A literature review to gather the scientific evidence for an African Swine Fever virus (ASFV) exposure assessment of US domestic pigs raised in total confinement and/or with outdoor access to ASFV-infected feral swine

Funded by the National Pork Board, Award #23-042

May 17, 2023

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List of Abbreviations

ASF African Swine Fever

ASFV African Swine Fever Virus

CSF Classical Swine Fever

DFA Direct Fluorescent Antibody

DIVA Differentiate Naturally Infected from Vaccinated Animals

DNR Department of Natural Resources

Dpi Days post infection

EFSA European Food Safety Authority

ELISA Enzyme-linked Immunosorbent Assays

FAO Food and Agriculture Organization

GSM Global System for Mobile communications

HAD HemAdsorption Dose

IFA Indirect Fluorescent Antibody

IPT Immunoperoxidase Test

Km Kilometer

MEM Minimum Essential Medium

NR Not reported

NVSL FADDL National Veterinary Services Laboratory Foreign Animal Disease Diagnostic Laboratory

PCR Polymerase Chain Reaction

PDNS Porcine Dermatitis and Nephropathy Syndrome

PremIDs or PINs National Premises Identification Numbers

PRRS Porcine Reproductive and Respiratory Syndrome

TCID Tissue Culture Infectious Dose

US United States

USDA United States Department of Agriculture

VEB Vegetative Environmental Buffer

VI Virus Isolation

WOAH World Organization for Animal Health (formerly OIE)

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1. Feral Swine and Domestic Pig Populations in the US

1.1 Historical points of feral swine introduction

The swine family (*Suidae*) is not native to any part of the US. Chinese-origin swine were brought to Hawai'i around AD 400 and Spanish-origin swine were first introduced to the continent by Hernando de Soto in 1539 when his expedition landed on the Florida peninsula.[1] These points of introduction likely led to the establishment of the feral hog populations in these areas. Other US introductions were made by the English in 1607 and Dutch in 1625.[1] During the colonial period, harvested grains were scarce and prioritized for feeding humans over livestock.[1] Swine in the colonies were largely free-range and scavenged from forest and woodlands, much like how pigs were produced in England at the time.[1] Other modifications of early colonial production were turning out pigs on a grain field following harvest and then to the forest to feed on mast.[1]

In free-range systems with no fencing for confinement, pigs could thrive with little human input because of their abilities to live in a variety of habitats, omnivorous diets, proboscises (i.e., snouts) for digging and rooting, and tusks and fast speed for defense from predators.[1] Pigs were collected from the forests for harvesting of meat, but some of them were lost likely joining other wild pig groups.[1]

Borrowing from the Enlightenment period of Europe, nineteenth century America saw the rise of a more structured and ordered approach to agricultural production.[1] With its favorable climate, the southern US was the pork center of the US.[1] However, the Civil war decimated not only the hog population, but also its farms, which never fully recovered.[1] The Civil war was also a notable point of human interference that led to the release of additional domesticated pigs into this region.[1]

Genetically, domestic pigs (*Sus scrofus domesticus*) are the domesticated subspecies that originated from the Eurasian wild boar (*Sus scrofus*).[2] In the US, the word “feral” is preferred over the word “wild.” “Feral” refers to domestic pigs, hogs, or swine, that have escaped from farms (or even their intentional release) since their original introduction in the 1500s. To that end, in the US, there are pigs, hogs, boars, razorbacks, piney woods rooter swine that can be feral. There are also hybrids of feral swine in the US that have mated with Eurasian or Russian wild boars.[3] There are even feral swine in the US that are of full Eurasian descent.[2,4] These Eurasian wild boars have been imported into the US and released onto private hunting reserves for hunting since 1890.[5] Some farms also produce farmed wild hog meat which they sell in niche markets and restaurants.[6] For the purpose of this literature review and to match the language used in many US state laws and regulations[7], the term “feral swine” will be used to refer to any members of the *Sus scrofa* genus and species that live in the wild.

1.2 Current Feral Swine Situation

1.2.1 Population and Geography

Feral swine have been reported in at least 35 states and are estimated to have a population of more than 6 million[8] individuals (**Figure 1**). There are also feral pigs in Canada, with many of them in the plains Prairie Provinces[9] (i.e., Alberta, Saskatchewan, and Manitoba). Research performed in 2015 suggested that the geographic distribution of wild pigs was “most strongly limited by cold temperatures and the availability of water.” However, a note made by the authors indicated that since historical distribution data was used, feral swine found in warmer climates may have been overrepresented and further research was needed to determine if colder climates were a limitation for the presence of feral swine[10]. It may be quite likely that pigs may be able to survive in colder climates of the US, just as they do in Canada[11], by burrowing in the snow and developing thicker coats of fur.[12]

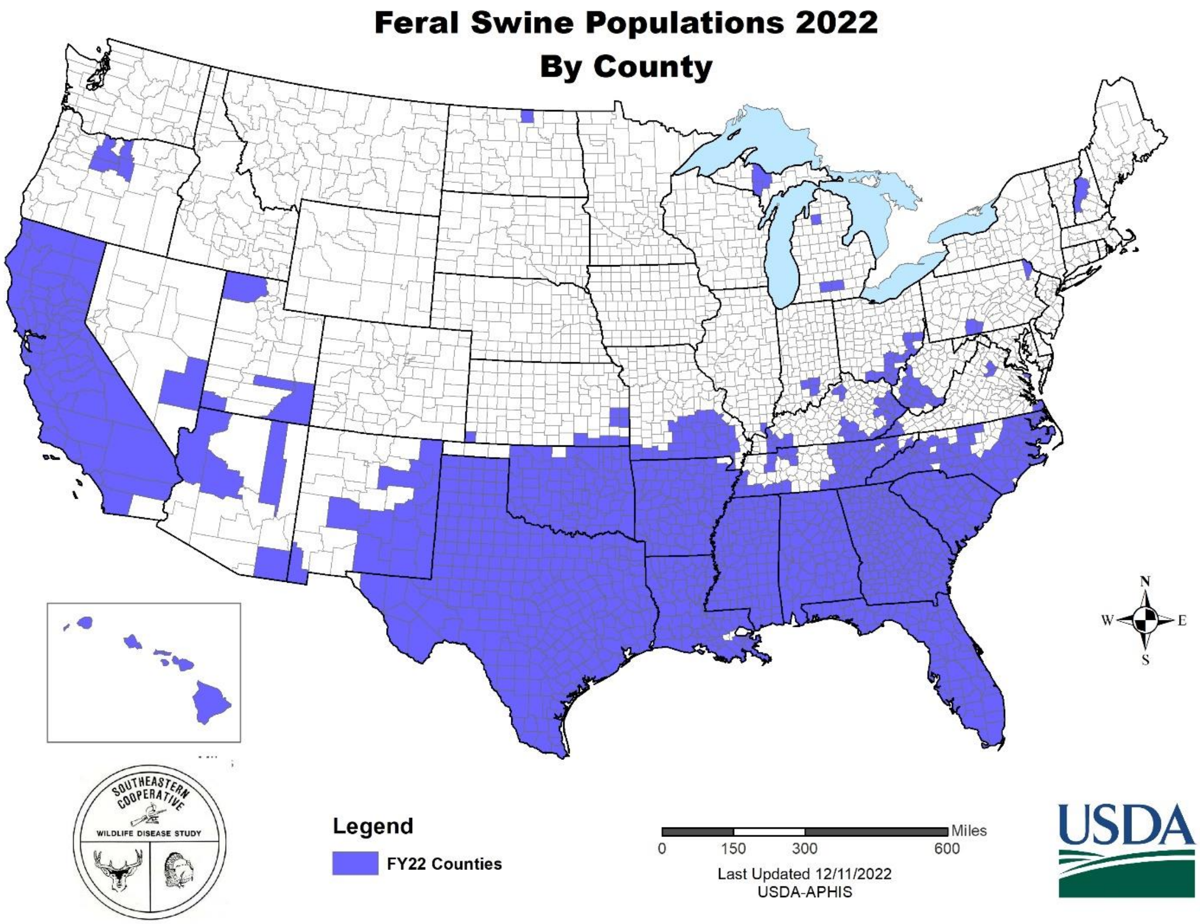

Figure 1 Feral swine population by county in the US, 2022.
Source: USDA, APHIS History of Feral Swine in the Americas, 2023.[13]

The estimated home range of feral swine in the US is 1.18 to 2.6 km (0.7 to 1.6 miles)[14] (**Figure 1**). Although feral swine are estimated to increase their geographical spread by 12.6 km per year[15] and have a high fecundity rate whose population rate may exceed 2.0 each year[16], their rapid spread in the US cannot be explained by these biological factors alone. Another explanation for their rapid spread is that it is the result of human interference. There have been numerous anecdotal reports of people capturing pigs from one region or state and (illegally) transporting them to another for release for hunting purposes[17].

Hog hunting is popular sport in the US, especially in the South. However, the legality of hunting feral swine is dependent on the state laws and regulations. For example, in California they are considered game animals and require a permit for hunting[18], whereas in Texas, as long as you have prior consent, no permit is needed for hunting on private property[19]. In Minnesota, it is illegal to hunt or trap feral swine, unless prior authorization has been given by the Department of Natural Resources (DNR) commissioner of the for feral swine control or eradication. If a person in Minnesota is in possession of feral swine, it must be surrendered to the DNR commissioner within 24 hours, and if in violation of this statute, is guilty of a misdemeanor[7].

Feral swine are an invasive species that cause damage to agriculture land and livestock, natural resources including water and land, property, cultural and historic sites, and can injure pets and people. The USDA estimates that feral swine cause an estimated $1.5 billion each year in damages and control in the US[20].

In addition to the environmental and land damage, feral swine can and may harbor a plethora of infectious pathogens, both swine-specific and zoonotic, that are not commonly found in their domestic counterparts. Some of these diseases are on the World Organization for Animal Health (WOAH) and United States Department of Agriculture (USDA) list of reportable diseases (**Table 1**). African Swine Fever Virus (ASFV), the causative agent of African Swine Fever (ASF) is arguably the major concern. ASF is a foreign animal disease of high consequence, and although never diagnosed in the US, its introduction and subsequent spread would have myriad negative consequences for US domestic pig populations and the pork industry. Therefore, this review will focus on the available mitigations described in the literature that decrease the transmission of ASFV from feral swine to domestic pigs.

Table 1. Endemic Diseases of Concern to Swine in the US.

	Diagnosed since 2013	
Disease Susceptibility[21]	**Feral Swine**	**Domestic Pigs**
Brucellosis (*Brucella suis*)*^	⦿[22]	⦿[23]
Leptospirosis*†	⦿[24]	⦿[25]
Pathogenic E. Coli*	⦿[26]	⦿[27]
Salmonellosis*	⦿[28]	⦿[29]
Tuberculosis*†	⦿[30]	
Tularemia*†	⦿[22]	
Influenza A*	⦿[30]	⦿[31]
Porcine Circovirus Type 2	⦿[32] Not tested since 2012 in the US	⦿[33]
Porcine Epidemic Diarrhea	⦿[34]	⦿[35]
Porcine Reproductive & Respiratory Syndrome	⦿[30]	⦿[36]
Pseudorabies*^	⦿[37]	
Toxoplasmosis*	⦿[38]	
Q Fever*†		
Trichinellosis*^	⦿[39]	

Table 1. Endemic swine diseases of concern in the US, including reportable and zoonotic diseases. Information includes whether these diseases have been diagnosed in feral swine and/or domestic pigs in the US since 2013. *Zoonotic Pathogens; ^WOAH and USDA notifiable diseases[40]; †Notifiable disease in some US states[41].

1.3 Domestic Pig Production in the US - An Overview

1.3.1 Indoor Raised Domestic Pigs

In 2019, the United States ranked as the world's second largest global pork producer[42], with pork ranked as the fourth leading U.S. food export commodity and valued at a record $7.7 billion in 2020.[43] As of March 25th, 2021, the pig inventory in the U.S. was estimated at 74.8 million head, which consisted of 6.21 million head of breeding stock and 68.6 million head of market hogs.[44] The total number of pig operations in the U.S. at the end of 2017 was estimated at 68,300, with the majority of operations consisting of fewer than 1,000 head (n= 56,000).[45]

However, pig operations with over 5,000 head made up approximately 93% of the total U.S. pig population.[45]

In 2023, pig production operations are located throughout the U.S., but the majority of indoor operations are generally concentrated in the Upper Midwest (northernmost Corn Belt states)[44] near abundant feed supplies and slaughtering facilities (**Figure 2**). Since 1990, pig production has grown significantly in North Carolina, Oklahoma, Colorado, Utah, and Texas.[46] A 2021 analysis of the percentage of U.S. hogs and pigs inventory in each state or regions follows: Iowa, 31.4%; North Carolina, 12.0%; Minnesota, 11.6%; Illinois, 6.9%; Indiana, 5.7%; Nebraska, 4.9%; Missouri, 4.9%; Ohio, 3.6%; Oklahoma, 2.8%; Kansas, 2.8%; and small percentages in the Mountain and Pacific regions.[44]

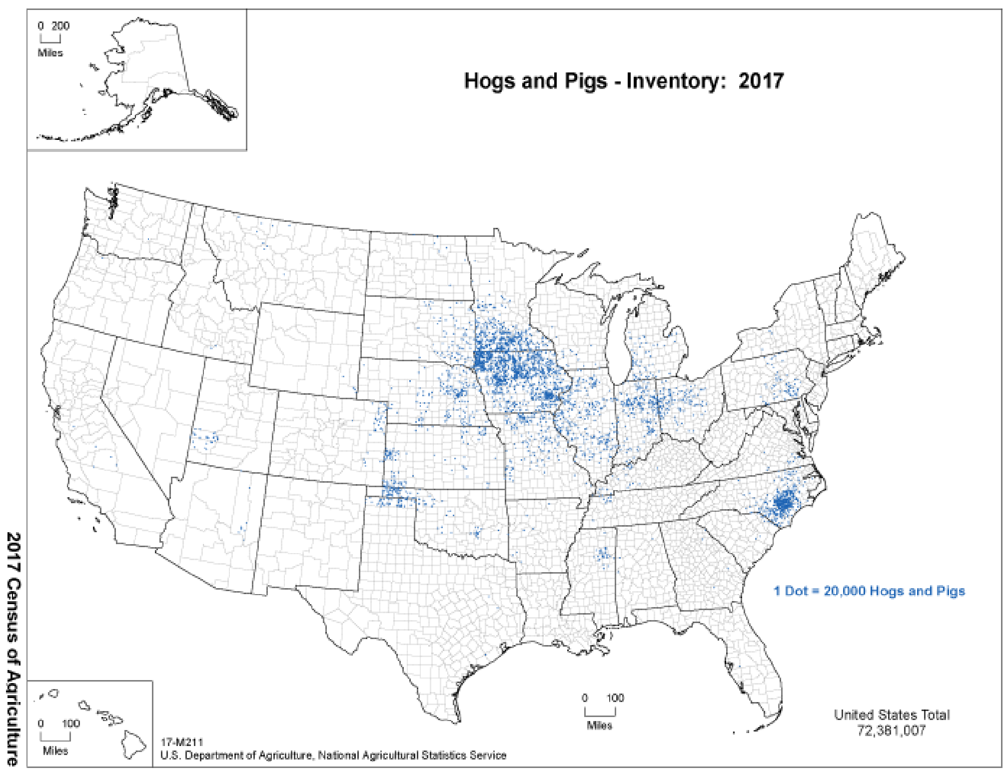

Figure 2. Distribution of U.S. hogs and pigs
Source: USDA National Agricultural Statistics Service, 2017 Ag Atlas Maps, Map # 17-M211

The modern pig production process of the commercial systems in the U.S. is typically divided into four phases, each with specific requirements in regard to housing, feeding, and animal care needs. They are: (1) breeding and gestation, (2) farrowing, (3) nursery, and (4) finishing.[46] While the breeding and farrowing phases may be located on the same premises, most large-scale operations utilize "multi-site" systems when transitioning to the nursery and finishing phases.[47] These production phases are geographically separated on separate premises with different National Premises Identification Numbers (PremIDs or PINs) and may be miles or states apart.[48]

The prevalence and type of pig producer vary by region but often depend on the regional infrastructure and available resources.[49] Most U.S. pig production has historically occurred within farrow-to-finish operations, where all production phases occur within a single building or location. Over the past several decades, pig production has transitioned from mostly farrow-to-finish operations to those that specialize in only a specific phase of production. In 1992, over 50% of U.S. pig operations used a farrow-to-finish approach, but by 2009 less than 25% were farrow-to-finish producers.[49] Pig producers specializing in only finishing hogs (feeder-to-finish) represented less than 20% of producers in 1992 but grew to nearly 50% by 2009.[49] This separation of phases necessitates the routine intrastate, interstate, and international shipment of pigs at various stages of production.

In 2009, the number of pigs moved into a state for feeding or breeding purposes totaled 41 million head, representing the largest share of livestock inshipments in the U.S.[46] In the event of an ASF outbreak, a widespread stop order on the routine movement of pigs could result in overcrowding conditions on farms. This overcrowding may lead to animal health and welfare concerns which could necessitate the euthanasia of pigs or cessation of breeding programs.

Efficient and timely movement of pigs is critical for each stage of production, and any disruption in this flow severely limits the capacity to maintain animal health and welfare, continuity of business, and domestic markets.[50]

1.3.2 Outdoor Raised Domestic Pigs

Pig production in the United States (US) in the first decade of the 2000's has had a resurgence of the extensive production and management practices typical of the 1800's and early 1900's, most notably, raising pigs outdoors. In the 2017 USDA agriculture census, 78.5% of farms reported having fewer than 100 pigs (n=52,123).[45]

Being raised "outdoors" may mean outdoor access which allows pigs to have access to the outdoors for a certain period of day or all day or just seasonally part of the year. For example, in colder climates, breeding farms might concentrate farrowing events between March and May to protect piglets from cold environmental temperatures and hypothermia.[51] Others may choose to move their pigs indoors for part of the year, or seasonally produce animals such that there are no pigs on the farm in the winter.[52]

In 2002, the United States Department of Agriculture (USDA) required that all organic certified farms meet USDA's organic standards, including pig producers.[53] These standards include requirements for outdoor access, type of feed, and limitation on the types of medical treatments. According to the 2021 USDA Certified Organic Survey the top 5 states producing USDA organic certified pork are Iowa (n=7,703 pigs), Wisconsin (n=1,853 pigs), Michigan (n=1,853), Pennsylvania (n=1,318 pigs), and Minnesota (n=1,318).[54] In addition to USDA certified organic farms that allow their pigs outdoor access, there are other farms that are not certified but allow their pigs to have outdoor access.

Figure 3. Picture of a Hoop Barn.
Source: Hoop Barns for Grow-Finish Swine, Agricultural Engineers Digest, 2004.[55]

Other housing for which outdoor access may be implied are hoop barns with deep bedding within which one side of the barn is open to the outside (**Figure 3**). Flooring on outdoor access farms may be grass pasture, dirt, forest land, straw or straw-like bedding, or concrete.[56] Although many of the pigs that are raised on pasture or dirt lots may be marketed as “free-range”, this is not the same “free-range” system as the colonial period or what is even found in other countries[57,58]. Many state laws require that all pigs be enclosed using fencing[59]. In other words, it is illegal to have free-ranging or roaming domestic pigs, whether they be for pork production or as pets. Therefore, for this literature review, any domestic pigs that are not fenced-in are considered illegal and are not included in the pathways nor in the mitigations. Examples of fencing materials that can be used to restrict free-roaming pigs include electric, barbed wire, and galvanized wire netting[60] and will be discussed further in the section on fencing (**section 4.3.2**.)

1.4 Overview of ASF transmission

There are four independent but overlapping cycles of ASFV transmission: 1) *sylvatic cycle* between the natural reservoirs, the soft tick *Ornithodoros sp.* and warthogs 2) *tick-pig cycle* between the soft tick *Ornithodoros sp.* and domestic pigs 3) *domestic cycle* between wild swine and/or domestic pigs and/or contaminated pig products and 4) *wild boar-habitat cycle* between wild boars and their habitat such as contaminated carcasses and intraspecies scavenging.[61] The sylvatic cycle only exists in Africa, and outside of Africa, the tick-pig cycle was implicated in the Iberian Peninsula outbreak[62], but now the majority of transmission occurs via the domestic cycle, with wild boars being the maintenance host of the virus[63]. Therefore, the scope of this literature review will be limited to the potential pathways of transmission FROM feral swine TO domestic pigs in the US, both indoor and out (**section 3**). Pigs that are owned but not fenced (i.e., free-range), farmed Eurasian or Russian boars, pet pigs, zoo animals[64], and pigs used in research or medical settings will not be considered.

1.5 Summary of Literature Review Approach, Scope, and Purpose

In many countries where ASF is endemic, backyard and outdoor pig farms are heavily implicated in continued ASFV transmission[65] because of their contact with the wild boar population[66] and decreased biosecurity practices[67]. In the US, the presence of feral swine (**Figure 1**) overlaps the presence of domestic pigs (**Figure 2**). It is imperative that preventative measures are in place to prevent contact between feral swine and domestic pigs, especially when considering the possibility of transmission of ASFV from feral swine to domestic swine. Therefore, following identification of potential pathways of transmission, mitigations described in the literature to decrease and/or eliminate transmission pathways will be presented (**section 4**).

2. African Swine Fever Disease and Virus

2.1 Susceptible hosts of ASF in the US

African Swine Fever is a highly contagious viral disease affecting wild and domestic pigs across all ages and all domestic breeds.[68,69] The virus is also found within soft-bodied ticks.[68] The disease presentation is determined by the virulence of the virus strain, with highly virulent strains of ASFV clinically manifesting as hemorrhages of the skin and organs, fever, loss of appetite , and high mortality .[69] Low virulent strains generally produce mild clinical signs including slight fever, reduced appetite, and depression.[70] Other reported clinical signs for NH/P68, a low virulent ASFV strain, reportedly caused high fever, necrotic skin areas, and joint swelling.[71] As of 2019, no virulence markers had been identified.[72] All forms of the disease result in decreases in productivity and cause serious economic impacts for pork producers, especially when ASF infections limit or remove access to international trade of pigs and pork products.[73]

As of 2020, North America (i.e. the United States, Canada, and Mexico) has never experienced an incidence of ASF.[74,75] Since January 2021, ASF has been reported in 45 countries in five different world regions within which more than 870,000 pigs have been affected and a reported 1,120,000 animal losses globally.[76] There is substantial concern about incursions of ASF into the United States' domestic pig and/or feral swine populations because of the rapid expansion of ASF outbreaks into previously ASF-free countries, especially countries in the Western Hemisphere like the Dominican Republic and Haiti. In 2022, of the 19 countries that reported ASF in wild boar, 15 also reported ASF in domestic pigs.[77]

Given the potential for and demonstrated transmission of ASFV from feral swine to domestic pigs, ASFV in the feral swine population in the US creates an immediate threat to the domestic pig population. The potential risks and impacts of ASF have been demonstrated by the severity of consequences of ongoing outbreaks, including those in China, where, as of mid-2019, industry estimates that 50-70% of commercial pigs have been infected.[73] The consequences of these ASF outbreaks reinforce the need for ASF awareness and evaluation of the possible pathways by which ASFV can spread via feral swine to domestic pigs, contaminate food sources, and infiltrate pork production systems and pig farms, both indoors and out.

2.2 African Swine Fever Virus

The ASFV is a member of the *Asfivirus* genus and the sole member of the Asfarviridae family.[2] Being multi-enveloped and measuring 175-215 nm in diameter when including the extracellular envelope, the virus has icosahedral symmetry and contains a molecule of linear covalently close-ended double-stranded DNA. The DNA is 170-194 kilo base pairs long, forming two inverted and complimentary terminal loops.[78] The variation in DNA length is reportedly due to differences in the presence or absence of various multi-gene families.[79,80]

As of 2019, there are eight known serogroups of ASF viruses based on hemadsorption inhibition assay assessment [81,82], and 23 molecular genotypes have been identified based on phylogenetic analysis of the p72 capsid protein gene (*B646L*).[82,83] However, the p72 ASFV phylogenetic analysis used for identifying these various genotypes does not accurately describe the actual serotype of the virus.[83] Due to the sylvatic cycle that is maintained in Africa, genetic diversity of ASFV occurs only in that continent. Across all the genotypes, Genotypes I and II are the most prevalent of the ASF viruses[79] Genotype I viruses are endemic to West and Central Africa and have demonstrated lower virulence in comparison to the Genotype II viruses, which were introduced to Eastern Europe in the mid-2000's.[72,81]

Different strains and genotypes have varying abilities to produce disease. Cross-immunity across genotypes exists to some extent, with an association between cross-protectiveness and the CDv2 protein (*EP402R* gene)[84] and a C-type lectin protein (*EP153R* gene)[85] However, as of 2020, characterization of the exact mechanisms that are involved in cross-protection between strains is still needed.[86] Additionally, antigenic diversity has been related to potential changes in genes, including MGF, E183L, and B602L, based on a review by Malogolovkin & Kolbasov (2019).[82] The exact details behind the drivers of genetic and antigenic diversity require further research as of 2020.

2.3 Clinical Signs

There are four clinical forms of ASF including peracuate , acute, subacute, and chronic. Each form ranges in severity based on many factors including the infectious dose, route of exposure, swine species, virus virulence, endemic status in the area, and concurrent infections. For example, African Wild Boars (*Sus scrofa algira*) tend to be subclinical (unapparent/silent) carriers of the disease, whereas domestic pigs tend to have peracute or acute presentation of clinical signs.[63]

Peracute presentation is characterized by a high fever 105°F (40.5°C), loss of appetite and inactivity. Sudden death will typically occur within 1-3 days, which may occur before any other clinical signs develop. [63]

Acute presentations have overlapping clinical signs similar to peracute but generally develop additional signs such as increased respiratory rate; ocular and nasal discharge; bloody froth from the nose/mouth; hemorrhages (spot-like or extended) on the ears, abdomen, and/or hind legs; reddening of the skin on the chest, abdomen, perineum, tail, and legs; constipation or diarrhea, which may become mucoid to bloody; vomiting; and abortion in sows at all stages of pregnancy. Death may occur anywhere between 6-15 days depending on the virulence of the virus isolate, with a mortality rate of 90-100% in domestic swine.[63]

Subacute presentations share similar clinical signs with acute presentations although the signs are generally less severe. The exception are the vascular changes such as hemorrhages and edema, which are more severe in subacute presentations of ASF. Edema may cause serous pericarditis, swollen joints, and subsequent pain when walking. Fever, depression, and loss of appetite also tend to fluctuate throughout the course of disease. The mortality rate of subacute presentations of ASF is 30-70% with death usually occurring within 7-20 days of the first appearance of clinical signs, and survivors may recover after one month.[63]

Chronic presentations are generally present in those regions where ASF is endemic. Mortality rates are typically less than 30%, and clinical signs occur 14-21 days following exposure. Clinical signs tend to be milder, e.g., lower fevers, less depression, suppression of appetite rather than anorexia, with a majority of infected pigs recovering from the disease.[63]

2.4 Diagnostics and Sampling Strategies

Testing for ASFV can be classified into two categories. These categories are: 1) direct detection of viral antigen, which consists of virus isolation (VI), direct fluorescent antibody testing (DFA), sequencing, and real-time polymerase chain reaction (PCR); and 2) serology for detection of antibodies, which consists of enzyme-linked immunosorbent assays (ELISA), indirect fluorescent antibody (IFA), and immunoperoxidase test (IPT).[69,87]

Viral antigen testing is currently based on individual animal sampling because it is the only validated method to detect ASFV. However, the National Veterinary Services Laboratory Foreign Animal Disease Diagnostic Laboratory (NVSL FADDL) as of April 2020 is in the process of validating aggregate sampling, such as oral fluid testing via rope sampling.[87]

Although recommended tissue samples for testing by WOAH include lung and bone marrow[88], the only approved sample types for testing by the NVSL FADL in the US are whole blood and fresh tissue (spleen, lymph nodes, and tonsil), blood spot, blood swab, and spleen swab.[69,87]

In the US, where there is no available vaccine for ASFV, the presence of antibodies indicates previous infection or persistent infection in subacute and chronic forms.[69,87] In Vietnam where an ASF vaccine is currently under field evaluation, an accompanying ELISA that differentiates naturally infected from vaccinated animals (DIVA) is needed.[89] An additional vaccine being developed for wild boar and domestic pigs in the EU is expected to have an accompanying DIVA antibody ELISA available at the time of vaccine release, although no confirmed date of release is available.[90] Confirmatory testing for samples positive for antibodies by ELISA should be performed using IFA, IPT, or immunoblotting.[69]

Under the US Swine Hemorrhagic Fevers Integrated Surveillance Plan, feral swine in very high-risk counties in high-risk states and territories of the US (e.g., Texas, Louisiana, Georgia, Florida, Puerto Rico, and the U.S. Virgin Islands) will have whole blood and serum tested for ASF.[91] Additionally, where sick or dead swine resulting from non-traumatic events are found, tonsil and spleen samples (or lymph nodes if tonsils and spleen aren't available) will be submitted for ASF testing.[91]

The FAD PReP Manual suggests prioritizing pigs for testing by the following criteria:[87]

- Abnormal mortalities should be prioritized first.
- The next targeted group is sick pigs with ASFV compatible clinical signs.
- Pigs with high probability of disease, such as decreased production or feed intake, under high stress, appear unhealthy, or may have had exposure to the disease, should be prioritized next.

- Finally, pigs that are apparently healthy are to be targeted last.

2.5 Virus Survival

As previously discussed, ASFV is structurally complex and thus resistant to a range of environmental factors such as pH and temperature. Depending on the virus strain, temperature, pH, substrate, and other conditions, the virus can persist for considerable periods of time in the environment, tissues, secretions, and excretions. **Table 2** summarizes the amount of virus detected in various porcine secretions and excretions. ASFV has also been detected in semen at 2 days following experimental infection with the semen quality remaining largely unaffected.[92]

Table 2. Amount of ASF virus shed through different excretions/secretions by pigs

Secretion/ excretion type	Titer	Environmental survival of the virus	References
Aerosol	Max 2.3/1.8/2.1 $\log_{10}$ genome copies/5L snout/floor/1m*	No viral DNA detected in corridor air between pens	Olesen et al., 2017[93]
	~2.7-3.2 $\log_{10}$ $TCID_{50}eq/m^{3}$^	14 min (experimental aerosol)	de Carvalho Ferreira et al., 2013[94]
Oropharyngeal fluids	~3.0-4.3 $\log_{10}$ $TCID_{50}$eq/swab	NR	de Carvalho Ferreira et al., 2012[95]
	~4.5 genome copies/ml	NR	Guinat et al., 2014[96]
Nasal swab	~3.0 $\log_{10}$ $TCID_{50}$eq/swab	NR	de Carvalho Ferreira et al., 2012[95]
	4 $\log_{10}$ HAD_{50}/ml ~6 genome copies/ml	NR	Guinat et al., 2014[96]
Ocular swab	~1.5 $\log_{10}$ $TCID_{50}$eq/swab	NR	de Carvalho Ferreira et al., 2012[95]

Feces	4.83 log_{10} $TCID_{50}$/g	8.48 days+ at 4ºC 3.71 days+ at 37ºC	Davies et al., 2017[97]
	~2.7-3.8 log_{10} $TCID_{50}$eq/g	NR	de Carvalho Ferreira et al., 2012[95]
	1.9-3.6 log_{10} genome copies/ml	≤7 days	Fischer et al., 2020[98]
Rectal swab	2 log_{10} HAD_{50}/ml ~5.5 genome copies/ml	NR	Guinat et al., 2014[96]
Urine	2.94 log_{10} $TCID_{50}$/g	15.33 days+ at 4ºC 2.88 days+ at 37ºC	Davies et al., 2017[97]
	2.3-3.9 log_{10} genome copies/ml	≤7 days	Fischer et al., 2020[98]
Vaginal swab	~1.7 log_{10} $TCID_{50}$eq/swab	NR	de Carvalho Ferreira et al., 2012[95]

*Measured at 1-2 cm from pig snout, just above pen floor, and 1 m above pigs
^Measured 1 m above pen floor
#Data values extrapolated from graphs
+Estimated from initial viral titer and half-life value
NR: Not reported
HAD_{50}/mL: ASF viral titer expressed as 50% hemadsorption doses per milliliter
$TCID_{50}$: ASF viral titer expressed as 50% tissue culture infectious dose

In the acute phase of disease, during which time period the viral titer is highest, the maximum reported viral load of different ASFV strains ranges from 6 to 9 log_{10} HAD_{50}/ml blood.[99] As illustrated in **Table 3**, ASFV remains stable in organs and tissues for appreciable amounts of time, with temperature strongly influencing survivability.

Table 3. ASFV persistence in organs and tissues

Tissue/organ	**Duration of survival/ infectivity**	**Methods**	**Reference**
Spleen	Predicted survival* 483 days at -20°C 126 days at 4°C 15 days at 23°C	Organs from naturally infected pigs	Mazur-Panasiuk & Woźniakowski, 2020[100]
	≥ 2 years at -20°C 7-30 days at 4°C < 7 days at 21°C	Organs from infected pigs stored on various matrices	Fischer et al., 2020[98]
	≥105 weeks at -70°C and -20°C	Spleen portions from infected pigs stored in screw cap bottles	Plowright & Parker, 1967[101]
Kidneys	Predicted survival* 353 days at -20°C 35 days at 4°C 17 days at 23°C	Organs from naturally infected pigs	Mazur-Panasiuk & Woźniakowski, 2020[100]
Lungs	Predicted survival* 714 days at -20°C 136 days at 4°C 9 days at 23°C	Organs from naturally infected pigs	Mazur-Panasiuk & Woźniakowski, 2020[100]
Bone marrow	2-3 months at -20°C <1-30 days at 4°C ≤ 7 days at 21°C	Organs from infected pigs stored on various matrices	Fischer et al., 2020[98]
Muscle	18-24 months at -20°C 2-3 months at 4°C <1 day at 21°C	Organs from infected pigs stored on various matrices	Fischer et al., 2020[98]
Skin	3 months at -20°C 3-6 months at 4°C 7 d to 3 months at 21°C	Organs from infected pigs stored on various matrices	Fischer et al., 2020[98]

*Predicted survival times based on linear regression formula

While **Table 3** demonstrates marked persistence of infective ASFV in various organs and tissues, especially at lower temperatures, one study recovered no viable virus from infected wild boar carcasses buried for 18 to 440 days at 20 different sites.[102] Since the burial period included summer months for all sampled carcasses, the authors propose that higher temperatures, as well as other local environmental factors, likely inactivated the virus. **Table 4** illustrates the variability in ASFV survival under different environmental conditions.

Table 4. Environmental survival of ASFV under various conditions

Substrate	**Duration of viability/ infectivity**	**Methods**	**Reference**
Water/straw/hay	≥ 56 days at 4°C < 7 days at 23°C	Infectious spleen tissue incubated in different substrates for 56 days	Mazur-Panasiuk & Woźniakowski, 2020[100]
Soil/grain	28 days at 4°F < 7 days at 23°C	Infectious spleen tissue incubated in different substrates for 56 days	Mazur-Panasiuk & Woźniakowski, 2020[100]
Leaf litter	8-13 days at 4°C < 7 days at 23°C	Infectious spleen tissue incubated in different substrates for 56 days	Mazur-Panasiuk & Woźniakowski, 2020[100]
Straw & feces in pen	1 day	Healthy pigs introduced 1, 3, 5, or 7 days post removal of infected pigs	Olesen et al., 2018[103]
Yard soil (pH=6.7)	7 days at 4° and 25°C	Spiked with infectious blood	Carlson et al., 2020[104]
Swamp mud (pH=5.2)/ beach sand	<3 days at 25°C	Spiked with infectious blood	Carlson et al., 2020[104]
Forest soil (pH=3.2/pH=4.1)	0 days at 25°C	Spiked with infectious blood	Carlson et al., 2020[104]
Wheat/barley/rye/ triticale	<2 hrs at room temperature	Contaminated with infectious blood	Fischer et al., 2020[105]

Corn/peas	0 hr at room temperature	Contaminated with infectious blood	Fischer et al., 2020[105]
	$T_{1/2}$ (days)		Stoian et al., 2019[106]
Soybean meal	9.6-12.9	Feed/ingredients inoculated with ASFV, incubated at 0-26^{o}C, humidity 20-100%	
Choline	11.9		
Soy oil cake	12.4		
Complete feed	14.2		

2.6 Cleaning and Inactivation

Due to the complexity of its structure, the ASF virus is generally more resistant to inactivation than other enveloped viruses.[107] It persists across a wide range of temperatures and pH. AFSV is inactivated after exposure to 133°F (56°C) for 70 minutes and 140°F (60°C) for 20 minutes, but is very resistant to low temperatures and can survive many freeze-thaw cycles.[99,108] When ASFV was held at -4°F (-20°C) in porcine spleen, kidneys, and lungs, the half-life ranged from 20 to 32 days.[99] The virus survives in serum-free medium at a pH range of 3.9 to 11.5.[108] In the presence of serum, the virus can persist at a pH of 13.4 for 7 days.[108]

In a study comparing the effects of time and temperature on ASFV in alkaline (pH=10.2) minimum essential medium (MEM) to plasma, the virus was inactivated much more readily in MEM than in plasma.[107] Another study measured the persistence of ASFV in serum-containing media and pig slurry at different temperatures over time.[109] The results demonstrate that ASFV remained stable for 24 hours at moderate temperatures [39°F (4°C) and 72°F (22°C)], and inactivation was more rapid in slurry than in the study medium.

Krug et al. (2011) dried ASFV at 86°F (30°C) to mimic the more likely viral state in environmental contamination; this resulted in approximately 2.4 mean $\log_{10}$ reduction in infectivity of ASFV on steel and plastic surfaces.[110] Additional information on chemical inactivation of ASFV and approved disinfectants can be found in **Appendix A** (https://www.aphis.usda.gov/animal_health/emergency_management/downloads/asf-virus-disinfectants.pdf).

In addition to the disinfectants listed in **Appendix A**, a recent study found sodium hydroxide, phenol, glutaraldehyde, benzalkonium chloride, and acetic acid to effect a greater than 4 log_{10} reduction in ASFV titer with a contact time of 30 minutes at 50°F (10°C).[111]

2.7 Viral Shedding and the Course of Infection in Suids

The incubation period for ASFV, i.e., the period between infection to the onset of clinical signs, is 4-19 days.[69] For control purposes, the WOAH considers the incubation period to be 15 days.[112] The latent period, i.e., the period between infection to the onset of infectiousness, is 1-7 days.[95] The duration of the incubation and latent periods is dependent on the strain, infectious dose, and transmission route. Studies have found that pigs infected with ASFV can shed virus before viremia occurs and even before the development of clinical signs.[95,96]

ASFV has been found to initially infect and deplete monocytes and macrophages in lymphoid organs, especially lymph nodes and spleen.[113] As the course of infection progresses, the virus then spreads to other cell types, such as megakaryocytes, tonsillar epithelial cells, hepatocytes, kidney cells, and endothelial cells in various organs.[113]

ASFV is present in multiple tissues of infected pigs, with spleen and lymph nodes typically having the highest amount of virus. The virus is also shed in the secretions, and excretions, such as blood, oral fluids, feces, urine, and aerosols, of infected suids. Blood has consistently been found to contain high amounts of virus.[113] A study by Howey, et al. suggests that the virus is shed in both nasal and oral secretions in similar quantities.[114] Pigs infected with low to moderately virulent virus strains have been found to shed virus from the oropharynx for up to 70 days post infection (dpi), although the amount of virus is present is lower and intermittently shed after 30 dpi.[94] de Carvalho Ferreira, et al. discovered a positive association between the amount of virus excreted in feces and the virus titers in air, for which infectious ASFB can be detected in feces and urine for up to 5 days at room temperature.[97]

2.8 Differential Diagnosis

The clinical signs of ASF cannot be distinguished from Classical Swine Fever (CSF). Other differential diagnoses include Porcine Reproductive and Respiratory Syndrome (PRRS), Porcine Dermatitis and Nephropathy Syndrome (PDNS), Erysipelas, Aujeszky's Disease (in young pigs), Salmonellosis, bacterial septicemias, and acute febrile haemorraghic syndrome.[69,87,115]

2.9 Host Range (*note: Host range and clinical presentation are described more thoroughly in Section 1 but repeated briefly here for context*.)

African wild suids are the natural hosts of ASFV, with the *Ornithodoros* soft ticks serving as the vector in the sylvatic disease cycle, where present. Feral pigs, European wild boar (*Sus scrofa feus*) and all members, and all ages, of the *Suidae* family are susceptible to ASFF, with domestic pigs acting as accidental hosts. All suids are susceptible to all serotypes of ASFV. In the US, the collared peccary (*Pecari tajacu*) which may resemble a small pig, is not part of the Suidae family, but a member of the Tayassuidae family and is considered to be resistant to ASFV[116].

The severity of illness may differ depending on the specific infecting virus serotype and the species that is infected. The ASFV is not readily transmissible to humans and thus should not be considered a zoonotic disease. It cannot be spread to humans via contact with infected pigs, nor through the consumption of infected pork.[63]

2.10 Purpose of Literature Review

The purpose of this literature review is to identify ASF pathways of transmission. Knowing the viral characteristics of ASFV is important to better identify potential transmission pathways in the US. For example, ASFV has been shown to survive in yard soil for up to seven days at both 4°C and 25°C[104], and the virus is known to be transmitted oronasally. This identifies contaminated yard soil as a potential link for viral transmission from infected and shedding feral swine to susceptible domestic herds. Specifically, if feral swine have access to or share an environment with domestic swine then viral exposure may occur when pigs root around in the same area.

The next section of this review will identify transmission pathways that have been identified in ASF positive countries where wild boars have either directly, or indirectly transmitted ASF to domestic pigs. Other, more theoretical pathways via which ASFV could be transmitted but that have not been demonstrated either experimentally or epidemiologically will also be discussed. Collectively, what is known about the virus, its host range, and what has happened in other countries will inform the US pig industry about risk but will not estimate risk.

This review will focus on potential pathways and provide examples of mitigations in ASF-positive regions. However, this literature review will not consider preventative measures or mitigations currently in place that may make ASF transmission through these pathways less likely for US pigs. This last step is essential in for a complete risk estimation. An example of risk assessment for ASF in the US that is in progress that includes entry and exposure assessments in addition to risk estimations with and without mitigations in place is the "Assessment of the Risk Associated with the Movement of Liquid, Cooled Boar Semen Within, Into, and Outside of a Control Area During an ASF Outbreak in the US." The Boar Semen RA is limited in scope to boars raised in total confinement and, while there are some pathways that may be applicable to the broader pig industry in the USA, many are unique to boar studs and mitigated only by boar stud-specific practices.

3. Pathways of Transmission of ASFV from Feral Swine to Domestic Pigs in the US
(*Pathways that have either been shown or may be transmitted from the wild suids of Africa, Asia, and Europe to domestic populations of pigs*)

Wild boars (*Sus scrofa*) in Europe and Asia, and warthogs (*Phacochoerus africanus*), giant forest hogs (*Hylochoerus meinertzhageni*), red river hogs (*Potamochoerus porcus*), and bushpigs (*Potamochoerus larvatus*) in Africa, are all susceptible to ASFV[117], and have all been suspected in the spread of ASFV to domestic pigs in their native regions[118]. It is important to note that in many of the exposures, the domestic pigs were housed in farms with "low biosecurity" where wild suids and domestic pigs had opportunities for interaction[117].

To provide information regarding the ways infected feral swine could transmit ASFV to domestic pigs, this pathways analysis approach, similar to the approaches used in the risk assessment process by the Secure Food Systems team, has been completed. The following pathways have been identified in the "Assessment of the Risk Associated with the Movement of Liquid, Cooled Boar Semen Within, Into, and Outside of a Control Area During an ASF Outbreak in the US" that is in development as potential pathways for ASFV transmission (**Table 5**). We will use these pathways to further explore the transmission of ASFV from feral swine to domestic pigs.

Pathway	**Description or example**
Wild and peri-domestic animals	3.1.1 Feral Swine: Direct transmission 3.1.2 Other Wild Animals: Indirect transmission as fomites 3.1.3 Peri-domestic animals: Indirect transmission as mechanical vectors
Mortality and cull management	3.2.1 Direct Transmission: Contact with contaminated carcasses 3.2.2Indirect Transmission: Hunting

Domestic animals	3.3 Indirect Transmission - Fomite
Insects and arthropods	3.4.1 Arthropods: Indirect Transmission via Sylvatic Cycle 3.4.2 Insects: Indirect Transmission as Mechanical Vectors
Water	3.5 Ingestion of contaminated water
Feed and Bedding	3.6.1 Feed: Ingestion of contaminated feed 3.6.2 Bedding: Ingestion or inhalation of contaminated bedding
Aerosols	3.7 Inhalation of virus-laden air or ingestion of air particles
Vehicles and equipment	3.8 Fomite transmission of ASFV to domestic pigs if pigs come in contact with contaminated vehicles and equipment
People	3.9 Fomites transmission of ASFF to domestic pigs if pigs come in contact with people either wearing contaminated outwear/shoes or people with viable ASFV on their body surfaces.
Biological materials	3.10 Direct transmission of ASFV if the biological material is contaminated with or contains viable ASFV and is injected into, ingested by, or inhaled by a domestic pig.

Table 5. Potential Pathways and their examples of ASFV Transmission from feral swine to domestic pigs in the US

3.1. Wild and peri-domestic animals

3.1.1 Feral Swine

Infected feral swine can transmit ASFV to domestic pigs via direct contact, or indirectly through their excreta in the environment[119]. Understanding feral swine ecology[120] and behavior[117] at the wild/domestic interface would explain the main drivers that result in feral swine having direct contact with domestic pigs (or resulting in indirect contact should the domestic pigs' environment be contaminated by feral swine). In Europe, three key drivers of contact between wild boar and domestic pigs are: I. food availability, II. sexual attraction, and III. overlap of habitat.[117] The following are examples of these main drivers but written in the context of US feral swine ecology and behavior:

I. In a food scarce environment, feral swine can become attracted to the available feed at a pig farm.

II. Feral swine, especially boars, may be sexually attracted to domestic sows and gilts (such sexual behavior mainly occurs in Autumn in Europe[117]).

III. Wherever the presence of feral swine and domestic pigs overlap, there exists the potential for these two populations have direct contact or indirect contact via the environment in their shared habitats.

<u>3.1.2 Other wild animals</u>

It is possible that other wild animals could serve as mechanical vectors in the spread of ASFV. One way is the movement of contaminated feral swine carcass from one place to another during scavenging. In South Korea, leopard cats are known to scavenge on wild boar carcasses.[121] In Germany, a study to identify the role of scavengers in the spread of ASFV was performed, for which Raccoon dogs (*Nyctereutes procyonoides*) were identified as having scavenged on wild boar carcasses.[122] Since most of the carcass material was consumed on the spot by these wild carnivores, the authors concluded that these scavengers only "present a minor risk…for spreading [ASFV]". [122] In the same study though, it was also noted that very rarely a small piece of the meat would be taken from the area by these scavengers[122], which is a potential ASFV pathway that should not be completely discounted.

Another potential pathway of indirect transmission is the spread of ASFV in the feces of scavengers and predators. However, this transmission pathway seems unlikely since results from a study of wolf feces in ASFV positive areas of Poland suggests that ASFV does not survive passage through the wolf's intestinal tract.[123] Finally, it is also possible that any wild animal's body could become contaminated from an ASFV contaminated environment, but ASFV transmission via this particular wild animal ASFV pathway has not been evaluated.[124]

3.1.3 Peri-domestic animals

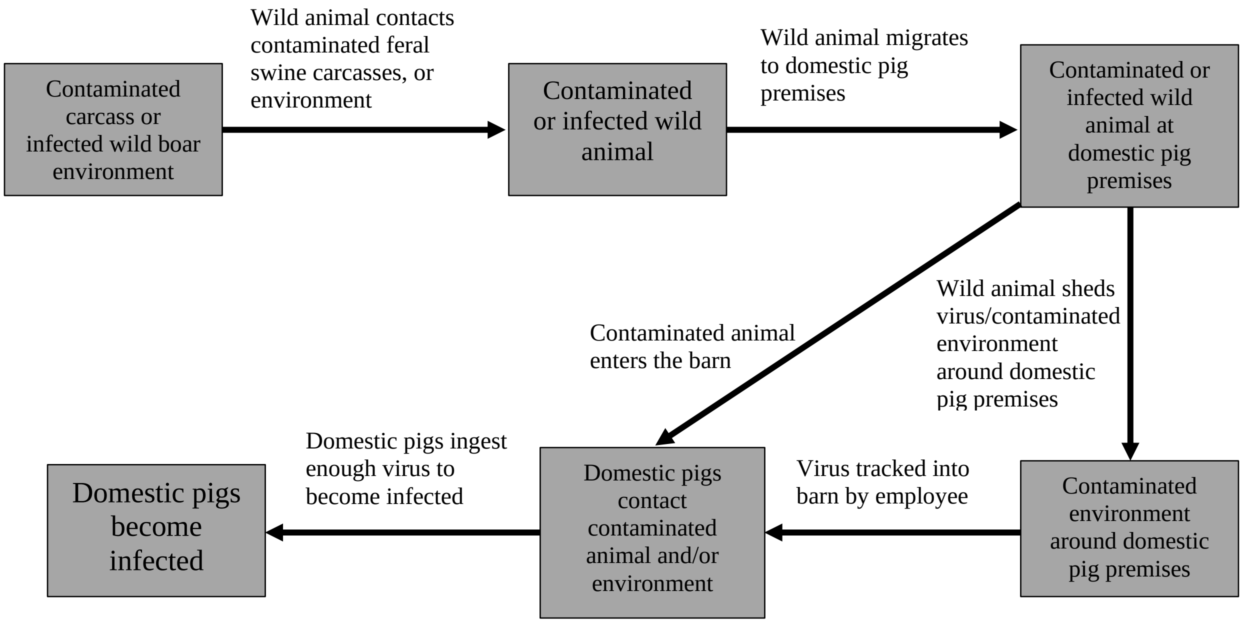

Figure 4. Examples of pathways for which wild animal can indirectly transmit ASFV from feral swine to domestic pigs.

It is also possible for peri-domestic animals, such as synanthropic birds (e.g., house sparrows, rock doves (pigeons), crows, etc.) or rodents to serve as mechanical vectors from the movement of ASFV contaminated carcass, or because their body surfaces become contaminated [124]. In Germany, peri-domestic animals such as red foxes (*Vulpes vulpes*), buzzards (*Buteo buteo*), ravens (*Corvus corax*), and white-tailed eagles (*Haliaeetus albicilla*) were seen scavenging wild boar carcasses.[122] In South Korea, large-billed crows, golden eagles, grey-backed thrushes, grey-backed thrushes, eyebrowed thrushes, crows, cinereous vultures, raccoons and rodents were also seen visiting wild boar carcasses and even stayed there for a while.[121] Blood collected from birds and rodents from ASF positive farms in Lithuania and Russia were tested for ASFV antibodies and all were negative suggesting this is an unlikely pathway.[125] A study in Vietnam, funded by the Swine Health Information Center, in suggests that rodents may not be risk for spreading ASFV.[126] Despite peri-domestic animals presence on positive ASFV farms in Europe, their scavenging behavior, and having been implicated in spreading pathogens including ASFV in Spain[124], the role of peri-domestic animals in the transmission of ASFV is unclear, and additional research is needed.[124]

3.2. Mortality and Cull Management

Other than contact with live wild boars, direct contact with ASFV contaminated wild boar carcasses can also be a pathway of transmission to domestic pigs. Infectious ASFV can be maintained in carcasses for months, especially in cold and moist climates, like those found in Eastern and Central Europe[117]. Even following decomposition, a contaminated environment may still serve as a pathway for ASFV transmission, due to the long ASFV survival times and the rooting behavior of suids. Additionally, any field dressing of recently hunted feral swine, or the inappropriate disposal of their offal, could also contaminate the environment with ASFV.[117,127] The duration of infectiousness in the environment is highly dependent on soil pH, structure, and ambient temperature.[104] For example infectious ASFV can be found in yard soil for one week, swamp soil for three days, and beach sand for up to two weeks.[104] Therefore, an environment contaminated by ASFV contaminated carcass (or offal) could serve as a pathway of transmission to domestic pigs. Similarly, if the environment becomes contaminated with the carcasses of improperly disposed of ASFV-infected domestic pigs, then it follows that this is a potential ASF pathway if other susceptible domestic pigs come into direct or indirect contact with that contaminated environment.

3.3 Domestic Animals

There is no direct evidence to support the transmission pathway of ASFV via domestic animals such as dogs and cats[128], or poultry and other livestock species. It is possible that domestic animals could serve as mechanical vectors in the spread of ASFV, either by contaminating their body, or the movement of contaminated carcass. Transmission is especially possible if they have access to or travel between both feral swine and domestic pigs or their environments. Another potential pathway of transmission could be from scavenging on ASFV contaminated feral swine carcass or grazing in ASFV contaminated environments. This pathway is unlikely given that wolf feces from wolves allowed to scavenge on contaminated wild boar carcasses tested negative for infectious ASFV, suggesting that the virus does not survive passage through the intestines of non-suids.[123] Although these pathways of ASFV transmission via domestic animals are possible, more information is needed to fully extrapolate their role in ASF transmission.

3.4 Insects and Arthropods

3.4.1 Arthropods

Ticks have been established as a vector for ASFV on the African continent, specifically *Ornithodoros* spp. soft ticks,[61] in other words, the sylvatic cycle in which ASFV moves between soft ticks and African warthogs.[61] Therefore, it is possible that soft ticks could become infected from feral swine and domestic pigs become an "accidental host" if infested by the tick vectors[120,129]. In an outbreak in southern Spain and Portugal in 1960 *Ornithodoros erraticus* ticks acted as vectors.[117] Ticks were found on infected farms that used "traditional pig housing" and transmission to naïve pigs continued to occur a year after the initial outbreak. The ticks involved in this outbreak were shown to maintain the virus for up to five years following natural infection. Viral replication has been shown to occur in other *Ornithodoros* species[117] as well.

Current outbreaks in Europe have not been attributed to the transmission of ASFV via the sylvatic cycle because wild boars do not have permanent resting places in which ticks are also present making this pathway unlikely. For example, in the Portuguese outbreak, ticks were rarely found in rabbit burrows surrounding the infected farms, they were only found in farms using "traditional pig housing"[117]. In Ukraine, *Ornithodoros verrucosus* was only found in limestone cliffs on riverbanks, to which wild boars have limited access[117]. In Africa, despite the presence of the sylvatic cycle, there is "limited evidence on the role of soft ticks in ASFV persistence and transmission in domestic pig areas"[117]. Where there is clear overlap in the habitats of the warthogs and ticks with those of the domestic pigs, transmission via ticks is very possible, but in regions of Africa where these areas of overlap are non-existent, tick transmission is infrequent[117]. In Mozambique, domestic pigs that were positive for anti-tick antibodies were housed in areas close to national parks, and similar ASFV viruses were isolated from the positive pigs and the ticks involved in that sylvatic cycle[117].

The four *Ornithodoros* ticks found in the US *Ornithodoros coriaceus*, *O. turicata, O. puertoricensis,* and *O. parkeri,* have all been experimentally infected with ASFV in published studies. In addition, with the exception of *O. parkeri*[130,131], the infected ticks were also deemed competent hosts and resulted in ASFV transmission to the susceptible pigs in the experimental studies.[130,131] Other *Ornithodoros* ticks that exist in the US have not yet been identified as competent vectors because experimental studies have yet to be performed with them[130],(**Appendix B**). In 2019, Wormington et. al., identified areas of the United States in which the competent ticks have been found and where feral swine are present (**Figure 5**)[130]. Using domestic pig population data from 2012, the authors also identified areas of the United States in which spillover risk from the sylvatic cycle to domestic pigs is higher, due to the presence of domestic pigs and the co-occurrence of competent ticks and feral swine (See Figure 2 in the original literature).[130]

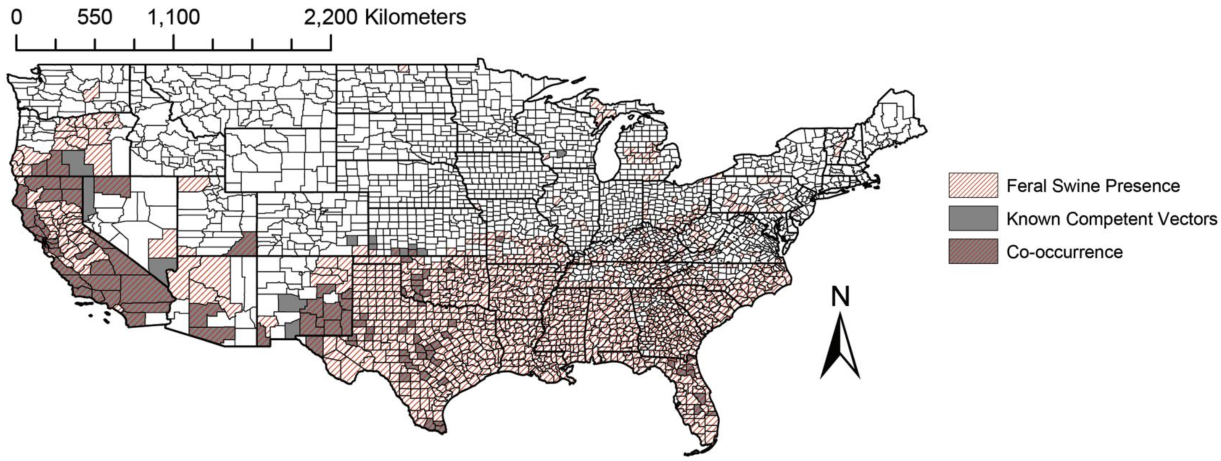

Figure 5. Map of counties in the US that have both competent tick vectors for ASFV and the presence of feral swine.
Source: *Wormington et. al. (2019), Figure 1.*

Two hard bodied ticks from Europe, the common tick (*Ixodes Ricinus*) and the meadow tick (*Dermacentor reticulatus*), have been evaluated as biological vectors of ASFV, but no viral replication occurs in these ticks, though they may be potential mechanical vectors as the virus can survive in these ticks for six to eight weeks.[132,133] These genera of hard bodied ticks exist in the US; however, no literature was found identifying them as potential biological or mechanical vectors for ASFV. Additional information is therefore needed to evaluate whether hard bodied ticks could play a role in the transmission of ASFV in the US.

3.4.2 Insects

Flies can potentially serve as mechanical vectors in the spread of ASFV between feral swine and domestic pigs. In Europe, *Stomoxyxs* and *Tabinaidae spp.* flies are suspected in transmission of ASF cases in domestic pigs during summer months[117]. Although flies cannot acquire, maintain, or transmit ASFV[117], pigs were experimentally infected following ingestion of blood-feeding stable flies (*Stomoxyxs calcitrans*) that had recently been fed ASFV positive blood.[134] Authors did note though that larger Horseflies (*Tabanidae* spp.) would be more likely to spread the virus because of their propensity to travel further.[134] These larger flies are also able to consume and carry larger volumes of blood.[132–136] Other fly species such as houseflies (*Muscidae* spp.) and blowflies (*Calliphoridae* spp.) that are not hematophagous, but feed on and/or lay eggs in decaying pig carcasses,[137] have been hypothesized to be mechanical vectors for ASFv as well.[132,133]

In South Korea (Republic of Korea), other insect types were collected from ASF positive farms and tested for ASFV-DNA. There were a total 28,718 insects collected from 14 farms and included flies, mosquitos, cockroaches, and an uncategorized group.[138] None of the insects showed any trace of ASFV-DNA, but authors concluded that the possibility of the spread of ASFV by these insects shouldn't be completely discounted[138]. In Romania, biting midges (*Culicoides* spp.) collected from ASF positive farms, tested positive for the presence of ASFV-DNA.[139] Although ASFV-DNA was present, the infectiousness wasn't evaluated, therefore further evaluation is needed for biting midges and their role as a vector in the spread of ASFV.

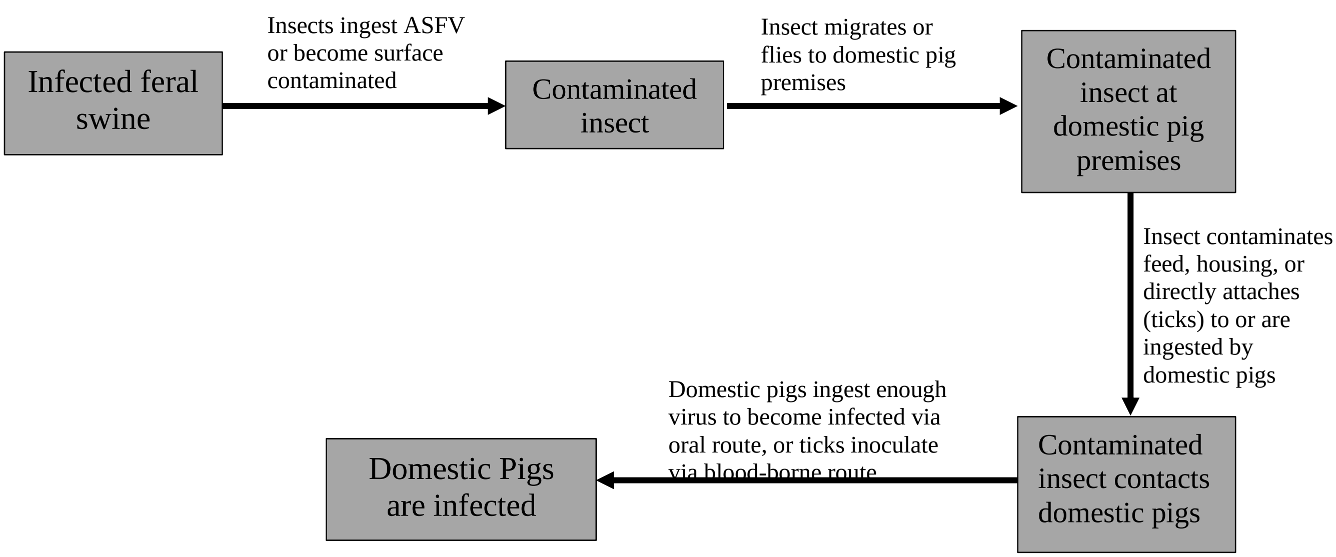

Figure 6. Example of a pathways for which insects and arthropods can indirectly transmit ASFV from feral swine to domestic pigs.

Finally, hematophagous hog lice (*Haematopinus suis*) have been suggested as potential vectors.[132,140,141] In an experimental setting, hog lice feeding on viremic pigs were able to transmit virus to healthy pigs.[142] However, other experimental studies failed to demonstrate the ability of hog lice to transmit virus between pigs.[143–145] Even if hog lice demonstrate substantial ability to transmit disease within-barns, these lice do not survive outside of their host for more than a few hours,[132] it is unlikely for lice to be carried between farms via farm workers, vehicles, or any other means aside from moving pigs between farms. Furthermore, the capacity of hog lice to act as a competent vector was rated between unlikely and probable by subject matter experts.[140]

3.5. Water

There is evidence that water can serve as an indirect pathway in which ASFV is transmitted from feral swine to domestic pigs. Experimental studies have shown that the virus can "remain infectious in stagnant water from 50 to 176 days"[117] and when water is contaminated by ASFV infectious blood, the viability of the virus is preserved for at least 60 days when stored at 4°C[99]. Niederwerder et al. also experimentally infected pigs orally through drinking and 40% of these pigs were exposed to the liquid inoculum that contained as little at 1 $TCID_{50}$[106]. In an epidemiological study in Romania, it was hypothesized that the Danube River may have played a part in an outbreak of ASFV in the region[129], including infection of a high-biosecurity, large breeding farm[99].

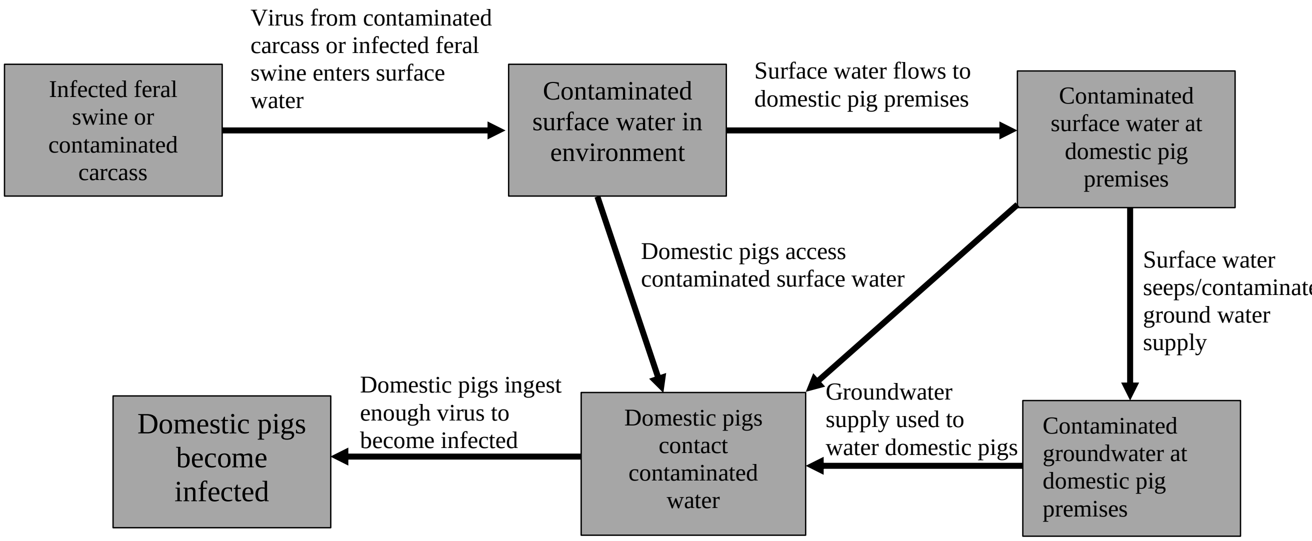

Figure 7. Example of pathways for which water can indirectly transmit ASFV from feral swine to domestic pigs.

3.6. Feed and Bedding

3.6.1 Feed

Transmission of ASFV is possible via the natural consumption of contaminated feed that is plant-based, especially after repeated consumption. Were the feedstuffs to be contaminated by infected feral pigs or their contaminated carcasses, feed could serve as an indirect transmission pathway for feral pigs to transmit ASFV to domestic pigs[120]. There are a few feedstuffs that have been experimentally contaminated with ASFV to determine how long they may remain infectious, many of these durations will also be affected by storage temperature. Compound feed contaminated with ASFV stored at room temperature was positive for virus isolation for less than 5 days, but was positive for virus isolation at least 60 days when stored frozen.[146] According to a systematic literature review performed in 2021 by the European Food Safety Authority (EFSA), viral persistence for cereals, oil seeds, and legumes were published. No data was available tubers, forage and roughage, or tote bags.[147]

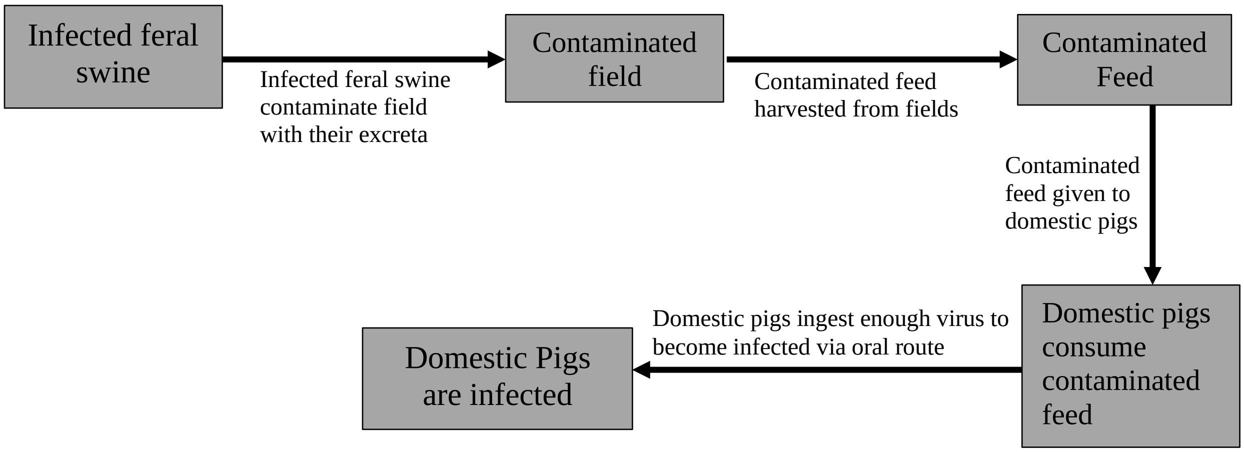

Figure 8. Example of a pathway for which feed serves as an indirect pathway for transmission of ASFV from feral swine to domestic pigs.

3.6.2 Bedding

Transmission of ASFV is possible via naturally derived bedding such as straw[133] or the hulls/husks of rice or other cereals[146]. In Poland and other Baltic states, fresh grain and grass contaminated by infected wild boars was implicated as a direct source of infection of ASFV, because infected wild boars were found in the same areas as the domestic pig outbreaks[148]. In a risk factor analysis study of the ASF incursion in Romania in 2019, 21 of the ASFV positive farms reported that "wild boars had access to the bedding storage".[149] According to a systematic literature review performed in 2021 by EFSA, other bedding material such as dust, wood chips, turf or hulls/husks of rice or other cereals, were not available.[146]

3.7. Aerosols

Aerosol spread of ASF is limited to short distances[125] and can be a form of indirect transmission of ASFV. A report from China noted positive ASFV in aerosols, dusts and air outlet samples, but these samples were limited to those of the piggeries.[150] Another study determined the half-life of ASFV in air to be 19 minutes when tested by PCR.[94] There was no identified literature that explored aerosol spread between feral swine and domestic pigs, nor were there epidemiological accounts of aerosol spread over long distances. Although long-distance cannot be completely discounted, it is more likely that if transmission were to occur from feral swine via aerosols, that feral swine and domestic pigs would need to be in close contact with each other.

3.8. Vehicles and Equipment

3.8.1 Vehicles

Given that AFSV survives in secretions and excretions of swine such as saliva, nasal fluids, urine, and feces, they may be important sources of contamination coming from feral swine and their environments. If those materials contaminate equipment and vehicles, they can then serve as fomites to spread disease to susceptible swine. Contaminated livestock vehicles have been implicated as a source of ASFV in outbreaks[151], although according to the EFSA, no data exists for ASFV survival on or in vehicles used for live pig transport, vehicles visiting pig farms, or any other vehicle types.[147] In Vietnam, ASFV-DNA was isolated from feed delivery vehicles, but samples were not tested for live virus.[152] Infected feral swine could also, theoretically, contaminate vehicles if a collision[153] or other contact occurs.

3.8.2 Equipment

Equipment and its surfaces may be contaminated with ASFV when in contact with infectious material from feral swine. Although there is no evidence to support this exposure pathway between feral and domestic swine it remains possible. ASFV-DNA has been isolated from an animal contact surface in Vietnam and it is possible that feral swine could contaminate feeders and waterers with their saliva. In Vietnam, ASFV from dried infectious blood persisted on various porous and non-porous fomites, between 2 days on paper and more than 7 days on glass[154].

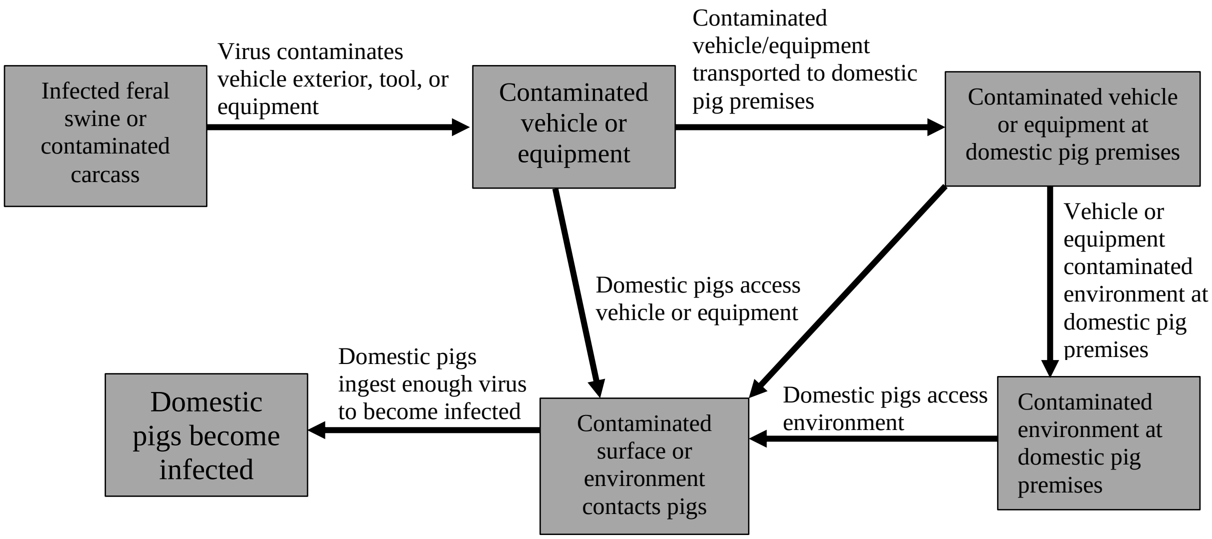

Figure 9. Example of pathways for which vehicles and equipment can indirectly transmit ASFV from feral swine to domestic pigs.

3.9. People

People can serve as a means of indirect transmission of ASFV from feral swine to domestic pigs. The clothing or shoes of a person following contact with infected feral swine, their carcass, or a contaminated environment may serve as a pathway of transmission[155], especially during normal hunting or slaughter practices[120].

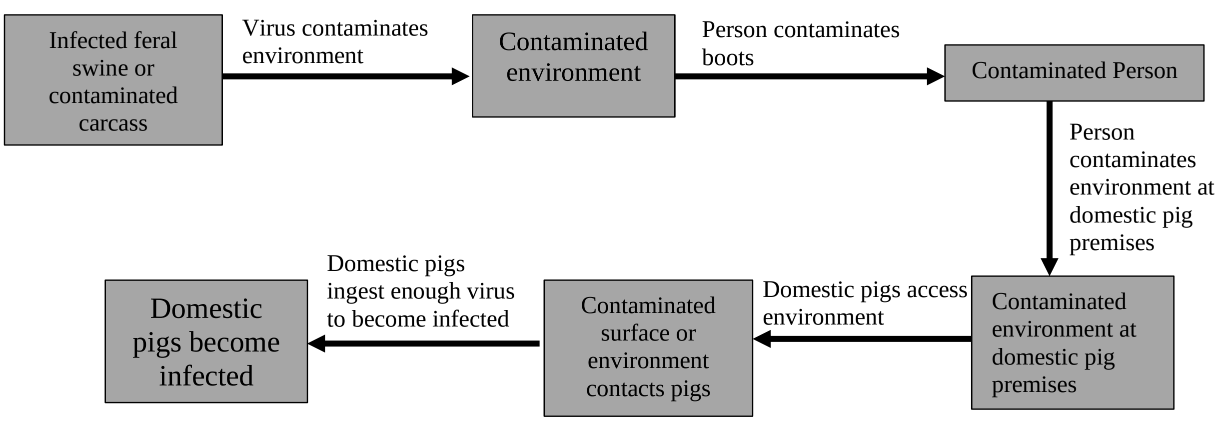

Figure 10. Example of a pathway for which people can serve as indirect pathway for transmission of ASFV from feral swine to domestic pigs.

Another possibility is the movement of contaminated feral swine meat or offal, or other sub-products such as skins, skulls, tusks, and other hunting trophies.[119] Hunters that eviscerate hunted feral swine on the spot may introduce additional virus into the environment.[127] Hunters that dress their hogs elsewhere and improperly dispose of offal could introduce ASFV into the environment, as has been described in areas of Europe.[117]

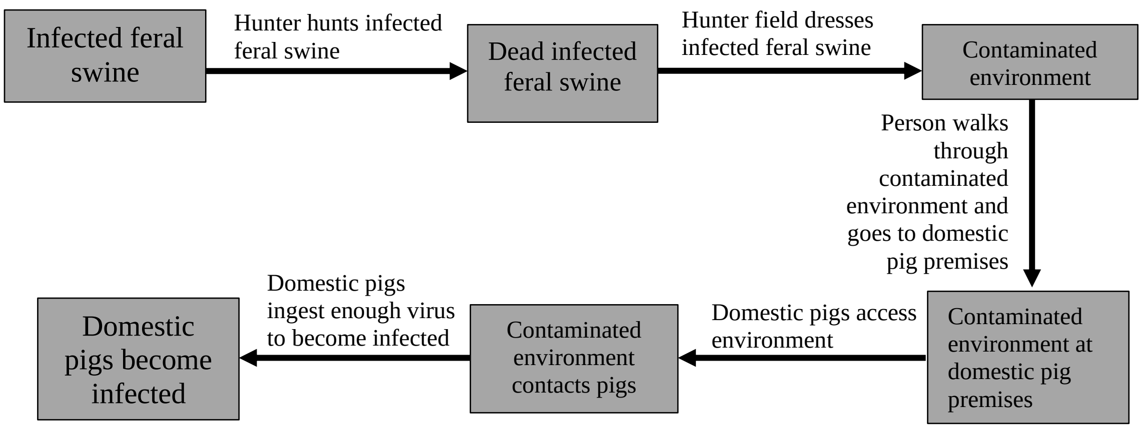

Figure 11. Example of a pathway for which hunters (i.e., people) can serve as an indirect pathway for transmission of ASFV from feral swine to domestic pigs.

3.10. Biological Materials

Biological materials are those that are derived from a variety of natural sources or produced by biotechnological processes.[156] Products may be composed of sugars, proteins, nucleic acids, cells, tissues, or combinations of these.[156] The biological materials that may be used on domestic pig farms include vaccines, bacterins, diagnostic kits, and oral, injectable, or topical medications. Any of the ingredients that are used in these products, that are derived from infected feral swine or infected domestic pigs could serve as an indirect pathway transmission. Another form of biological materials is semen for the use in artificial insemination. ASFV has been detected in semen at 2 days following experimental infection[92], semen from infected feral swine being used for artificial insemination could serve as an indirect pathway of indirect transmission from feral swine to domestic pigs.

4. Mitigation Measures for ASFV Transmission from Feral Swine to Domestic Pigs

This section will review mitigation measures that have been proposed or are in use outside the US in countries that are ASFV positive, have eradicated ASFV, and/or have borders with ASFV positive countries. The mitigation measures reviewed are designed to either decrease or eliminate the likelihood of indirect or direct transmission of ASFV from wild boars/feral swine to domestic pigs. In addition to the detailed information on the mitigation measures, regulations and recommendations from the USDA to mitigate disease transmission between feral swine in the US and domestic pigs are included where appropriate.

In Section 3, the potential pathways of transmission for ASFV transmission from feral swine to domestic pigs in the US were presented. Some of the pathways overlap and therefore one mitigation measure may interrupt more than one transmission pathway. For example, educating hunters on the proper disposal of entrails or offal could not only mitigate transmission via the mortality and cull management pathway (**Section 3.2**) but also the wild/peri-domestic animals (**Section 3.2**) and people (**Section 3.9**) pathways. Therefore, instead of repeating information for each pathway, the different mitigations measures identified in the literature will be described, along with the various pathways they mitigate. For reference, the following pathways in Section 3 are (**Table 6**).

Table 6. Potential pathways of ASFV transmission from feral swine to domestic pigs in the US.

Pathways of Transmission
3.1 Wild and peri-domestic animals 3.1.1 Feral Swine 3.1.2 Other Wild Animals 3.1.3 Peri-domestic animals
3.2 Mortality and cull management
3.3 Domestic animals
3.4 Insects and arthropods 3.4.1 Arthropods 3.4.2 Insects

3.5 Water
3.6 Feed and Bedding 3.6.1 Feed 3.6.2 Bedding
3.7 Aerosols
3.8 Vehicles and equipment 3.8.1 Vehicles 3.8.2 Equipment
3.9 People
3.10 Biological materials

4.1 Prevention, Surveillance, and Communication

4.1.1 Prevention

4.1.1.1 Vaccination

The vaccination of domestic pigs, wild boars, and feral swine against ASFV have all been proposed as preventative measures. Vaccination that targets domestic pigs would help mitigate transmission from feral swine by increasing the domestic pig population's resistance to infection and decreasing the severity of clinical disease. Vaccination of wild boars and feral swine will mitigate this pathway by reducing the amount of virus shed which should then decrease the probability of virus transmission to domestic pigs. Although vaccines would be a great preventative tool, there are no commercially available ASFV vaccines in the United States.

Although there are no approved vaccines for use in the US, there are a few vaccines being considered as potentially effective (and safe) to control ASFV in domestic pigs in Vietnam[90], China[157], and EU[90]. The proposed EU vaccine is also being considered for use in wild swine[90]. There are limitations to the use of vaccines in free ranging populations. A vaccine for wild animals must be orally administered, must be effective after a single dose, and must be stable in the environment, especially under extreme conditions.[117] A suitable delivery device for oral administration is also necessary, most likely in the form of bait.[117] Finally, this bait must be safe and traceable, not only for wild boars, but also for non-target species.[117]

Pathways Mitigated: 3.1.1 Feral Swine

Another type of vaccine that has been proposed for the prevention of ASFV is an anti-tick vaccine. There are currently two anti-tick vaccines that have been used in Australia, Cuba, Mexico, and Columbia using *Rhipicephalus microplus* antigens for use in cattle[158], but none against *Ornithodoros spp*[159]. It is also important to note, that an anti-tick vaccine would only prevent transmission of ASFV from ticks and would not be effective against other transmission pathways of ASFV from feral swine to domestic pigs.

Pathways mitigated: 3.4.1 Arthropods

4.1.2 Surveillance

An important part of preventing transmission of ASFV from feral swine to domestic pigs is to first recognize its presence in the feral swine population. Surveillance is a tool that has been used by previously negative and currently positive ASFV countries for early detection of infections in wild swine populations.[117] Depending on the ASFV status in a region or country, passive or active surveillance of wild boars that were found dead or hunted, has been effective.[160] Active or passive surveillance of ticks may also be performed in tick habitats or pig farms.[160] The US has targeted active surveillance of feral swine in certain regions. More information of this program can be found https://www.aphis.usda.gov/animal_health/downloads/animal_diseases/swine/hemorrhagic-fevers-integrated-surveillance-plan.pdf.

Pathways Mitigated: 3.1.1 Feral Swine; 3.2 Mortality and cull management

4.1.2.1 Diagnostics

Diagnostic assays are necessary for the detection of ASFV. Many of the validated diagnostic methodologies in use have been validated for use in wild suids, some of which may have better sensitivity in this population. For example, in a comparative study of the most widely used ASF diagnostic assays in the EU, the confirmatory IPT might be a better serological tool for evaluating prior viral infection in wild boars compared to three commercial ELISAs available in Europe.[161] They were Ingenasa-Ingezim PPA Compac K3 (Ingenasa, Madrid, Spain), IDvet-ELISA (ID Screen African swine feer indirect assay; Grabels, France), and indirect Svanova-ELISA (Svanovir ASFV-Ab; Boehringer Ingelheim Svanova, Uppsala, Sweden).[161] In the US, any samples submitted for testing of ASFV is currently performed at the National Veterinary Services Laboratory of the USDA.

Pathways Mitigated: 3.1.1 Feral Swine; 3.2 Mortality and cull management

4.1.3 Communication

Communication offers a way to provide information on recommendations for mitigation measures used to reduce the likelihood of ASFV spread from feral swine to domestic pigs. Although state and federal regulators are charged with controlling ASFV, control cannot be achieved without the engagement, cooperation, and efforts from the individuals who own or handle wild swine or domestic pigs. To foster the public/private partnership necessary to prevent and respond to ASFV, appropriate communication campaigns are needed that provide, at the very least, basic information about the disease and preventative measures.[155] In addition to pig producers, all those involved in the pig production and marketing chain should be involved, including backyard holdings, transporters, service providers and slaughterhouses.[155]

Following the outbreak in China in August 2018, South Korea started education on ASF through seminars, posters, and specific education for livestock farmers. Their purpose was to indicate "the importance of rapid reporting of pig diseases".[162] Education was not only for farmers but also for the public to discourage bringing or leaving of "dangerous foods or food wastes in areas where wild boars are present".[162] It was also noted that communication is needed to raise awareness of hunters, especially with their involvement in detecting potentially infected wild swine and contaminated carcasses.[119]

Pathways Mitigated: 3.1.1 Feral Swine; 3.2 Mortality and cull management; 3.9 People

4.2 Containment of ASFV in native wild suid species

4.2.1 Outbreak Zones

Following positive ASF cases in wild swine, many countries have used containment zoning. Each of the zones in a nation will have specific regulations in place, regarding hunting or surveillance. For example, in South Korea positive wild boar areas have been zoned into occurrence areas (outbreak areas), buffer zones, and border zones.[162] In Belgium and the Czech Republic, the zones that were created were the infected zone, buffer zone, and a control zone.[117] In 2008, South Africa declared an ASFV control zone where *Ornithodoros* spp. ticks and potentially infected warthogs were present.[163]

Pathway(s) Mitigated: 3.1.1 Feral Swine; 3.2 Mortality and cull management; 3.9 People

4.3 Mitigation Measures Targeting the Wild Boar/Feral Swine Populations

4.3.1 Reduction of Population Density

Decreasing the population density of wild swine has been proposed as a mitigation measure for ASFV transmission from wild swine to domestic pigs. It is thought, that by decreasing the susceptible population of wild swine, there will be less disease pressure exerted on the susceptible domestic pig population.[164] There are many ways to decrease population density, the following are examples of wild swine population reduction methods.

<u>*Pathway(s) Mitigated: 3.1.1 Feral Swine*</u>

4.3.1.1 Hunting

A 2014 scientific report of EFSA reported that "no evidence was found in scientific literature proving that wild boar populations can be drastically reduced by hunting…in Europe".[164] This extrapolation was based on the adaptive behavior of wild boars in which these practices may disperse groups of boars, potentially increasing the geographic spread of ASFV.[164] Driven hunts of wild boar in active areas of ASFV circulation is performed have recently shown to be ineffective because these types of hunts resulted in the dispersal of the wild boars, especially when dogs are used, potentially spreading ASFV even further.[119]

The Food and Agriculture Organization (FAO) recommends that in areas where hunting biosecurity compliance is difficult a total ban on hunting should be considered, especially if proper surveillance or ASFV testing is unlikely and/or proper disposal of carcasses is impossible.[119] This mitigation measure decreases the disturbance and dispersal of infected and susceptible wild swine and also places a restriction on the movement of potentially contaminated killed animals.[119]

In the Czech Republic, strict hunting practices to reduce disturbance of the wild boar population as much as possible was deployed as part of a depopulation strategy in the control zone.[117] The hunting involved hunting communities who were only allowed to hunt under special permit and only outside the second-layer fences.[165] While it is possible that driven hunts could be effective, the experience in the Czech Republic suggests that hunting should be done over a very large area and involve multiple hunters, hunting clubs, and landowners.

Some countries have used armed forces for hunting wild swine.[119] The Czech Republic used trained snipers with thermal vision methods and silencers[166]. It has been noted that hunters with knowledge of a targeted area and wild boar behavior are best suited to the activity.[119] Belgium used trained local forest officers to assist in finding wild boar, night vision equipment, and Global System for Mobile Communications (GSM) camera traps at bait sites to cull the last wild boars in their ASFV infected area.[119]

In northern and eastern European countries, selective hunting of reproducing females can be more effective at reducing population density in those regions, but may lead to adaptive behavior of the wild swine groups in the future; however, information is incomplete and may differ regionally.[119]

For hunting to be effective, the location of the hunt must be considered. Aerial hunting over dense forest or in areas with high human population might not be possible[119]. Seasonality can also affect hunting effectiveness; cold temperatures may make it easier to hunt wild swine during the day because they are more likely to forage at this time compared to the spring and summer when they are nocturnal.[167] Farrowing season can also complicate tracking efforts as sows will build farrowing nests in areas of dense foliage and will stay in or near the nest for approximately 3 weeks following farrowing.[167] In Europe, wild boar become more difficult to track during farrowing because the dense green foliage of the forests complicate identification of their location.[119]

4.3.1.2 Trapping

According to a report by EFSA, "the success of trapping depends on a variety of factors, including topography, time of year, type of trap used, number and density of traps deployed, trap location, number of nights each trap is used, type of bait used and duration of pre-feeding before the traps are set”.[164] They also noted that trapping is considered to be more costly and less efficient when compared to hunting.[164] Cage traps were used inside a second layer fence in South Korea.[165] The use of traps have been employed in the US, and Gaskamp et. al, (2021) has published information on the different types of traps and their effectiveness and efficiency for capturing wild pigs in the state of Oklahoma.[168]

When used in conjunction with euthanasia, and when managed appropriately, trapping can be a highly effective method of reducing wild swine populations.[119] Trapping and euthanasia was used effectively in small classical swine fever-affected populations in Bulgaria and Belgium.[119]

4.3.1.3 Contraception

Contraception has been proposed as a method of reducing the wild boar population in Europe.[119] Of course, there are numerous criteria that must be met for contraception use.[119] To date (c. 2022), there are no effective oral contraceptives that are available and marketed, only injectable, making it logistically difficult to administer to wild suids.[119] Although the use oral contraceptives in feral swine is desirable, more research on its formulation and application is necessary.[119]

4.3.1.4 Poisoning (Biocides)

Poisoning, or the use of biocides, has been proposed to control wild boar populations.[164] This strategy has not been applied in the EU, as regulations restrict biocide use in this manner. This tool has been used for other wildlife in the Americas and Oceania with mixed results.[119] In Australia, poisoning has been effective at managing pig populations, using toxin 1080 (sodium fluoroacetate) or sodium nitrite-dosed baits.[169] Most successful applications of poisoned bait follow pre-feeding with untreated bait.[169] A general downside of using bait is that once boars have perished, carcasses may be difficult to locate for disposal.[169] Like oral contraception, there are many considerations needed for the use of biocides as a tool for the reduction of wild swine populations.[119]

4.3.1.5 Artificial Feeding Stations

The use of artificial feeding stations has been proposed to keep wild boar in a specific geographic area to restrict their movement, and subsequently the geographical spread of diseases such as ASF.[164] Outside of restricting wild boar movement, they have also been used to facilitate trapping, hunting, or as a deterrent or distraction from agricultural fields.[164] Although there are benefits of artificial feeding stations for the restriction of movement and other mitigations to decrease population density, it has also been noted that they increase the concentration of wild boar in a given area which might facilitate spread of ASFV within the wild boar population.[164] Additionally, artificially feeding could also increase wild suid populations through "improved survival during winter and reproductive output".[164]

The FAO has proposed regulating supplementary feeding as a more appropriate mitigation.[119] This can either be a complete ban on supplementary feeding, or a restriction on the total amount used in an area.[119] For example, the EU set a limit of ten kilograms per square kilometer per month in most parts of northern and eastern Europe.[119] This is especially important for hunters who use supplementary feed as an attractant for wild boar.[119] An alternative that was suggested was using salt licks instead of feed.[119]

4.3.1.6 Manipulation of Habitat (of wild boar) Carrying Capacity

The manipulation of the carrying capacity of wild boar habitats was also proposed during a European World Café workshop.[166] The general purpose of manipulating habitats is generally targeted at reducing the population of wild boar in a given geographical area. Examples proposed are the fencing off of water or food sources or clearing away bushes and trees.[166] In the Czech Republic, farmers were not allowed to harvest their agricultural fields in an attempt to attract wild boars so they remain in the area. The strategy seemed to be effective in reducing further spread of ASFV .[166] This manipulation of a wild boar habitat may only be effective in a small ASFV affected area.[166]

4.3.1.7 Comparing Mitigations for the Reduction of Population Density

There are many contradictions for the effectiveness of the mitigation strategies presented (e.g., hunting, trapping, fencing) for the reduction of wild swine populations. For example, hunting has been known to scatter groups of wild swine, potentially furthering the spread of ASFV. Yet, in a few accounts, hunting has been successful in helping eradicate ASFV from the wild swine population. Other mitigations like trapping could be less effective than hunting but could also be highly effective. There are important caveats to consider when ensuring a measure employed is effective for the reduction of wild swine population density. The caveats and considerations include, but are not limited to, these three areas.

I. Clear Objectives. There must be clear objectives in the mitigation measure used. Capturing a whole group of wild swine would be more effective than only hunting/trapping a few of them, as the wild swine that were not successfully captured could scatter.[119]

II. Wild Swine Ecology and Behavior. It is important to consider wild swine ecology and behavior when choosing which mitigation measure to use. Location (e.g., dense forests or open range), seasonality (e.g., winter or spring), and wild swine population can all play an important part in how successful a mitigation measure can be.[164]

III. Layering of mitigation measures. All the population reduction strategies presented should not be considered exclusive measures and could be used in conjunction with one another to make their use more effective. An example of this in the literature was the use of artificial feeding to get wild swine to concentrate in a smaller area before trapping.[164]

4.3.2 Fencing

4.3.2.1 To restrict movement of wild swine

In South Korea, fences were installed at the boundaries between ASF outbreak areas and wild boar buffer zones.[162] In the Czech Republic, fences were used to physically isolate the infected zone to decrease the further spread of ASF.[117] Although there are numerous accounts for the use of fencing to restrict the movement of wild boar, it may not be useful in all situations. Information on the spatial distribution of wild boar populations is necessary to identify the locations wherein fencing can be effective.[164] Fencing was used a control measure in South Korea to limit the spread of ASFV in wild boar.[165] A three-layer fencing system was used. The first layer was an electrical fence 1-2 kilometer (km) away from a confirmed wild boar carcass.[165] The second was "semi-rigid 1.5 meter high wire mesh" about 5-10km away from the electrical fence. The third was a 250km long fence, about 20-30km away from the second fence.[165]

The FAO recommendations regarding permanent boar-proof fencing are to use fences made of woven wire mesh at a minimum of 1.5-1.8 meters high, fixed to the ground, and with barbed wire on the sides and along the top.[119] To mitigate local spread and implement more focused control of ASFV spread, temporary fencing, such as that used in the Czech Republic and Belgium[119], is an option. The FAO also noted that electric fencing alone is not sufficient to completely block animal movements but could be used as a deterrent, especially to protect smaller areas of land. Other examples of deterrents that have been used include chemical, visual, and acoustic methods, all of which may have non-target effects, especially when used over a long term and for large areas.[119]

Pathway(s) Mitigated: 3.1.1 Feral Swine

4.3.2.2 To exclude feral swine from domestic pig farms (**Appendix C**)

The EU Commission requires that animals should be "kept in a way that ensures that no direct nor indirect contact occurs with wild boar or feral pigs".[170] Wild boar- and feral swine-proof fences have been described, and have been in used for hunting preserves (to keep wild boar in) and also in agricultural or ecological environments (to keep wild swine out).[164] Therefore in the US, these wild boar-proof fences, either single or double, might also be used on domestic pig farms to keep feral swine from entering the premises or pasture by preventing direct contact between feral swine and domestic pigs[170].

The EU commission prescribes that any perimeter fence should "delimitate the commercial holding" and that for outdoor farms fences be "preferably doubled, at least 1m apart, and proofed against wild boar and pigs. Fences should be at least 2m high of which 50cm should be under the ground".[170] In the ASFV control area of South Africa in 2008, the only pig farms allowed were those that used double-fence barriers and other biosecurity measures to prevent contact between domestic pigs and the wild swine.[163] The Veterinary Services of South Africa required domestic pigs be kept in secure, pig-proof pens, with cement flooring.[171] These pens had to be surrounded by pig-proof fencing that was at least 1.3 meters high and anchored into the ground with concrete.[171] In South Korea, pig farms are required to have double fences.[165] In

Sardinia, regulations state that farms "should have perimeter barriers of at least 1.5m high and wild boar proofed and fenced fields have a maximum extension of 3 hectares[1]".[170]

Pathway(s) Mitigated: 3.1.1 Feral Swine; 3.2 Mortality and cull management

4.3.3 Confinement of Domestic Pigs

Bellini et. al (2016) recommended that domestic pigs in backyard holdings be confined in stables to minimize the risk of ASFV spread.[155] In 2015, the EU Commission banned outdoor keeping of pigs.[170]

In certain countries where ASFV is considered epidemic, in an attempt to decrease further spread of the disease, the establishment of backyard herds has been prohibited, although such discriminatory practices should be carefully evaluated due to high risk of poor compliance.[155]

4.3.4 Hunting Practices

In the EU, hunters play an important part in helping contain and further prevent the spread of ASFV. Recreational hunters in the US could also play an important part in the mitigation of ASFV transmission from feral swine to domestic pigs; however, as in the EU, the hunting must be properly designed and overseen. Given this caveat, if hunting is ineffective, unguided, or poorly regulated, hunters may increase the risk of ASFV spread when they contact infected wild swine, their carcasses, and environment. Therefore, the following are mitigation strategies specific to hunters that would make hunters an asset rather than a liability.

[1] *hectare*. Defined as a metric unit of square measure, equal to 2.471 acres or 10,000 square meters.

4.3.4.1 Education

A few biosecurity recommendations specific to hunting practices have been proposed by Bellini et al. (2016) and include:

- "Hunters shall be authorized to hunt in the area only after a specific training on basic hygiene and biosecurity practices."[155]
- "Hunting suits, including boots/shoes should be kept in specific bags. Boots are worn in the dressing room before hunting and re-placed in the same bag after hunting."[155]

In a survey in Serbia, Montenegro, and Kosovo, hunters reported "regularly disinfect[ing] their hands, clothing, footwear and equipment after the manipulation or handling of offal or dead animals (72% in Kosovo, 83% in Montenegro and 89% in Serbia)".[127]

Pathway(s) Mitigated: 3.1.1 Feral Swine; 3.2 Mortality and Cull Management; 3.8.2 Equipment; 3.9 People

4.3.4.2 Dressing facilities

Special, dedicated carcass dressing facilities for hunting have been proposed to be available for hunters, to assist with biocontainment of potentially contaminated wild boar carcasses following hunting. A few biosecurity recommendations specific to dressing facilities by the EU Commission[172] and further addressed by Bellini et al. (2016) and include:

- "Transport of hunted animals to the dressing facility is carried out using dedicated vehicles. Private cars shall be parked outside the hunting house, possibly on the main road."[155]
- "The use of the dressing facilities should be authorized only in case its available: tap water, electricity, waste water collection and freezers."[155]

- "Animal dressing should be performed using appropriate aprons which must remain in the facility. Working tools cannot be transported to other places."[155]
- "Boots and apron shall be cleaned and disinfected after each use."[155]
- "Dressing rooms are to be equipped with effected disinfectants."[155]
- "Offals should be stored in proper containers inside the dressing areas and before storing, containers shall be cleaned and sprayed with effective disinfectants."[155]
- "Ground pits for offal disposal should be at least 1.5 meters deep, fenced and closed with a locked closure. Pits should be located in close proximity to the dressing room."[155]

In a study of hunting environments in the Balkan countries, only in Serbia (not in either Montenegro or Kosovo) is disinfection widely used to clean and disinfect these facilities.[127]

Pathway(s) Mitigated: 3.1.1 Feral Swine; 3.2 Mortality and Cull Management; 3.8.1 Vehicles; 3.8.2 Equipment; 3.9 People

4.3.4.3 Quarantine Testing (of hunted wild swine)

These dedicated dressing facilities could have a holding facility where the freshly hunted wild boar carcasses are quarantined while awaiting the results from ASFV testing.[155] A few biosecurity recommendations specific to quarantine and carcass testing from the EU Commission[172] that has been laid out by Bellini et al. (2016) and include:

- "Hunted wild boar should never leave the hunting area unless checked and tested and the carcasses released only when resulted negative to ASFV."[155]
- "Wild boar carcasses shall be individually identified before storing. In case of ASFV positive outcome all stored carcasses have to be disposed under veterinary supervision and the whole dressing room cleaned and disinfected."[155]

Pathway(s) Mitigated: 3.2 Mortality and Cull Management

4.3.5 Cleaning & Disinfection

In a study evaluating the stability of ASFV in different soil matrices, the use of citric acid or calcium hydroxide resulted in complete inactivation of the virus after 1 hour of treatment, although the authors noted that the depth of carcass fluid drainage might affect the efficacy of treatment.[104]

Pathway(s) Mitigated: 3.1.1 Feral Swine; 3.2 Mortality and Cull Management

4.3.6 Reporting, Testing, and Removal of Wild Swine Carcasses

The European Union (EU) Commission has recommended procedures for the control of ASFV. One of those recommendation is the reporting of all dead boars and the testing of wild boars hunted/killed in ASF control zones.[162] In South Korea, all hunted wild boars were tested for the presence of ASFV[162] in addition to the active search for carcasses. In the Czech Republic, boars found dead were collected and rendered "under strict biosecurity measures".[117]

Pathway(s) Mitigated: 3.2 Mortality and Cull Management

4.3.7 Domestic Animals

A minimum biosecurity requirement of the EU commission is that all farm buildings should "be built in such a way that no…other animals (e.g. dogs and cats) can enter the stable". The US also has regulations for dogs and cats that are imported from ASFV positive countries in that the dogs and cats must be bathed at the US post-entry point(s) within two days of arrival.[173]

Pathway(s) Mitigated: 3.3 Domestic Animals

4.3.8 Insects and Arthropods

Stable flies can be restricted with the use of "sanitation, biological, and chemical controls". An example is the elimination of "fly breeding sites in combination with placing insecticide-treated nets".[170]

In Spain and Portugal where *Ornithodoros erraticus*, a known ASFV vector, is present, it is recommended that pig-housing facilities used in outdoor production are kept in good repair to help prevent tick harborage and thus ASFV. If the pig-housing facility is no longer in use, the facility should be fenced off to keep domestic swine from entering. If ticks are present, the pig-housing facility should be destroyed.[170] If in use and in good repair, methylene bromide should be applied to the facilities and/or the domestic pigs treated with ivermectin. If infected ticks are found, it is recommended to keep a building empty of swine for six years.[170]

Pathway(s) Mitigated: 3.4 Insects and Arthropods

4.3.9 Water

Fencing has been used to exclude wild swine from highly sensitive environmental areas, such as mound springs and freshwater lagoons.[169] Virkon S® (active components: sodium chloride and potassium peroxymonosulfate) can be used to disinfect watering equipment against ASFV.[119] The location and depth of surface and ground water should be considered when disposing of ASFV contaminated wild swine carcasses[119], to prevent leeching of ASFV into these bodies of water.

Pathway(s) Mitigated: 3.5 Water

4.3.10 Feed and Bedding

In the EU, in regions where there may be a risk of ASFV contamination on locally harvested grasses or grains, there is a ban on feeding fresh grass or untreated grains to pigs.[146] Drying for two hours of dry wheat, barley, rye, triticale, corn and peas inactivates ASFV.[146] In the EU, in regions where there may be a risk of ASFV contamination on locally harvested grasses or grains, the use of straw bedding for pigs is discouraged, unless a treatment for inactivation is used, or the straw has been stored for at least 90 days before use.[146]

Pathway(s) Mitigated: 3.6 Feed and Bedding

4.3.11 Vehicles and Equipment

In South Korea, the government started a “ban of vehicle entrance close to pig-raising barns program that prohibits related vehicles from entering the area of pig houses”.[165] These vehicles could be for the transport of feed, pigs, and manure. When this was enacted, some farms needed to be reconfigured to make this possible.[165] It is recommended that “cleaning and disinfection protocols are established and periodically performed on every farm facility, vehicle, and piece of equipment”[170] to mitigate the transmission of ASFV from these surfaces.

It is recommended that “sharing of equipment between holdings should be discouraged.[170]

Pathway(s) Mitigated: 3.8 Vehicles and Equipment

4.3.12 People

In addition to routine biosecurity measures, a mitigation measure recommended by the EU Commission[172] is that footbaths “should be used at the entrance of every unit where animals are held” [170] and that “organic material should be removed from footwear prior to disinfecting” [170].

Pathway(s) Mitigated: 3.9 People

4.3.13 Biological Materials

The EU commission minimum biosecurity requirements for breeding farms are that semen, ova, or embryos should come from free-ASFV certified farms.[170]

Pathway(s) Mitigated: 3.10 Biological Materials

4.4 Other Mitigation Measures

There were no specific mitigation measures specifically found in the literature for the transmission of ASFV from wild swine to domestic pigs by wild animals other than wild swine, peri-domestic animals, or aerosols. General mitigation strategies applicable for these pathways, but non-specifically for wild swine, include the following.

4.4.1 Other Wild Animals and Peri-domestic Animals

The US currently has recommended industry biosecurity practices in swine operations regarding wildlife include pest/rodent-control plans and exclusion of birds and other wildlife species by bird proof walls and fencing.[46] EFSA recommends the prompt removal of animal feed or any other elements that might attract certain types of birds.[124]

4.4.2 Aerosols

In Spain, in an effort to decrease the spread of infectious disease in pigs, a minimum distance of 1-2km (dependent on pig production type) between pig farms is required by royal decree.[174] Planting trees around premises is another suggested biosecurity measure in the livestock industry, for poultry farms in particular, as greenery may act as a vegetative environmental buffer (VEB), or natural filter of airborne particles going into and out of a farm.[175,176] There is evidence for U.S. sow farms of a protective effect from disease on premises located in an area of shrubs and trees.[177]

4.5 Conclusion

This section has provided mitigation measures that have been proposed or are currently in action in the US or internationally. The mitigation measures presented are designed to either decrease or eliminate the likelihood of indirect or direct transmission of ASFV from wild swine to domestic pigs. There are many considerations that should be made when deciding which mitigations are more appropriate to be implemented, which will be discussed in the next section (S**ection 5**).

5. Final Considerations and Conclusion

The purpose of this literature review is to identify the pathways by which feral swine might transmit ASFV to domestic pigs in the US. Using literature from current or previously ASFV infected countries, mitigations that have been used to address these pathways are also described. A risk estimation is not included since this requires gathering additional information on US practices, climate, pig density, etc. A risk assessment, if completed, would provide a risk estimation, therefore a risk assessment is a clear next step.

In the literature identified and reviewed, there are many reports from the EFSA in which specific knowledge gaps related to pathways of ASFV transmission from wild swine to domestic pigs have already been identified, including feed[146] and insects[124]. Additionally, anecdotal reports are included to provide the evidence that water[129] and peri-domestic animals[121,122] are pathways for ASFV[121,122,129]. However, there are no confirmatory studies available regarding water and peri-domestic animals. Therefore, additional work is needed to provide more definitive evidence of some of the potential pathways of ASFV transmission from feral swine to domestic pigs to really understand their importance in the US.

As expected, mitigations to prevent wild suid to domestic pig exposure collected from various countries facing different types of ASFV challenges cover a range of recommendations. The following eight considerations may be used to guide development of US standards while keeping in mind the situations or settings in which the use of specific interventions might be implemented.

I. Geography or Topology: While the use of electric fences to deter wild swine may be appropriate for a small area, electric fencing might be difficult for a larger area or in areas with dense forests.[119]

II. Control versus Eradication: Some countries like the Czech Republic and Belgium were aggressive in their actions to eradicate ASFV from feral swine, whereas Poland has created zones with the intent to control ASFV in the wild suids in their country rather than pursue eradication.

III. ASFV strain characteristics: Some virus strains are more virulent than others. Strain virulence has an impact on morbidity and mortality in the wild swine population and subsequently the effectiveness of surveillance in the population.[160] In other words, highly virulent strains that cause high mortality are easier to detect due to the increased numbers of dead suids present for testing. In contrast, moderately or mildly virulent strains may go undetected if sublethal and surveillance efforts are focused only on dead animal testing. In addition, strains that are mildly virulent in wild suids may spread more easily due to the movement of subclinically infected suids.

IV. Funding: Many countries discussed the high cost of fencing for zoning purposes and the remarkable expense of building border fences to restrict the movement of wild boars. Economics may limit the use of fences.

V. Regulations or Policy: Some of the mitigation measures in effect in other countries are written in their regulatory or governmental policy. These policies may require or restrict certain on-farm practices that mitigate the spread of ASFV from wild swine to domestic pigs. For example, the farming of free-ranging domestic swine is illegal in Sardinia[129], and South Korea has a national program prohibiting vehicles from entering areas where pigs are held[165].

VI. Proximity to "risk": The type of preventative or mitigation measures carried out in a country were primarily dependent on risk. South Korea began education campaigns in response to the outbreak in neighboring China[162]. However, other mitigation measures, such as the program prohibiting vehicles from entering areas where pigs are held[165], were not enacted until after ASFV was confirmed in the country itself.

VII. Culture: The use of some mitigations may become contentious, especially when it pertains to the regulations on how pigs are raised. For example, in Sardinia, the production of free-range pigs is a traditional way of farming in the mountainous regions[129]. Although the Sardinian government has made the farming of free-ranging pigs illegal, the removal of these traditional practices has been difficult because this practice is deeply-rooted in these farmers' cultural identities.[129]

VIII. Protection of animals and trade: Many countries decided to build border fences to keep wild swine moving into their country from neighboring ASFV positive countries. Denmark is a large exporter of pork and built a fence along the border with Germany to protect Danish trade status with non-European countries.[178]

In conclusion, there are many considerations to make when choosing which mitigation strategy (or strategies) may be the most effective at reducing the transmission of ASFV from feral swine to domestic pigs in the US. It is important to understand that feral swine ecology and behavior in North America differs from that of wild boars in Europe and Asia. Additionally, the different wild swine and tick species in Africa make the sylvatic transmission cycle a unique exposure pathway particularly for countries with competent tick vectors, susceptible hosts, and hospitable climates. In the North American ecosystem, some pathways will be less important and some of the mitigations that work where ASFV currently is located might not be effective in the

US. Additional knowledge gaps exist for the potential pathways of ASFV transmission from wild swine to domestic pigs in other countries that also need further elucidation. In particular, there is a lack of information regarding environmental contamination from ASFV shedding.[146]

Additionally, some mitigation strategies in Section 4 are currently in place in the US regarding the control of feral swine population density. Although US pork producers may have little input in the success or failure of feral swine population reduction efforts, the very existence of federal feral swine control efforts should be considered a protective measure to keep in place indefinitely. Finally, it is expected that risk of transmission from feral swine to domestic pigs will vary across the regions and sectors of the US pork industry. Clear and targeted mitigations are needed in the US that are specifically targeted to meet regional and sector risks and should be considered in future work.

Acknowledgements

The guidance of Drs. Carol Cardona, Marie Culhane, Cesar Corzo and the efforts of the entire University of Minnesota Secure Food Systems team were necessary and important for the completion of this literature review. Drs. Chris Rademacher and Montserrat Torremorell recognized the need for this work and provided the inspiration and structure. The intellectual support of the aforementioned people and the financial support from the National Pork Board are greatly appreciated.

References

1. Anderson JL. *Capitalist Pigs: Pigs, Pork, and Power in America*. First edition. West Virginia University Press; 2019.

2. USDA, APHIS. Feral Swine-Managing an Invasive Species. Accessed April 16, 2023. https://www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/operational-activities/feral-swine

3. Smyser TJ, Tabak MA, Slootmaker C, et al. Mixed ancestry from wild and domestic lineages contributes to the rapid expansion of invasive feral swine. *Mol Ecol*. 2020;29(6):1103-1119. doi:10.1111/mec.15392

4. Helcel J. The Origin of the Wild Pig Species. Texas A&M Natural Resources Institute Journal. Published January 2, 2018. Accessed April 16, 2023. https://nri.tamu.edu/blog/2018/january/the-origin-of-the-wild-pig-species/

5. Mississippi Department of Wildlife, Fisheries, and Parks. Wild Hog Program-History of Introduction. Wildlife & Hunting. Accessed April 16, 2023. https://www.mdwfp.com/wildlife-hunting/wild-hog-program/about-wild-hogs/history-of-introduction/

6. Wild Game For Retail Sale. Published online October 2016. Accessed April 29, 2023. https://www.fdacs.gov/content/download/65482/file/wildgameforretailsale.pdf

7. *Game and Fish, M.N. Statute*. Vol 97A.56.; 2015. Accessed April 17, 2023. https://www.revisor.mn.gov/statutes/cite/97A.56

8. USDA, National Invasive Species Information Center. Wild Boar. Terrestrial Invasives, Terrestial Vertebrates. Accessed April 16, 2023. https://www.invasivespeciesinfo.gov/terrestrial/vertebrates/wild-boar

9. Aschim RA, Brook RK. Evaluating Cost-Effective Methods for Rapid and Repeatable National Scale Detection and Mapping of Invasive Species Spread. *Sci Rep*. 2019;9(1):7254. doi:10.1038/s41598-019-43729-y

10. McClure ML, Burdett CL, Farnsworth ML, et al. Modeling and Mapping the Probability of Occurrence of Invasive Wild Pigs across the Contiguous United States. *PLoS ONE*. 2015;10(8):e0133771. doi:10.1371/journal.pone.0133771

11. O'Brien P, Vander Wal E, Koen EL, et al. Understanding habitat co-occurrence and the potential for competition between native mammals and invasive wild pigs (Sus scrofa) at the northern edge of their range. *Can J Zool*. 2019;97(6):537-546. doi:10.1139/cjz-2018-0156

12. Robbins J. Feral Pigs Roam the South. Now Even Northern States Aren't Safe. *The New York Times*. https://www.nytimes.com/2019/12/16/science/feral-pigs-canada-texas.html. Published December 16, 2019. Accessed April 29, 2023.

13. USDA APHIS | History of Feral Swine in the Americas. Published March 9, 2023. Accessed April 29, 2023. https://www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/operational-activities/feral-swine/sa-fs-history

14. Gaston W, Armstrong JB, Arjo W, Stribling HL. Home Range and Habitat Use of Feral Hogs (Sus scrofa) on Lowndes County WMA, Alabama. In: *National Conference on Feral Hogs*. ; 2008:18.

15. Snow NP, Jarzyna MA, VerCauteren KC. Interpreting and predicting the spread of invasive wild pigs. *J Appl Ecol*. 2017;54(6):2022-2032. doi:10.1111/1365-2664.12866

16. Croft S, Franzetti B, Gill R, Massei G. Too many wild boar? Modelling fertility control and culling to reduce wild boar numbers in isolated populations. *PLoS ONE*. 2020;15(9):e0238429. doi:10.1371/journal.pone.0238429

17. Davis C. Feral Hogs in Missouri: Illegal release and transport has driven up damage over decades. *Missouri Department of Conservation*. https://mdc.mo.gov/newsroom/feral-hogs-missouri-illegal-release-transport-has-driven-damage-over-decades. Published February 6, 2020. Accessed April 29, 2023.

18. *Natural Resources, California Code of Regulations. Title 14*.; 1996.

19. *Parks and Wildlife, T.X. Code*. Vol 5-A-42.; 2019.

20. USDA, APHIS. Feral Swine Damage. Published November 10, 2020. Accessed April 16, 2023. https://www.aphis.usda.gov/aphis/ourfocus/wildlifedamage/operational-activities/feral-swine/feral-swine-damage

21. USDA, APHIS. Feral Swine Diseases Risks to Livestock. Published online February 13, 2018. Accessed April 29, 2023. https://permanent.fdlp.gov/gpo118917/fs-disease-risk-livestock.pdf

22. Peper ST, Hoffarth A, Athanasiou K, et al. Brucella spp. and Francisella tularensis from an invasive alien species (Sus scrofa) in the southcentral USA. *Ecosphere*. 2021;12(3):e03426. doi:10.1002/ecs2.3426

23. Havas KA, Yancey CB, Zhuang J, Braun C, Smith DC. Brucella suis and farm biosecurity: assessing risk in pigs raised outdoors in New York State. *J Am Vet Med Assoc*. 2022;260(S2):S87-S94. doi:10.2460/JAVMA.21.08.0399

24. Poudel A, Hoque MM, Madere S, et al. Molecular and Serological Prevalence of Leptospira spp. in Feral Pigs (Sus scrofa) and their Habitats in Alabama, USA. *Pathogens*. 2020;9(10):857. doi:10.3390/pathogens9100857

25. Anderson T, Hamond C, Haluch A, et al. Animals Exposed to Leptospira Serogroups Not Included in Bacterins in the United States and Puerto Rico. *Trop Med Infect Dis*. 2023;8(3):183. doi:10.3390/tropicalmed8030183

26. Lama JK, Bachoon DS. Detection of Brucella suis, Campylobacter jejuni, and Escherichia coli Strains in Feral Pig (Sus scrofa) Communities of Georgia. *Vector-Borne Zoonotic Dis*. 2018;18(7):350-355. doi:10.1089/vbz.2017.2187

27. Remfry SE, Amachawadi RG, Shi X, et al. Shiga Toxin–Producing Escherichia coli in Feces of Finisher Pigs: Isolation, Identification, and Public Health Implications of Major and Minor Serogroups. *J Food Prot*. 2021;84(1):169-180. doi:10.4315/JFP-20-329

28. Cummings KJ, Rodriguez-Rivera LD, Grigar MK, et al. Prevalence and Characterization of Salmonella Isolated from Feral Pigs Throughout Texas. *Zoonoses Public Health*. 2016;63(6):436-441. doi:10.1111/zph.12244

29. Elnekave E, Hong S, Mather AE, et al. Salmonella enterica Serotype 4,[5],12:i:- in Swine in the United States Midwest: An Emerging Multidrug-Resistant Clade. *Clin Infect Dis*. 2018;66(6):877-885. doi:10.1093/cid/cix909

30. Pedersen K, Miller RS, Musante AR, White TS, Freye JD, Gidlewski T. *Antibody Evidence of Porcine Reproductive and Respiratory Syndrome Virus Detected in Sera Collected from Feral Swine (Sus Scrofa) across the United States*. Vol 26.; 2018:41-44. http://www.aasv.org/shap.html.

31. Chamba Pardo FO, Alba-Casals A, Nerem J, Morrison RB, Puig P, Torremorell M. Influenza Herd-Level Prevalence and Seasonality in Breed-to-Wean Pig Farms in the Midwestern United States. *Front Vet Sci*. 2017;4. Accessed November 14, 2022. https://www.frontiersin.org/articles/10.3389/fvets.2017.00167

32. VerCauteren KC, Beasley JC, Ditchkoff SS, Mayer JJ, Roloff GJ, Strickland BK, eds. Chapter 5. Invasive Wild Pigs in North America: Ecology, Impacts, and Management. In: *Invasive Wild Pigs in North America: Ecology, Impacts, and Management*. 1st ed. CRC Press; 2020. doi:10.1201/b22014

33. Wang Y, Noll L, Lu N, et al. Genetic diversity and prevalence of porcine circovirus type 3 (PCV3) and type 2 (PCV2) in the Midwest of the USA during 2016–2018. *Transbound Emerg Dis*. 2020;67(3):1284-1294. doi:10.1111/tbed.13467

34. Bevins SN, Lutman M, Pedersen K, et al. Spillover of Swine Coronaviruses, United States. *Emerg Infect Dis*. 2018;24(7):1390-1392. doi:10.3201/eid2407.172077

35. Bjustrom-Kraft J, Woodard K, Giménez-Lirola L, et al. Porcine epidemic diarrhea virus (PEDV) detection and antibody response in commercial growing pigs. *BMC Vet Res*. 2016;12:99. doi:10.1186/s12917-016-0725-5

36. Paploski IAD, Corzo C, Rovira A, et al. Temporal Dynamics of Co-circulating Lineages of Porcine Reproductive and Respiratory Syndrome Virus. *Front Microbiol*. 2019;10. Accessed November 14, 2022. https://www.frontiersin.org/articles/10.3389/fmicb.2019.02486

37. Feral Swine Captured Tests Positive for Pseudorabies. *State of Vermont - Agency of Agriculture Food and Markets*. https://agriculture.vermont.gov/agency-agriculture-food-markets-news/feral-swine-captured-tests-positive-pseudorabies. Published May 9, 2019. Accessed April 29, 2023.

38. Dubey JP, Cerqueira-Cézar CK, Murata FHA, et al. Genotyping of viable Toxoplasma gondii from the first national survey of feral swine revealed evidence for sylvatic transmission cycle, and presence of highly virulent parasite genotypes. *Parasitology*. 2020;147(3):295-302. doi:10.1017/S0031182019001586

39. Holzbauer SM, Agger WA, Hall RL, et al. Outbreak of Trichinella spiralis Infections Associated With a Wild Boar Hunted at a Game Farm in Iowa. *Clin Infect Dis*. 2014;59(12):1750-1756. doi:10.1093/cid/ciu713

40. USDA, APHIS. Voluntary 2023 U.S. National Animal Health Reporting System (NAHRS) Reportable Diseases, Infections, and Infestations List. Published online April 6, 2023. Accessed April 29, 2023. https://www.aphis.usda.gov/animal_health/nahrs/downloads/nlrad-nahrs-disease-list.pdf

41. Reportable Diseases. Minnesota Board of Animal Health. Published October 2021. https://www.bah.state.mn.us/reportable-diseases/

42. *World Food and Agriculture - Statistical Yearbook 2020*. FAO https://www.fao.org/3/cb1329en/online/cb1329en.html

43. *2020 United States Agricultural Export Yearbook*. USDA-Foreign Agricultural Service; 2020. https://www.fas.usda.gov/sites/default/files/inline-files/2020-ag-export-yearbook.pdf

44. Quarterly Hogs and Pigs. Published online March 2021. https://downloads.usda.library.cornell.edu/usda-esmis/files/rj430453j/7p88db205/mw22w1890/hgpg0321.pdf

45. National Agricultural Statistics Service (NASS). *2017 Census of Agriculture*. USDA; 2019:1-820. https://www.nass.usda.gov/Publications/AgCensus/2017/Full_Report/Volume_1,_Chapter_1_US/usv1.pdf

46. USDA:APHIS:VS, Iowa State University Center for Food Security and Public Health. FAD PreP: Foreign Animal Disease Preparedness & Response Plan: Swine Industry Manual. Published online 2011.

47. Harris D. *Multi-Site Rearing Systems, in Multi-Site Pig Production*. Iowa State University Press; 2000.

48. Sanhueza J, Corzo C, Geary E. Foreign Animal Disease and Secure Pork Supply: The Importance of a Premises Identification Number (PIN). Published online January 19, 2018. https://www.vetmed.umn.edu/sites/vetmed.umn.edu/files/shmp_2017l18.29_importance_of_pins_-_science_page.pdf

49. McBride WD, Key N. *U.S. Hog Production From 1992 to 2009: Technology, Restructuring, and Productivity Growth*. USDA-Economic Research Service; 2013. https://www.ers.usda.gov/webdocs/publications/45148/40364_err158.pdf?v=1778.1

50. Goldsmith TJ, Culhane MR, Sampedro F, Cardona CJ. Proactive Risk Assessments and the Continuity of Business Principles: Perspectives on This Novel, Combined Approach to Develop Guidance for the Permitted Movement of Agricultural Products during a Foot-and-Mouth Disease Outbreak in the United States. *Front Vet Sci*. 2017;3:117. doi:10.3389/fvets.2016.00117

51. CME: Seasonal Patterns Determine Hog Production. The Pig Site. Published October 31, 2008. Accessed April 29, 2023. https://www.thepigsite.com/news/2008/10/cme-seasonal-patterns-determine-hog-production-1

52. Honeyman MS. Extensive bedded indoor and outdoor pig production systems in USA: Current trends and effects on animal care and product quality. *Livest Prod Sci*. 2005;94(1-2):15-24. doi:10.1016/J.LIVPRODSCI.2004.11.029

53. Greene C, Kremen A. U.S. Organic Farming in 2000-2001: Adoption of Certified System. Published online February 25, 2003. Accessed May 11, 2023. https://www.ers.usda.gov/webdocs/publications/42476/17400_aib780e_1_.pdf?v=0

54. USDA, NASS. *Certified Organic Survey 2021 Summary*.; 2022. Accessed April 17, 2023. https://downloads.usda.library.cornell.edu/usda-esmis/files/zg64tk92g/2z10z137s/bn99bh97r/cenorg22.pdf

55. Brumm M, Harmon J, Honeyman M, et al. Hoop Barns for Grow-Finish Swine. *Agric Eng Dig*. 2004;41. Accessed April 29, 2023. https://www.mwps.iastate.edu/sites/default/files/uploads/AED_41.pdf

56. Delsart M, Pol F, Dufour B, Rose N, Fablet C. Pig Farming in Alternative Systems: Strengths and Challenges in Terms of Animal Welfare, Biosecurity, Animal Health and Pork Safety. *Agriculture*. 2020;10(7):261. doi:10.3390/agriculture10070261

57. Bosch J, Barasona JA, Cadenas-Fernández E, et al. Retrospective spatial analysis for African swine fever in endemic areas to assess interactions between susceptible host populations. *PLOS ONE*. 2020;15(5):e0233473. doi:10.1371/journal.pone.0233473

58. Thomas LF, de Glanville WA, Cook EA, Fèvre EM. The spatial ecology of free-ranging domestic pigs (Sus scrofa) in western Kenya. *BMC Vet Res*. 2013;9(1):46. doi:10.1186/1746-6148-9-46

59. Rumley RW. States' "Fence Law" Statutes. The National Agricultural Law Center. Published September 2021. Accessed April 29, 2023. https://nationalaglawcenter.org/state-compilations/fence-laws/

60. USDA, APHIS. Fencing out Feral Swine Information Sheet. Published online February 2022. Accessed April 29, 2023.

https://www.aphis.usda.gov/publications/animal_health/fencing-out-feral-swine-infosheet.pdf

61. Chenais E, Ståhl K, Guberti V, Depner K. Identification of Wild Boar–Habitat Epidemiologic Cycle in African Swine Fever Epizootic. *Emerg Infect Dis*. 2018;24(4):810-812. doi:10.3201/eid2404.172127

62. Dixon LK, Chapman D. African Swine Fever Virus. In: *Encyclopedia of Virology*. Elsevier; 2008:43-51. doi:10.1016/B978-012374410-4.00358-7

63. Beltrán-Alcrudo D, Arias M, Gallardo C, Kramer S, Penrith ML. *African Swine Fever: Detection and Diagnosis – A Manual for Veterinarians. FAO Animal Production and Health Manual No. 19.* Food and Agriculture Organization of the United Nations (FAO); 2017. http://www.fao.org/3/a-i7228e.pdf

64. Martin C. African Swine Fever: Secure Zoo Strategy Plan. Published online February 1, 2022. Accessed April 29, 2023. https://zahp.org/wp-content/uploads/2021/12/ASF-in-Zoo-Response.pdf

65. Nielsen SS, Alvarez J, Bicout DJ, et al. African swine fever and outdoor farming of pigs. *EFSA J*. 2021;19(6). doi:10.2903/J.EFSA.2021.6639

66. Gervasi V, Guberti V. African swine fever endemic persistence in wild boar populations: Key mechanisms explored through modelling. *Transbound Emerg Dis*. Published online July 13, 2021:tbed.14194. doi:10.1111/TBED.14194

67. Mutua F, Dione M. The Context of Application of Biosecurity for Control of African Swine Fever in Smallholder Pig Systems: Current Gaps and Recommendations. *Front Vet Sci*. 2021;0:771. doi:10.3389/FVETS.2021.689811

68. Salguero FJ. Comparative Pathology and Pathogenesis of African Swine Fever Infection in Swine. *Front Vet Sci*. 2020;7:282.

69. World Organization for Animal Health (OIE). ASF (Infection with ASFv). *OIE Terr Man*. Published online 2019.

70. World Organisation for Animal Health (WOAH). Chapter 3.8.1. African Swine Fever (Infection with African Swine Fever Virus). In: *Terrestrial Manual 2019*. World Organisation for Animal Health (WOAH); 2019. https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/2.3.10_VHS.pdf

71. Sun E, Huang L, Zhang X, et al. Genotype I African swine fever viruses emerged in domestic pigs in China and caused chronic infection. *Emerg Microbes Infect*. 2021;10(1):2183-2193. doi:10.1080/22221751.2021.1999779

72. Sánchez-Cordón PJ, Montoya M, Reis AL, Dixon LK. African swine fever: A re-emerging viral disease threatening the global pig industry. *Vet J*. 2018;233:41-48. doi:10.1016/j.tvjl.2017.12.025

73. Mason-D'Croz D, Bogard JR, Herrero M, et al. Modelling the global economic consequences of a major African swine fever outbreak in China. *Nat Food*. 2020;1(4):221-228. doi:10.1038/s43016-020-0057-2

74. Healey B, Komal J, Gutierrez JG. *Canada, Mexico and U.S. Plan North American Strategy for African Swine Fever Prevention and Preparedness*.; 2020.

75. USDA APHIS VS. *Planning and Preparing for African Swine Fever: Quick Briefing*.; 2020.

76. *African Swine Fever (ASF) - Situation Report 32*. World Organisation for Animal Health (WAHIS); 2023.

77. Disease Situation. World Organisation for Animal Health - WAHIS. Published April 29, 2023. Accessed April 29, 2023. https://wahis.woah.org/#/dashboards/country-or-disease-dashboard

78. Alonso C, Borca M, Dixon L, Revilla Y, Rodriguez F, Escribano JM. ICTV virus taxonomy profile: Asfarviridae. *J Gen Virol*. 2018;99(5):613-614. doi:10.1099/jgv.0.001049

79. Dixon LK, Sun H, Roberts H. African swine fever. *Antiviral Res*. 2019;165:34-41. doi:https://doi.org/10.1016/j.antiviral.2019.02.018

80. Dixon LK, Chapman DAG, Netherton CL, Upton C. African swine fever virus replication and genomics. *Virus Res*. 2013;173(1):3-14. doi:10.1016/j.virusres.2012.10.020

81. Ge S, Li J, Fan X, et al. Molecular Characterization of African Swine Fever Virus, China, 2018. *Emerg Infect Dis J*. 2018;24(11):2131. doi:10.3201/eid2411.181274

82. Malogolovkin A, Kolbasov D. Genetic and antigenic diversity of African swine fever virus. *Virus Res*. 2019;271:197673. doi:10.1016/j.virusres.2019.197673

83. Malogolovkin A, Burmakina G, Titov I, et al. Comparative analysis of african swine fever virus genotypes and serogroups. *Emerg Infect Dis*. 2015;21(2):312-315. doi:10.3201/eid2102.140649

84. Rodríguez JM, Yáñez RJ, Almazán F, Viñuela E, Rodriguez JF. African swine fever virus encodes a CD2 homolog responsible for the adhesion of erythrocytes to infected cells. *J Virol*. 1993;67(9):5312-5320. doi:10.1128/jvi.67.9.5312-5320.1993

85. Galindo I, Almazán F, Bustos MJ, Viñuela E, Carrascosa AL. African swine fever virus EP153R open reading frame encodes a glycoprotein involved in the hemadsorption of infected cells. *Virology*. 2000;266(2):340-351. doi:10.1006/viro.1999.0080

86. Arias M, Torre AD la, Dixon L, et al. Approaches and Perspectives for Development of African Swine Fever Virus Vaccines. *Vaccines 2017 Vol 5 Page 35*. 2017;5(4):35. doi:10.3390/VACCINES5040035

87. USDA APHIS VS. *African Swine Fever Response Plan: The Red Book (April 2020)*. USDA; 2020.

88. Goonewardene KB, Onyilagha C, Goolia M, Le VP, Blome S, Ambagala A. Superficial Inguinal Lymph Nodes for Screening Dead Pigs for African Swine Fever. *Viruses*. 2022;14(1):83. doi:10.3390/v14010083

89. Contreras A. An Enzyme Linked Immuno-Sorbent Assay (ELISA) for Detecting African Swine Fever Antibodies and Differentiating Infected from Vaccinated Animals (DIVA) - BIOSTONE ANIMAL HEALTH LLC. USDA - Research, Education & Economics Information System. Published 2021. Accessed April 29, 2023. https://portal.nifa.usda.gov/web/crisprojectpages/1025742-an-enzyme-linked-immuno-sorbent-assay-elisa-for-detecting-african-swine-fever-antibodies-and-differentiating-infected-from-vaccinated-animals-diva.html

90. African Swine Fever. DISCONTOOLS. Published January 31, 2023. Accessed April 29, 2023. https://www.discontools.eu/database/35-african-swine-fever.html

91. *Swine Hemorrhagic Fevers: African and Classical Swine Fevers Integrated Surveillance Plan*. USDA-APHIS-VS; 2022. Accessed April 29, 2023. https://www.aphis.usda.gov/animal_health/downloads/animal_diseases/swine/hemorrhagic-fevers-integrated-surveillance-plan.pdf

92. Friedrichs V, Reicks D, Hasenfuß T, et al. Artificial Insemination as an Alternative Transmission Route for African Swine Fever Virus. *Pathogens*. 2022;11(12):1539. doi:10.3390/pathogens11121539

93. Olesen AS, Lohse L, Boklund A, et al. Transmission of African swine fever virus from infected pigs by direct contact and aerosol routes. *Vet Microbiol*. 2017;211:92-102. doi:10.1016/j.vetmic.2017.10.004

94. de Carvalho Ferreira HC, Weesendorp E, Quak S, Stegeman JA, Loeffen WLA. Quantification of airborne African swine fever virus after experimental infection. *Vet Microbiol*. 2013;165(3):243-251. doi:https://doi.org/10.1016/j.vetmic.2013.03.007

95. de Carvalho Ferreira HC, Weesendorp E, Elbers ARW, et al. African swine fever virus excretion patterns in persistently infected animals: A quantitative approach. *Vet Microbiol*. 2012;160(3):327-340. doi:https://doi.org/10.1016/j.vetmic.2012.06.025

96. Guinat C, Reis AL, Netherton CL, Goatley L, Pfeiffer DU, Dixon L. Dynamics of African swine fever virus shedding and excretion in domestic pigs infected by intramuscular inoculation and contact transmission. *Vet Res*. 2014;45(1):93. doi:10.1186/s13567-014-0093-8

97. Davies K, Goatley LC, Guinat C, et al. Survival of African Swine Fever Virus in Excretions from Pigs Experimentally Infected with the Georgia 2007/1 Isolate. *Transbound Emerg Dis*. 2017;64(2):425-431. doi:10.1111/tbed.12381

98. Fischer M, Hühr J, Blome S, Conraths FJ, Probst C. Stability of African swine fever virus in carcasses of domestic pigs and wild boar experimentally infected with the ASFV "Estonia 2014" isolate. *Viruses*. 2020;12(10). doi:10.3390/v12101118

99. Mazur-Panasiuk N, Żmudzki J, Woźniakowski G. African swine fever virus - persistence in different environmental conditions and the possibility of its indirect transmission. *J Vet Res Pol*. 2019;63(3):303-310. doi:10.2478/jvetres-2019-0058

100. Mazur-Panasiuk N, Woźniakowski G. Natural inactivation of African swine fever virus in tissues: Influence of temperature and environmental conditions on virus survival. *Vet Microbiol*. 2020;242:108609. doi:10.1016/j.vetmic.2020.108609

101. Plowright W, Parker J. *The Stability of African Swine Fever Virus with Particular Reference to Heat and PH Inactivation*.; 1967.

102. Zani L, Masiulis M, Bušauskas P, et al. African swine fever virus survival in buried wild boar carcasses. *Transbound Emerg Dis*. Published online March 2020. doi:10.1111/tbed.13554

103. Olesen AS, Lohse L, Boklund A, et al. Short time window for transmissibility of African swine fever virus from a contaminated environment. *Transbound Emerg Dis*. 2018;65(4):1024-1032. doi:10.1111/tbed.12837

104. Carlson J, Fischer M, Zani L, et al. Stability of African Swine Fever Virus in Soil and Options to Mitigate the Potential Transmission Risk. *Pathogens*. 2020;9(11):977. doi:10.3390/pathogens9110977

105. Fischer M, Mohnke M, Probst C, et al. Stability of African swine fever virus on heat-treated field crops. *Transbound Emerg Dis*. 2020;67(6):2318-2323. doi:10.1111/tbed.13650

106. Niederwerder M, Stoian AMM, Rowland RRR, et al. Infectious Dose of African Swine Fever Virus When Consumed Naturally in Liquid or Feed. *Emerg Infect Dis J*. 2019;25(5):891. doi:10.3201/eid2505.181495

107. Kalmar ID, Cay AB, Tignon M. Sensitivity of African swine fever virus (ASFV) to heat, alkalinity and peroxide treatment in presence or absence of porcine plasma. Published online 2018. doi:10.1016/j.vetmic.2018.04.025

108. World Organization for Animal Health (OIE). *Technical Card: African Swine Fever*.; 2019.

109. Turner C, Williams SM. Laboratory-scale inactivation of African swine fever virus and swine vesicular disease virus in pig slurry. *J Appl Microbiol*. 1999;87(1):148-157. doi:10.1046/j.1365-2672.1999.00802.x

110. Krug PW, Lee LJ, Eslami AC, Larson CR, Rodriguez L. Chemical disinfection of high-consequence transboundary animal disease viruses on nonporous surfaces. *Biologicals*. 2011;39(4):231-235. doi:10.1016/j.biologicals.2011.06.016

111. Juszkiewicz M, Walczak M, Mazur-Panasiuk N, Woźniakowski G. Effectiveness of Chemical Compounds Used against African Swine Fever Virus in Commercial Available Disinfectants. *Pathogens*. 2020;9(11):878. doi:10.3390/pathogens9110878

112. World Organization for Animal Health (OIE). Infection with African Swine Fever Virus. In: *Terrestrial Animal Health Code*. 28th ed. ; 2019:687.

113. Blome S, Gabriel C, Beer M. Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Res*. 2013;173(1):122-130. doi:https://doi.org/10.1016/j.virusres.2012.10.026

114. Howey EB, O'Donnell V, de Carvalho Ferreira HC, Borca M V, Arzt J. Pathogenesis of highly virulent African swine fever virus in domestic pigs exposed via intraoropharyngeal, intranasopharyngeal, and intramuscular inoculation, and by direct contact with infected pigs. *Virus Res*. 2013;178(2):328-339. doi:https://doi.org/10.1016/j.virusres.2013.09.024

115. Beltrán-Alcrudo D, Arias M, Gallardo C, Kramer S, Penrith ML. *African Swine Fever: Detection and Diagnosis – A Manual for Veterinarians. FAO Animal Production and Health Manual No. 19.* Food and Agriculture Organization of the United Nations (FAO); 2017.

116. Brown VR, Bevins SN. A Review of African Swine Fever and the Potential for Introduction into the United States and the Possibility of Subsequent Establishment in Feral Swine and Native Ticks. *Front Vet Sci*. 2018;5:11. doi:10.3389/fvets.2018.00011

117. Dixon LK, Stahl K, Jori F, Vial L, Pfeiffer DU. African Swine Fever Epidemiology and Control. *Annu Rev Anim Biosci*. 2020;8:221-246. doi:10.1146/annurev-animal-021419-083741

118. Jori F, Bastos ADS. Role of Wild Suids in the Epidemiology of African Swine Fever. *EcoHealth*. 2009;6(2):296-310. doi:10.1007/s10393-009-0248-7

119. Guberti V, Khomenko S, Masiulis M, Kerba S. *African Swine Fever in Wild Boar: Ecology and Biosecurity*. FAO, World Organisation for Animal Health (WOAH) (founded as OIE), European Commission; 2022. doi:10.4060/cc0785en

120. Wieland B, Dhollander S, Salman M, Koenen F. Qualitative risk assessment in a data-scarce environment: A model to assess the impact of control measures on spread of African Swine Fever. *Prev Vet Med*. 2011;99(1):4-14. doi:10.1016/j.prevetmed.2011.01.001

121. Lim SJ, Han SH, Park JY, et al. Wildlife as Potential Vectors of African Swine Fever Virus. *J For Environ Sci*. 2022;38(1):55-63. doi:doi.org/10.7747/JFES.2022.38.1.55

122. Probst C, Gethmann J, Amler S, Globig A, Knoll B, Conraths FJ. The potential role of scavengers in spreading African swine fever among wild boar. *Sci Rep*. 2019;9(1):11450. doi:10.1038/s41598-019-47623-5

123. Szewczyk M, Łepek K, Nowak S, et al. Evaluation of the Presence of ASFV in Wolf Feces Collected from Areas in Poland with ASFV Persistence. *Viruses*. 2021;13(10):2062. doi:10.3390/v13102062

124. Authority (EFSA) EFS, Nielsen SS, Alvarez J, et al. Research priorities to fill knowledge gaps in the control of African swine fever: possible transmission of African swine fever virus by vectors. *EFSA J*. 2021;19(6):e06676. doi:10.2903/j.efsa.2021.6676

125. Guinat C, Gogin A, Blome S, et al. Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions. *Vet Rec*. 2016;178(11):262-267. doi:10.1136/vr.103593

126. SHIC Grant-Funded Study in Vietnam Indicates Rodents may be Low Risk ASF Vector. Swine Health Information Center. Accessed April 30, 2023. https://www.swinehealth.org/shic-grant-funded-study-in-vietnam-indicates-rodents-may-be-low-risk-asf-vector/

127. Orrico M, Hovari M, Beltrán-Alcrudo D. A Novel Tool to Assess the Risk for African Swine Fever in Hunting Environments: The Balkan Experience. *Pathogens*. 2022;11(12):1466. doi:10.3390/pathogens11121466

128. USDA. *Federal Order for Importation of Live Dogs for Resale From Regions Where African Swine Fever Exists Or Is Reasonably Believed to Exist DA-2021-001.*; 2021.

129. Bellini S, Casadei G, De Lorenzi G, Tamba M. A Review of Risk Factors of African Swine Fever Incursion in Pig Farming within the European Union Scenario. *Pathogens*. 2021;10(1):84. doi:10.3390/pathogens10010084

130. Wormington JD, Golnar A, Poh KC, et al. Risk of African Swine Fever Virus Sylvatic Establishment and Spillover to Domestic Swine in the United States. *Vector Borne Zoonotic Dis*. 2019;19(7):506-511. doi:10.1089/vbz.2018.2386

131. Golnar AJ, Martin E, Wormington JD, et al. Reviewing the Potential Vectors and Hosts of African Swine Fever Virus Transmission in the United States. *Vector-Borne Zoonotic Dis*. 2019;19(7):512-524. doi:10.1089/vbz.2018.2387

132. Bonnet SI, Bouhsira E, De Regge N, et al. Putative role of arthropod vectors in African swine fever virus transmission in relation to their bio-ecological properties. *Viruses*. 2020;12(7):778.

133. Fila M, Woźniakowski G. African swine fever virus–the possible role of flies and other insects in virus transmission. *J Vet Res*. 2020;64(1):1.

134. Olesen AS, Lohse L, Hansen MF, et al. Infection of pigs with African swine fever virus via ingestion of stable flies (Stomoxys calcitrans). *Transbound Emerg Dis*. 2018;65(5):1152-1157. doi:https://doi.org/10.1111/tbed.12918

135. Olesen AS, Hansen MF, Rasmussen TB, Belsham GJ, Bødker R, Bøtner A. Survival and localization of African swine fever virus in stable flies (Stomoxys calcitrans) after feeding on viremic blood using a membrane feeder. *Vet Microbiol*. 2018;222(May):25-29. doi:10.1016/j.vetmic.2018.06.010

136. Herm R, Tummeleht L, Jürison M, Vilem A, Viltrop A. Trace amounts of African swine fever virus DNA detected in insects collected from an infected pig farm in Estonia. *Vet Med Sci*. 2020;6(1):100-104.

137. Amendt J, Richards CS, Campobasso C Pietro, Zehner R, Hall MJR. Forensic entomology: applications and limitations. *Forensic Sci Med Pathol*. 2011;7(4):379-392.

138. Yoon H, Hong SK, Lee I, Lee ES. *Insects as Potential Vectors of African Swine Fever Virus in the Republic of Korea*. Preprints; 2020. doi:10.22541/au.158930924.49716985

139. Balmoș OM, Supeanu A, Tamba P, et al. African Swine Fever Virus Load in Hematophagous Dipterans Collected in Outbreaks from Romania: Risk Factors and Implications. *Transbound Emerg Dis*. 2023;2023:e3548109. doi:10.1155/2023/3548109

140. Saegerman C, Bonnet S, Bouhsira E, et al. An expert opinion assessment of blood-feeding arthropods based on their capacity to transmit African swine fever virus in Metropolitan France. *Transbound Emerg Dis*. 2021;68(3):1190-1204.

141. EFSA Panel on Animal Health and Welfare. Scientific Opinion on the Role of Tick Vectors in the Epidemiology of Crimean-Congo Hemorrhagic Fever and African Swine Fever in Eurasia. *EFSA J*. 2010;8(8):1703.

142. Sanchez Botija C, Babiola C. African swine fever virus in Haematopinus suis. *Bull L'office Int Epizoot*. 1966;(66):699-705.

143. Montgomery RE. On a form of swine fever occurring in British East Africa (Kenya Colony). *J Comp Pathol Ther*. 1921;34:159-191.

144. Heuschele WP, Coggins L. Studies on the transmission of African swine fever virus by arthropods. In: *Proceedings of the United States Livestock Sanitary Association*. ; 1965:94.

145. Kovalenko YR, Sidorov MA, Burba LG. Experimental study of African swine fever. II. Pasture ticks and Haematopinus as possible reservoirs and vectors of African swine fever. Published online 1967:33, 76-90, and 91-94.

146. Authority (EFSA) EFS, Nielsen SS, Alvarez J, et al. Research objectives to fill knowledge gaps in African swine fever virus survival in the environment and carcasses, which could improve the control of African swine fever virus in wild boar populations. *EFSA J*. 2021;19(6):e06675. doi:10.2903/j.efsa.2021.6675

147. Nielsen SS, Alvarez J, Bicout DJ, et al. Ability of different matrices to transmit African swine fever virus. *EFSA J*. 2021;19(4). doi:10.2903/J.EFSA.2021.6558

148. Depner KR, Staubach C, Probst C, et al. Die Afrikanische Schweinepest – epidemiologische Betrachtungen und Konsequenzen für die Tierseuchenbekämpfung. *Tierärztl Umsch*. 2016;71(3):72-78.

149. Boklund A, Dhollander S, Chesnoiu Vasile T, et al. Risk factors for African swine fever incursion in Romanian domestic farms during 2019. *Sci Rep*. 2020;10(1):10215. doi:10.1038/s41598-020-66381-3

150. Li X, Hu Z, Fan M, et al. Evidence of aerosol transmission of African swine fever virus in piggeries under field conditions: a case repor. Published online 2022. doi:10.21203/rs.3.rs-1608163/v1

151. Kosowska A, Barasona JA, Barroso-Arévalo S, Rivera B, Domínguez L, Sánchez-Vizcaíno JM. A new method for sampling African swine fever virus genome and its inactivation in environmental samples. *Sci Rep*. 2021;11(1):21560. doi:10.1038/s41598-021-00552-8

152. Gebhardt JT, Dritz SS, Elijah CG, Jones CK, Paulk CB, Woodworth JC. Sampling and detection of African swine fever virus within a feed manufacturing and swine production system. *Transbound Emerg Dis*. 2022;69(1):103-114. doi:10.1111/tbed.14335

153. Mayer JJ, Johns PE. Characterization of Wild Pig - Vehicle Collisions. In: Nolte DL, Arjo WM, Stalman DH, eds. *Characterization of Wild Pig - Vehicle Collisions*. ; 2007:175-187.

154. Nuanualsuwan S, Songkasupa T, Boonpornprasert P, Suwankitwat N, Lohlamoh W, Nuengjamnong C. Persistence of African swine fever virus on porous and non-porous fomites at environmental temperatures. *Porc Health Manag*. 2022;8(1):34. doi:10.1186/s40813-022-00277-8

155. Bellini S, Rutili D, Guberti V. Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems. *Acta Vet Scand*. 2016;58(1):82. doi:10.1186/s13028-016-0264-x

156. U.S. FDA Center for Biologics Evaluation and Research. What Are “Biologics” Questions and Answers. Published online 2018.

157. Chen W, Zhao D, He X, et al. A seven-gene-deleted African swine fever virus is safe and effective as a live attenuated vaccine in pigs. *Sci China Life Sci*. 2020;63(5):623-634. doi:10.1007/s11427-020-1657-9

158. Kasaija PD, Contreras M, Kirunda H, et al. Inspiring Anti-Tick Vaccine Research, Development and Deployment in Tropical Africa for the Control of Cattle Ticks: Review and Insights. *Vaccines*. 2023;11(1):99. doi:10.3390/vaccines11010099

159. Lv T, Xie X, Song N, et al. Expounding the role of tick in Africa swine fever virus transmission and seeking effective prevention measures: A review. *Front Immunol*. 2022;13. Accessed March 10, 2023. https://www.frontiersin.org/articles/10.3389/fimmu.2022.1093599

160. Guinat C, Vergne T, Jurado-Diaz C, Sánchez-Vizcaíno JM, Dixon L, Pfeiffer DU. Effectiveness and practicality of control strategies for African swine fever: what do we really know? *Vet Rec*. 2017;180(4):97-97. doi:10.1136/vr.103992

161. Gallardo C, Nieto R, Soler A, et al. Assessment of African Swine Fever Diagnostic Techniques as a Response to the Epidemic Outbreaks in Eastern European Union Countries: How To Improve Surveillance and Control Programs. *J Clin Microbiol*. 2015;53(8):2555-2565. doi:10.1128/JCM.00857-15

162. Yoo DS, Kim Y, Lee ES, et al. Transmission Dynamics of African Swine Fever Virus, South Korea, 2019. *Emerg Infect Dis*. 2021;27(7):1909-1918. doi:10.3201/eid2707.204230

163. Costard S, Wieland B, de Glanville W, et al. African swine fever: how can global spread be prevented? *Philos Trans R Soc B Biol Sci*. 2009;364(1530):2683-2696. doi:10.1098/rstb.2009.0098

164. Authority EFS. Evaluation of possible mitigation measures to prevent introduction and spread of African swine fever virus through wild boar. *EFSA J*. 2014;12(3):3616. doi:10.2903/j.efsa.2014.3616

165. Kim YJ, Park B, Kang HE. Control measures to African swine fever outbreak: active response in South Korea, preparation for the future, and cooperation. *J Vet Sci*. 2021;22(1):1-14. doi:10.4142/jvs.2021.22.e13

166. Jori F, Chenais E, Boinas F, et al. Application of the World Café method to discuss the efficiency of African swine fever control strategies in European wild boar (Sus scrofa) populations. *Prev Vet Med*. 2020;185:105178. doi:10.1016/j.prevetmed.2020.105178

167. Glow MP, Snow NP, VerCauteren KC. Feral Swine - Wildlife Damage Management Technical Series. Published online August 2020. Accessed May 10, 2023. https://www.aphis.usda.gov/wildlife_damage/reports/Wildlife%20Damage%20Management%20Technical%20Series/Feral%20Swine_WDM%20Technical%20Series_August%202020.pdf

168. Gaskamp JA, Gee KL, Campbell TA, Silvy NJ, Webb SL. Effectiveness and Efficiency of Corral Traps, Drop Nets and Suspended Traps for Capturing Wild Pigs (Sus scrofa). *Anim Open Access J MDPI*. 2021;11(6):1565. doi:10.3390/ani11061565

169. Gentle M, Wilson C, Cuskelly J. Feral pig management in Australia: implications for disease control. *Aust Vet J*. 2022;100(10):492-495. doi:10.1111/avj.13198

170. Jurado C, Martínez-Avilés M, Torre AL, et al. Relevant measures to prevent the spread of African Swine Fever in the European Union Domestic Pig Sector. *Front Vet Sci*. 2018;5(APR). doi:10.3389/fvets.2018.00077

171. Janse van Rensburg L, Etter E, Heath L, Penrith ML, van Heerden J. Understanding African swine fever outbreaks in domestic pigs in a sylvatic endemic area: The case of the

South African controlled area between 1977–2017. *Transbound Emerg Dis*. 2020;67(6):2753-2769. doi:10.1111/tbed.13632

172. European Commission. Strategic approach to the management of African Swine Fever for the EU. Published online April 29, 2020. Accessed May 9, 2023. https://food.ec.europa.eu/system/files/2020-04/ad_control-measures_asf_wrk-doc-sante-2015-7113.pdf

173. USDA, APHIS. USDA APHIS | USDA Announces Requirements for Importing Dogs from Countries Affected with African Swine Fever. Published August 4, 2021. Accessed May 3, 2023. https://www.aphis.usda.gov/aphis/newsroom/news/sa_by_date/sa-2021/asf-dog-imports

174. Ministerio de la Presidencia. *Real Decreto 324/2000, de 3 de marzo, por el que se establecen normas básicas de ordenación de las explotaciones porcinas*. Vol BOE-A-2000-4447.; 2000:9505-9512. Accessed May 10, 2023. https://www.boe.es/eli/es/rd/2000/03/03/324

175. Harmon JD, Hoff SJ. Basic Management Practices to Mitigate and Control Odors from Swine Operations. *Fact Sheet Pork Inf Gatew*. 2010;(PIG 10-02-11):1-3. http://agbiopubs.sdstate.edu/ar-

176. Malone GW, Abbott-Donnelly D. Environmental and Production Benefits of Trees for Poultry Farms. *Coop Ext Univ Del*. Published online August 2001:1-12. http://ag.udel.edu/extension

177. Arruda AG, Vilalta C, Perez A, Morrison R. Land altitude, slope, and coverage as risk factors for Porcine Reproductive and Respiratory Syndrome (PRRS) outbreaks in the United States. *PloS One*. 2017;12(4):e0172638-undefined. doi:10.1371/journal.pone.0172638

178. Denmark is building a border fence - to keep out wild boars. *NBC News*. https://www.nbcnews.com/news/world/denmark-building-border-fence-keep-out-wild-boars-n963801. Published January 29, 2019. Accessed May 7, 2023.

DISINFECTANTS APPROVED FOR USE AGAINST AFRICAN SWINE FEVER VIRUS IN FARM SETTINGS

This table is intended to assist persons in finding U.S. Environmental Protection Agency (EPA)-registered disinfectants that may be used against African swine fever virus (ASFv) in/on farm premises, structures and equipment; veterinary equipment and premises; and food processing equipment and premises. The information was obtained from the National Pesticide Information Retrieval System (NPIRS®)[1] on May 3, 2023. The list may not be an all-inclusive list of EPA-registered disinfectants. The inclusion of any product on this table does not imply endorsement of the product by United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS).

The biosecurity officer of the USDA APHIS or state partner incident management team responding to a foreign animal disease incident should use, as their first recourse, a commercially available EPA-registered disinfectant according to the State-registered container label. The use sites summarized on the table below for Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) section 3 registrations should appear on the EPA-registered labels which can be found on EPA's Pesticide Product Label System (PPLS) at Pesticide Product and Label System | US EPA. EPA-registered labels are often identical to the State-registered container labels; however, State labels may differ from EPA's label. Use the table below as a first step to identify a potential EPA-registered product to use against ASFv; next, using website NPIRS State Public (purdue.edu), locate the appropriate State-registered container label to verify all necessary uses are also on the container label.

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 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 on the APHIS VS website (USDA APHIS | Disinfectants).

Biosecurity officers should have with them a paper or electronic copy of the container label and, if applicable, section 18 label, and ensure that disinfectants are prepared and applied according to the appropriate label(s). Biosecurity officers using a section 18 disinfectant must record the amounts used and report this monthly to Nathan.G.Birnbaum@usda.gov and Samantha.Bates@usda.gov.
If a product is being used as permitted by FIFRA section 2(ee)(2), the product must be used according to all product container label instructions, with the exception that the targeted pests (microbes) need not be specified on the container label.

[1] The National Pesticide Information Retrieval System (NPIRS®) is a collection of pesticide-related databases, applications, and websites under the administration of the Center for Environmental and Regulatory Information Systems (CERIS) at Purdue University, West Lafayette, Indiana. The NPIRS® obtains product information on a monthly basis from the EPA Pesticide Product Information System (PPIS) website. This PPIS information, including registered use sites and pests listed on the EPA stamped approved label, is disseminated through the NPIRS® member and public websites and is provided for informational purposes only. Information derived from the NPIRS® does not constitute a label replacement or a recommendation. Before applying any pesticide, applicators must determine if the product under consideration is correct for the intended use site. Always check the container/package label to determine if the intended use site is included on the label. Read and follow label instructions before using any pesticide product. More information on the NPIRS® can be found at https://www.npirs.org/ppis/. Errors in the PPIS data can be reported at this website using the Data Quality menu option.

Reg. type	EPA Reg No	Product name	Manufacturer	Active ingredient(s)	Contact time	Registered use sites (according to NPIRS. Must confirm with label on container)	Website for EPA-approved label
FIFRA section 3	39967-137	Virkon S	Lanxess Corporation	Sodium chloride Potassium peroxymonosulfate	10 min	In/on animal feed equipment, livestock barns, livestock pens, livestock stalls, livestock stables, livestock equipment, hog farrowing pen premises, hog barns/houses/ parlors/pens, animal quarters, animal feeding and watering equipment, animal transportation vehicles, animal equipment, agricultural premises, agricultural premises and equipment, railroad boxcars, transportation vehicles, slaughter house instruments and premises, veterinary hospital premises, veterinary hospital instruments, food processing equipment and premises, and human footwear	US EPA, Pesticide Product Label, VIRKON S,10/22/2020
FIFRA section 3	66171-106*	Neogen Viroxide Super	Preserve International	Potassium peroxymonosulfate Sodium chloride	10 min	In/on swine production areas, farrowing units, nurseries, finisher houses, processing plants, and agricultural production equipment such as trucks, waterproof footwear, and associated livestock equipment and instruments. Companion animal facilities and facilities used for temporary confinement of animals.	US EPA, Pesticide Product Label, Neogen® Viroxide Super,02/27/2023
FIFRA section 3	69470-37	Clearon Bleach Tablets	Clearon Corp	Sodium dichloro-s-triazinetrione	30 min	In/on animal living quarters, farm premises, livestock premises, veterinary premises, veterinary clinics, veterinary hospitals, food processing plant and plant surfaces, and shoe baths	US EPA, Pesticide Product Label, CLEARON BLEACH TABLETS,03/18/2021

Reg. type	EPA Reg No	Product name	Manufacturer	Active ingredient(s)	Contact time	Registered use sites (according to NPIRS. Must confirm with label on container)	Website for EPA-approved label
FIFRA section 3	71355-1	Virocid	Ecolab, Inc.	Glutaral Alkyl dimethyl benzyl ammonium chloride Didecyl dimethyl ammonium chloride	10 min	In/on livestock pens, quarters, premises, stalls, barns, feeding equipment, watering equipment, transportation vehicles, hog barns/houses/parlors/pens, hog farms, animal cages, animal living quarters, animal feeding and watering equipment, animal equipment, farm equipment, trucks, veterinary hospital premises, veterinary hospital materials, and slaughter house premises	US EPA, Pesticide Product Label, VIROCID,10/14/2021
FIFRA section 3	71847-2	Klor-Kleen	Medentech Ltd.	Sodium dichloro-s-triazinetrione	30 min	In/on animal quarters, animal living quarters, and veterinary hospital premises	US EPA, Pesticide Product Label, KLOR-KLEEN, 08/05/2013
FIFRA section 3	71847-6	Klorsept	Medentech Ltd.	Sodium dichloro-s-triazinetrione	30 min	In/on livestock premises, farm premises, shoe baths, barns, animal quarters, animal feeding/watering, animal equipment, animal transportation vehicles, veterinary hospital premises, and food processing equipment and premises	US EPA, Pesticide Product Label, KLORSEPT,12/27/2018
FIFRA section 3	71847-7	Klorkleen 2	Medentech, Ltd.	Sodium dichloro-s-triazinetrione	30 min	In/on livestock premises, animal feeding/watering equipment, animal equipment, animal transportation vehicles, farm premises, food processing areas and premises, and shoe baths	US EPA, Pesticide Product Label, KLORKLEEN 2 ,08/06/2018
FIFRA section 3	74559-4*	Accel Concentrate Disinfectant Cleaner (aka INTERVention Farm Animal Care Disinfectant	Virox Technologies, Inc.	Hydrogen peroxide	5 min	In/on barns, farms, animal premises, animal housing facilities, agriculture premises and veterinary use, barn use, farm use,	US EPA, Pesticide Product Label, ACCEL (CONCENTRATE) DISINFECTANT CLEANER,09/08/2020

Reg. type	EPA Reg No	Product name	Manufacturer	Active ingredient(s)	Contact time	Registered use sites (according to NPIRS. Must confirm with label on container)	Website for EPA-approved label
		Cleaner & Deodorizer					
FIFRA section 18	N/A	various	various	Sodium hypochlorite	15 min nonporous 30 min porous	Federal, State, or private indoor or outdoor use sites, such as agricultural, transportation, quarantine, and laboratory equipment and facilities; and, footwear/personal protective equipment.	sodium-hypochlorite-label.pdf (usda.gov)
FIFRA section 18	N/A	various	Archer Daniels Midland Co.	Citric acid	15 min nonporous 30 min porous	Federal, State, or private indoor or outdoor use sites, such as agricultural and nonagricultural equipment and facilities; laboratory equipment and facilities; and footwear/personal protective equipment.	ENQL 7-1 CY06 (usda.gov)
FIFRA section 18	84683-3	Benefect Botanical Daily Cleaner Disinfectant Spray (and identical distributor products, such as AeroDis 7127, EPA Reg. No. 84683-3-67026))	Cleanwell, LLC	Thymol	15 min	USDA APHIS and its cooperators on hard nonporous surfaces inside and outside aircraft, as well as associated loading equipment including loading ramps	FIFRA Section 18 Emergency Exemption Label, Benefect Botanical Daily Cleaner Disinfectant Spray (usda.gov)

*Products not listed in NPIRS. Information is from the 10/20/22 EPA-approved Accel and the 2/14/23 EPA-approved Neogen Viroxide Super labels.

APPENDIX A

APPENDIX B

Table 2. Soft Tick Vectors Ranked by Their Relative Risk to Contribute to African Swine Fever Virus Transmission in the United States

Vector species	Vector competence	Vector–host associations in the United States by host species	Associated mammalian orders	U.S. counties with vector	Number of collections	Relative risk
Ornithodoros coriaceus	Competent	*Bos taurus*, *Falco* sp., *Lepus* sp., *Sus scrofa*, *Equus ferus*, *Homo sapiens*, *Odocoileus hemionus*	Lagomorpha Artiodactyla Chiroptera Perissodactyla Primates	31	20	1
Ornithodoros turicata	Competent	*Canis latrans*, *Cynomys ludovicianus*, *H. sapiens*, *Pecari tajacu*, *Sciurus niger*, *Sylvilagus audubonii*, *Crotalus atrox*, *Crotalus horridus*, *Gopherus agassizii*, *Gopherus polyphemus*, *Neotoma micropus*, *Sylvilagus* sp., *Gopherus* sp., *Neotoma* sp., *Otospermophilus beecheyi*, *Lepus* sp., *Taxidea taxus*, *Dipodomys* sp., *Cynomys* sp.	Carnivora Rodentia Primates Artiodactyla Cingulata	78	87	2
Ornithodoros puertoricensis	Competent	*Ctenosaura acanthura*	—	1	1	3
Otobius megnini	Unknown	*A. americanum*, *Aspidoscelis gularis*, *Brachylagus idahoensis*, *Cnemidophorus martyris*, *Gallus gallus*, *H. sapiens*, *Lepus townsendii*, *Lepus* sp., *Marmota monax*, *Ovis canadensis*, *S. audubonii*, *Sylvilagus bachmani*, *Tamias* sp., *C. latrans*, *Lepus californicus*, *Oreamnos americanus*, *Cervus canadensis*, *Odocoileus* sp., *Ovis aries*, *Equus caballus*, *O. hemionus*, *Odocoileus virginianus*, *E. ferus*, *Felis catus*, *Canis lupus*, *B. taurus*	Artiodactyla Lagomorpha Primates Rodentia Carnivora, Perissodactyla	148	271	4
Ornithodoros parkeri	Noncompetent	*C. latrans*, *Capra hircus*, *Cynomys gunnisoni*, *Dipodomys* sp., *H. sapiens*, *M. monax*, *Microtus longicaudus*, *Onychomys torridus*, *Otospermophilus variegatus*, *Petrochelidon pyrrhonota*, *T. taxus*, *Urocitellus washingtoni*, *Vulpes marcotis*, *Peromyscus maniculatus*, *Spermophilus* sp., *Sylvilagus* sp., *Urocitellus columbianus*, *Athene cunicularia*, *Cynomys leucurus*, *Lepus* sp., *Urocitellus richardsonii*, *O. beecheyi*, *Cynomys* sp.	Carnivora Artiodactyla Rodentia Primates Lagomorpha	70	210	5
Otobius lagophilus	Unknown	*Buteo swainsoni*, *C. lupus*, *Felis silvestris*, *H. sapiens*, *Spermophilus* sp., *Ochotona Unknown* sp., *Ochotona princeps*, *Sylvilagus* sp., *L. townsendii*, *L. californicus*, *Lepus* sp.	Carnivora Primates Rodentia Lagomorpha	33	51	6
Ornithodoros kelleyi	Unknown	*Euderma maculatum*, *H. sapiens*, *Myotis subulatus*, *Myotis thysanodes*, *Nycticeius humeralis*, *Pipistrellus subflavus*, *Pipistrellus* sp., *Antrozous pallidus*, *Myotis lucifugus*, *Pipistrellus hesperus*, *Eptesicus fuscus*	Chiroptera Primates	75	56	7
Ornithodoros hermsi	Unknown	*M. monax*, *Tamias* sp., *Urocitellus townsendii*, *Neotoma* sp., *Tamiasciurus hudsonicus*, *H. sapiens*	Rodentia Primates	29	13	8
Ornithodoros concanensis	Unknown	*Buteo jamaicensis*, *Petrochelidon fulva*, *Falco sparverius*, *H. sapiens*, *P. pyrrhonota*, *Aquila chrysaetos*, *Falco mexicanus*	Chiroptera Primates	34	39	9
Ornithodoros stageri	Unknown	*A. pallidus*, *H. sapiens*, *Myotis velifer*, *Tadarida* sp.,	Chiroptera Primates	11	17	10

(*continued*)

APPENDIX B

APPENDIX B

Table 2. (Continued)

Vector species	Vector competence	Vector–host associations in the United States by host species	Associated mammalian orders	U.S. counties with vector	Number of collections	Relative risk
Ornithodoros talaje	Unknown	*Cynomys* sp., *Neotoma albigula*, *O. beecheyi*, *Reithrodontomys fulvescens*, *Sauromalus ater*, *T. hudsonicus*, *Spermophilus* sp., *Neotoma florida*, *Neotoma lepida*, *N. micropus*, *Dipodomys* sp., *Neotoma* sp.	Rodentia	37	43	11
Argas sanchezi	Unknown	*Callipepla gambelii*, *H. sapiens*, *Lophortyx* sp., *G. gallus*	Primates	36	50	12
Ornithodoros yumatensis	Unknown	*E. fuscus*, *M. thysanodes*, *Nyctinomus mexicanus*, *P. hesperus*, *P. subflavus*, *A. pallidus*, *Plecotus rafinesquii*, *Tadarida brasiliensis*, *Plecotus townsendii*, *M. velifer*	Chiroptera	21	30	13
Ornithodoros sparnus	Unknown	*Neotoma cinerea*, *Neotoma floridana* sp., *Peromyscus crinitus*, *Neotoma* sp., *P. maniculatus*, *N. lepida*	Rodentia	12	49	14
Ornithodoros coprophilus	Unknown	—	Chiroptera	8	12	15
Ornithodoros dyeri	Unknown	—	Chiroptera	6	5	16
Ornithodoros rossi	Unknown	*A. pallidus*, *E. fuscus*, *Leptonycteris nivalis*, *Macrotus californicus*, *Myotis evotis*	Chiroptera	5	5	17
Ornithodoros dugesi	Unknown	*N. micropus*	Rodentia	1	2	18
Argas cooleyi	Unknown	*Gymnogyps californianus*, *Strix varia*, *Tyto Alba*, *P. pyrrhonota*, *C. latrans and Vulpes macrotis*	—	44	72	19
Argas radiatus	Unknown	*Ardea herodias*, *B. swainsoni*, *Coragyps atratus*, *Egretta thula*, *Pelecanus occidentalis*, *Meleagris gallopavo*, *G. gallus*	—	39	68	20
Argas persicus	Unknown	*G. gallus*	—	22	19	21
Carios capensis	Unknown	*A. herodias*, *Bubulcus ibis*, *Haematopus palliatus*, *Nycticorax nycticorax*, *Platalea ajaja*, *Platalea* sp., *S. varia*, *Egretta rufescens*, *P. occidentalis*	—	11	29	22
Argas giganteus	Unknown	*A. cunicularia*, *Gymnorhinus cyanocephalus*, *Icteria virens*, *Megascops asio*, *Oreoscoptes montanus*, *Passerella iliaca*, *Pipilo aberti*, *Poecile gambeli*, *Salpinctes obsoletus*, *Callipepla gambeli*, *Pipilo fuscus*, *Toxostoma crissale*, *Zonotrichia leucophrys*, *Pipilo erythrophthalmus*	—	10	21	23
Argas brevipes	Unknown	*Baeolophus inornatus*, *Campylorhynchus brunneicapillus*, *Colaptes chrysoides*, *F. sparverius*, *Glaucidium brasilianum*, *Megascops kennicotti*, *M. asio*, *Melanerpes formicivorus*	—	7	12	24
Carios denmarki	Unknown	*Larus occidentalis*, *Phalacrocorax penicillatus*, *Sterna fuscata*, *Uria aalge*	—	8	11	25
Argas ricei	Unknown	*Cathartes aura*, *C. atratus*	—	4	4	26
Argas miniatus	Unknown	*G. gallus*	—	2	2	27
Argas monolakensis	Unknown	*Larus californicus*	—	2	1	28
Ornithodoros aquilae	Unknown	*A. chrysaetos*, *Buteo regalis*, *F. mexicanus*	—	1	4	29

Relative risk and ranking was determined by organizing tick species by the following hazard levels: vector competence (1), association with the taxonomic family Suidae (2), association with the taxonomic order Artiodactyla (3), number of associated mammalian host orders (4), geographic prevalence in the United States (5), and number of reported collections (6). *Dashes* in cells indicate that no data were available to infer hazard.

APPENDIX B

Table 1: Data extraction of studies relevant to assess the feasibility to reduce wild boar populations by culling or trapping in Europe

Reference	Time period	Geographical area	Landscape	Population	Objective	Method for density estimation	Method of depopulation	Results	Maintenance issues
García-Jiménez et al. (2013)	2007–2012	Large hunting estate in Central Spain	Mediterranean forest	Wild boar and fallow deer	Assess bTB prevalence in wild boar and fallow deer	Population density based on hunting bag	Two hunting events (20 hunters plus dogs) un restricted hunting wild boar	2007-2008: 37 = 1.22 wild boar hunted per 100 ha 2011–2012: 18 0.59 wild boar hunted per 100 ha	Second season and third season increase in the wild boar hunting bag
Braga et al. (2010)	2005 – 2009	Alentejo, Portugal	Not reported	Wild boar	Investigated the sex ratio and age class structure in hunting bags of wild boar harvested by *espera*	Not estimated	*Espera* hunting - uses of bait (wheat grain and almonds) to attract wild boar to the shooting range of 15 elevated hunting stands at night	Number of wild boar harvested per 100 ha 2.83 - 7.60 *espera* hunting bags higher odds of harvesting an adult male	Removing adult males, however, may bias the population sex ratio towards females, reduce male life expectancy and raise the degree of polygyny.
Toigo et al. (2008)	1982-2004	Châteauvillain-Arc en Barrois, eastern France	Forest	Wild boars	Disentangling natural from hunting mortality in an intensively hunted wild boar population	Mark-recapture-recovery	Annual hunting	A wild boar had a > 40 % of chance of being harvested annually and this risk was as high as 70 % for adult males.	Despite high hunting mortality, the study population increased

APPENDIX C

Table 1: Data extraction of studies relevant to assess the feasibility to reduce wild boar populations by culling or trapping in Europe (continued)

Reference	Time period	Geographical area	Landscape	Population	Objective	Method for density estimation	Method of depopulation	Results	Maintenance issues
Hadjisterkotis (2004)	1997 - 2000	Cyprus	Forest	Wild boars illegally released in 1996	Eradicate wild boar (danger of transmitting diseases and environmental destruction)	Not estimated	Hunting was permitted and the game wardens were instructed to eliminate free-ranging animals	No reduction achieved	Consistent policy for eradication programme
Hadjisterkotis (2004)	1997 - 2004	Cyprus	Forest	Wild boars illegally released in 1996	Eradicate wild boar (danger of transmitting diseases and environmental destruction)	Signs of wild boar and interviews of foresters, farmers, hunters, monks	Hunting was permitted and the game wardens were instructed to eliminate free-ranging animals – improved ammunition	2001–2002: estimated 80 animals 2004- 2005: No sightings of boar	
Mentaberre et al. (2013)	2007-2011	Ports de Tortosa i Beseit National Game Reserve, Spain	abrupt calcareous mountain range, pine and oak forest	Wild boar	Effect of hosts management strategies on Salmonella serovar prevalence	Direct Abundance Index = wild boars/hunter and game season	Increase hunting and baited box trapping	Median = 0.47 ± 0.06 before management; Median = 0.32 ± 0.06, after management	
Sodeikat and Pohlmeyer (2003)	1998 - 2002	Lower Saxony, Germany	4000 ha 50 % forestland and 50 % farmland	4 - 5 wild boars per 100 ha	Movements after trapping	Hunting bag	Trapping baited with corn	No evaluation of trapping	Flight after trapping: 0.2 km – 4.6 km

Table 1: Data extraction of studies relevant to assess the feasibility to reduce wild boar populations by culling or trapping in Europe (continued)

Reference	Time period	Geographical area	Landscape	Population	Objective	Method Density estimation	Method of depopulation	Results	Maintenance issues
Boadella et al. (2012)	2008 - 2009	South-central Spain	Mediterranean ecosystem	10 control sites, 3 sites with culling	Abundance reduction through increased culling on the prevalence of two chronic infectious diseases	Presence frequency of wild boar faecal droppings on transects site 4, direct wild boar counts converted into kilometric abundance indices	Intense and year round wild boar culling strategy	Site 4, the mean estimated wild boar abundance (KAI) diminished by 47.5 % site 8, mean wild boar abundance (FBII) diminished by 56.8 % Site 9 not reported	Culling alone, especially in large areas, is likely not a sustainable long term option
Alexandrov et al. (2011)	08/2009 – 11/2009	Silistra region, Bulgaria	25-km^2 oak forest surrounded by crops (mainly maize)	Wild boar	Eradicate CSF from an area where hunting and vaccination alone might not be sufficient	Not described	Trapping as an addition to management by hunting	Approx. 6 animals per km^2 Reduced to below 2 animals per km^2	Not reported

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Table 1: Data extraction of studies relevant to assess the feasibility to reduce wild boar populations by culling or trapping in Europe (continued)

Reference	Time period	Geographical area	Landscape	Population	Objective	Method for density estimation	Method of depopulation	Results	Maintenance issues
Csanyi (1995)	1969 - 1992	Hungary		Wild boar	Trends in harvest rates between state enterprises and private hunting associations	Reported spring population size and number of wild boars shot in the year.	Hunting	Harvest rates ranging from 50 % to 30 % with highest harvest rates in the 1970s.	The harvest rate of wild boar populations was generally lower than that necessary to stabilise the population
Keuling et al. (2013)	1998–2009	Sweden, Poland, Germany, Belgium, France, Switzerland, Austria, Italy		Wild boar	Comparison of mortality rates in Central Europe	NA: Paper compares mortality rates from published papers.	Population control not assessed	mortality rates higher for males ($p = 0.019$) and especially male yearlings.	bias between reproductive and harvest rates leads to growing wild boar populations, high harvest rates required to regulate populations.
Keuling et al. (2009)	2002 - 2006	Southwestern Mecklenburg–Western Pomerania, Germany	Agriculture and grassland 63 % forest 34 %	The mean annual harvest increased from 2.83 individuals per 100 ha in 1999/2000 to 5.13 individuals per 100 ha in 2005/2006.	Test the impact of different hunting methods on seasonal home range sizes		Battues (8.3 hunters, 5.3 beaters and 2.7 dogs per 100 ha driven forest area)	Battues did not significantly influence the spatial utilisation before and after hunt.	To reduce populations and thus, damages, supplemental feeding should be reduced and hunting rates have to be increased especially for females, as all age classes of females are highly reproductive.

bTB: bovine tuberculosis; CSF: Classical Swine Fever; NA: not applicable.

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Table 2: Data extraction of studies relevant to assess the effect of fencing on movement of wild boars in Europe

Reference	Time period	Geographical area	Landscape	Population	Objective	Method movement estimation	Method of fencing	Results	Maintenance issues
Vidrih and Trdan (2008)	2005, from July until harvest of maize	Area of Smihel near Postojna, Slovenia	Agricultural land (maize)	Wild boar	Electric fence to prevent wild boar from entering a maize field	Boar tracks on the ground	Electric fence systems	No breaks through fencing were observed, although boar tracks on the outside of the fenced field were observed. Damage to arable fields in the vicinity of the protected field was also recorded.	Not reported
Santilli and Stella (2006)	1999-2003	Southern Tuscany, Italy	Agricultural land (maize)	Wild boar	Evaluate the effectiveness of 16.5 km linear electrical fence installed to farmland cultivated with maize	Not reported	Electric fence	93 % damage reduction was observed during the five years after fence installation without significant damage increase in the neighbouring areas	High price and intensive labour for installing and maintaining the electric fences

APPENDIX C