12]
- 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 nonpenetrating captive bolt are additional options available for pigs under 100 lbs.[78]
- 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).[78]
- USDA APHIS maintains a detailed table of suitable depopulation methods and the techniques and limitations of each method.[78]

Carcass disposal and environmental regulations differ by setting and state.[78]

Agricultural Setting[78]

- USDA APHIS maintains a detailed table with benefits and limitations of carcass disposal methods.[78] Options include:
  - Test negative animals to slaughter or non-infected disposal
  - Composting – likely to inactivate ASFV by multiple mechanisms
  - Above Ground Burial – likely to inactivate ASFV by multiple mechanisms
  - Deep Burial – unlikely to inactivate ASFV
  - Burning:
    - Open burning – likely to inactivate ASFV, but also potential to cause adverse human health impacts
    - Mobile incineration – safer than open burning but limited throughput
  - Landfill – negligible risk if leachate goes into wastewater treatment step; prioritize ASFV-negative herds for landfill to virtually eliminate risk
  - Rendering – prioritize ASFV-negative herds for rendering if renderers will accept non-infected material from infected premises or control area
  - Incineration/energy-from-waste – 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.[129]
- 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 over and above what is legally allowed, it is imperative that the competent authority be contacted to approve carcass disposal plans.[12]

Tools and standard operating procedures for depopulation exist.[125]

- USDA APHIS maintains a Carcass Management Dashboard, which contains information on disposal options for planning or response purposes.[125]
- NAHEMS guidelines/SOPs: Critical Activity Standard Operating Procedures (SOPs) — For planners and responders, these SOPs provide details for conducting 23 critical activities such as disposal, depopulation, cleaning and disinfection, and biosecurity that are essential to effective preparedness and response to an FAD outbreak. These SOPs provide operational details that are not discussed in depth in strategic documents or disease-specific response plans.[127]

What are the gaps?

- Are there other depopulation methods that would limit environmental viral transmission?
- Are there other carcass disposal methods that would limit environmental viral transmission?
- How long does the virus persist after various methods of disposal?
- Are current techniques effective in killing the virus and preventing spread to the environment?

Who is doing experiments in this area?

- **USA**: USDA APHIS[78], EPA, Iowa State University, University of Nebraska, Cornell University, DHS S&T PIADC[13]

Swine wastewater treatment on-farm – Is wastewater a significant risk of transmission?

What do we know about ASFV?

Surprisingly little is known about ASFV persistence in farm wastewater systems, though transmission via contaminated water (e.g., during seasonal rains) is suspected in several African communities.[39]

- 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.[40] Results, though, depend on temperature and tissue type,[40] and the study did not test wastewater *per se*.

Chemical disinfectants and heat are able to inactivate ASFV in pig slurry,[119] 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.[119] Alternatively, heating the slurry to 56°C for 90 seconds inactivated the virus;[119] 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).[124]
- 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 have not been tested explicitly with ASFV.[64]
- Pig slurry can harbor ASFV for 84-112 days, depending on temperature.[64]
- Contaminated water sources can harbor ASFV for 50-60 days, with colder temperatures facilitating persistence.[113]
- The Food and Agriculture Organization (FAO) does not have recommended decontamination procedures for water, but recommends burying, burning, alkalis, or acids for effluent and manure disposal.[52]

What are the gaps?

- Risk assessment to determine the overall risk of transmission via wastewater systems is needed.
- 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?

Who is doing experiments in this area?

- **International**: Germany (Friedrich Loeffler Institute),[40] Poland (Department of Swine Diseases),[131] Russia[113], United Kingdom (Pirbright Institute)[30]

Personal Protective Equipment (PPE) – Is any PPE necessary to limit spread or protect from decontamination efforts?

What do we know about ASFV?

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

- The Occupational Safety and Health Administration (OSHA) classifies PPE into four levels of protection. The levels range from D (lowest level of protection) to A (highest level). Most non-zoonotic foreign animal disease (FAD) events, including ASFV, will require Level C protection for biosecurity, which provides standard contact and droplet precautions. This includes protection for the body, hand, foot, eye, face, head, hearing, and for the respiratory system, and should be chosen based on potential 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.[127]
- 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[127]
- The Food and Agriculture Organization of the United Nations (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 (make sure they are the right size), 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.[15]

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 premises due to the differences in risk of exposure to a hazardous agent. For example, PPE may be different when working at an Infected Premises (IP) than when working on surveillance at a premises in a free area.[127]
- Commercially available PPE for prevention of the spread of infectious agents as well as protection from decontamination chemicals are available and marketed towards ASFV work, such as those offered by Dupont.[36]
- Additional general (non-ASFV specific) information can be found in FAD PReP Standard Operating Procedures (SOP): Health and Safety/Personal Protective Equipment (PPE).[127]

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 very 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.[127]

What are the gaps?

- Are there specific types/brands of PPE more effective for ASFV than for general use in animal disease responses?
- Effects of decontamination methods on PPE/personnel?
- Can we develop more effective PPE?

Who is doing experiments in this area?

- **USA**: Occupational Safety and Health Administration[127], USDA

Forensics – Natural vs intentional use? Test to be used for attribution?

What do we know about ASFV?

Due to the nature of ASFV (high morbidity and mortality, environmental stability, ease of spread), it is a candidate for intentional release,[22] but current testing methods should be able to discriminate between laboratory-derived and natural strains.

- Current methods of sample collection and testing are adequate for forensic studies to identify strain origin. Molecular characterization of ASFV isolates would follow standard procedures for genotyping based on the C-terminal end of p72 coding protein gene, the intergenic region and the central variable region and the full E183L-gene encoding the p54 protein. The central variable region is noted as the genome target of choice when determining origin and spread of related virus.[26]
- Existing real-time PCR methods using a primer set and TaqMan probe provide rapid identification of isolates across all 24 known genotypes.[26] Real-time PCR using a commercially available universal probe library ensures detection of isolates in the different p72 viral genotypes. Conventional PCR can be used but is noted as having a lower sensitivity than real-time PCR for animals infected with genotype II ASFV strains.[25]

What are the gaps?

- Need to establish efficient sample collection and transport for clinical and environmental samples that preserves live virus and nucleic acids.
- Need to advance capability to distinguish ASFV cultured from various animals.
- Can diagnostics distinguish between emerging strains (either by whole or targeted next-generation sequencing)?
- Can diagnostics differentiate between natural/intentional release strains and strains used for ASFV vaccines (e.g., attenuated live virus strains)?

Who is doing experiments in this area?

- **USA**: USDA APHIS
- **International**: France (World Organisation for Animal Health [OIE]), Italy (Food and Agriculture Organization of the United Nations [FAO]), Spain (Centro de Investigación en Sanidad Animal)[45]

Genomics – How does the virus compare to previous strains?

What do we know about ASFV?

ASFV is a DNA virus, and the sole member of its family (*Asfarviridae*).[86]

There are 24 known genotypes of ASFV, but only two (genotypes I and II) 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. [2, 14, 21, 102]

The strain of current potential pandemic concern is classified as genotype II, and emerged in the country of Georgia in 2007.[106]

The Georgia 2007 strain has spread through Eastern and Western Europe, Russia, China and Southeast Asia, but has not yet reached the United States.[106]

- Genotype II is still present in Africa and responsible for outbreaks in pigs and present in the host *Ornithodoros* tick, creating a continuous cycle of the disease as well as representing a source for new genotypes of virus. [102]
- In Africa, ASFV is maintained in a tick and warthog cycle where warthogs are not adversely affected by the disease. [28, 94] The spread of genotype II outside of Africa has shown the high virulence of the virus to not only domestic pigs but to the large population of wild boar in Russia and Europe. [43]
- Genomic analysis of genotype II strains over time show high overall identity between type II strains in different regions.[42] Despite the similarity, in vivo studies have shown variability in virulence, [46, 83, 111, 136] suggesting more work is needed to understand the genomic markers related to virulence.[9, 45]
- 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. [84-85, 104]

What are the gaps?

- How is the virus evolving within the most widespread genotypes and are there any similarities or differences between those genotypes present in Africa?
- 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 interact with host proteins to influence transmissibility, virulence, etc.?

Who is doing experiments in this area?

- **USA**: DHS S&T PIADC[85, 104], USDA APHIS[84]
- **International**: Armenia (Armenian National Agrarian University),[111] Canada (University of Victoria),[123] Germany (Friedrich Loeffler Institute[16, 42, 136], South Africa (Tshwane University of Technology[21]; University of Pretoria[14]; Onderstepoort Veterinary Institute[102]), Spain (Universidad Complutense de Madrid, INI-CISA [Centro de Investigación en Sanidad Animal])[9, 47]

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