The epidemiology of African swine fever virus and its potential for introduction into Australia

Final Report

APL Project 2014/483

March 2013

Eric Neumann, Director
60 Buick Crescent
Palmerston North 4412
New Zealand
ericjneumann@gmail.com
Phone: +64 27 485 8486

Adjunct Associate Professor of Medicine and Epidemiology
Massey University
e.neumann@massey.ac.nz

Table of Contents

Abbreviations

ASF	African swine fever
CSF	Classical swine fever
NVD	National Vendor Declaration
AAHL	Australian Animal Health Laboratory
AN	Assessed negative
APF	Approved processing facility
ARP	At-risk premises
AUSVETPLAN	Australian Veterinary Emergency Plan
CA	Control area
CCEAD	Consultative Committee on Emergency Animal Diseases
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	Chief veterinary officer
DC	Direct contact
DCP	Dangerous contact premises
DCPF	Dangerous contact processing facility
EAD	Emergency animal disease
EADRA	Emergency Animal Disease Response Agreement
EADRP	Emergency Animal Disease Response Plan
EDTA	Ethylene diamine-tetra-acetic acid (anticoagulant for whole blood)
ELISA	Enzyme-linked immunosorbent assay
GP	General permit
IETS	International Embryo Transfer Society
IM	Intramuscular
INP	Intra-nasopharyngeal
IOP	Intra-oropharyngeal
IP	Infected premises
LCC	Local control centre
NASOP	Nationally agreed standard operating procedure
NMG	National Management Group
OA	Outside area
OIE	World Organisation for Animal Health
PCR	Polymerase chain reaction
PE	Post-exposure
PI	Post-inoculation, or post-infection
POR	Premises of relevance
RA	Restricted area
RP	Resolved premises
SCC	State coordination centre
SP	Suspect premises
SpP	Special permit
TP	Trace premises
UP	Unknown status premises
ZP	Zero susceptible species premises

Executive summary

A systematic review of the scientific literature on African swine fever was conducted. The worldwide English-language literature was surveyed using PubMed and supplemented by other sources of peer-reviewed papers. The non-peer-reviewed literature on African swine fever was included infrequently when important or unusual data could not be identified elsewhere. The review includes published papers from 1921 through October 2014.

This literature review was summarized using the AUSVETPLAN format to ease its revision when considered appropriate by APL and/or Animal Health Australia. The existing text in AUSVETPLAN was removed and replaced in its entirety to avoid any potential bias in this current review of the literature. The review is comprehensive and overly detailed for AUSVETPLAN but summary extracts of sections under each heading or sub-heading can be easily derived and inserted into AUSVETPLAN when required.

Following is a summary of the key features of African swine fever:

1. Since ASF was detected in Georgia in 2007 and predictably spread to other countries in Central Asia, the Caucasus, the Russian Federation, and countries in the eastern part of the EU, a number of publications and reviews on the disease have recently been published. Several in addition to this current report may be useful as ancillary reference documents:
    a. **AUSVETPLAN:**
    Anonymous. (2014). *Disease strategy: African swine fever (Version 4.0)*. Primary Industries Ministerial Council: Canberra, ACT, Australia, 76 pages. Retrieved from http://www.animalhealthaustralia.com.au/wp-content/uploads/2011/04/ASF4.0-FINAL1Oct14.pdf on November 6, 2014.
    b. **Qualitative risk assessment for the European Union:**
    EFSA Panel on Animal Health and Welfare (AHAW). (2014). Scientific Opinion on African Swine Fever. *EFSA Journal*, 12(4), 77. DOI: 10.2903/j.efsa.2014.3628. Retrieved from http://www.efsa.europa.eu/en/efsajournal/doc/3628.pdf on November 6, 2014.
    c. **Control options for Great Britain:**
    Anonymous. (2014). *Disease Control Strategy for African and Classical Swine Fever in Great Britain*. United Kingdom Department for Environment, Food and Rural Affairs: London, England, 63 pages, (August 2014). Retrieved from https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/343406/swine-fever-asf-control-strategy-140812.pdf on November 6, 2014.
2. Virtually every successful ASF eradication programme has required destruction of all domestic pigs in an affected area. Once a feral suid population becomes infected, the infection is likely to persist though perhaps at a very low level. In these instances, the feral population can be expected to be the source of periodic incursions into the domestic pig population through direct exposure, carcass scavenging, or tick vectors moving between the populations.
3. African swine fever (ASF) is a highly contagious disease of wild and domestic pigs. The disease is highly contagious amongst domestic pigs and produces highly variable severe clinical signs including fever, hyperaemia of the skin, anorexia and loss of condition, pneumonia, and death. The virus is not zoonotic.
4. Sub-lethal strains of the virus can cause clinical signs and lesions that are indistinguishable from classical swine fever. The most characteristic (but not pathognomonic) lesion(s) in affected pigs is haemorrhage, particularly in abdominal lymph nodes, spleen, liver, kidneys, and the serosal surface of many abdominal organs.

5. African swine fever is caused by infection with the only known member of the viral family Asfarviridae. The virus is a large, double-stranded DNA arbovirus.
6. There are three transmission cycles for the virus: One is related to circulation between inapparently infected warthogs (and other wild suids) and an argasid tick vector, two is a domestic pig-tick cycle in which warthogs play no apparent role, and three is a cycle in which the virus is transmitted directly amongst domestic pigs without need for either the wild suid or tick host. There are no other known mammalian hosts besides domestic and wild suids.
7. The virus is currently endemic in much of Africa and Italy (Sardinia). Parts of Euro-Asia are currently experiencing outbreaks of the disease (Russian Federation, Estonia, Latvia, Lithuania, Ukraine, and Poland.
8. Clinical signs of infection can be highly variable and both virus and host characteristics contribute to this variability. Four forms of the disease are recognized: Peracute (sudden death without clinical signs), acute (rapid onset of system illness followed by death within one week), subacute (similar to acute both with death in one to three weeks), and chronic (multiple organ system involvement including arthritis and pneumonia with death a common outcome in weeks to months post-infection).
9. Relative to many other viruses, ASF virus is very stable across a range of temperatures and pH, particularly when held in a protein rich environment (blood, serum, meat, etc.). The virus is notorious persistent in processed meats such as Parma hams, salami, and sausage casing for many months. Detailed tables of survivability in these products are available.
10. Argasid (soft) ticks are an important vector for ASF virus, particularly in sylvatic cycles. A number of soft ticks (including at least two in Australia) are known to be competent vectors. The role of ticks as a reservoir species is well-described in Africa but appear to be less epidemiologically relevant in outbreaks of the disease outside of Africa where *Sus scrofa* are the predominant pig host involved. However, their importance even in these situations cannot be ignored.
11. The virus can be passsaged in some species of argasid tick between moulting stages (transtadial), transovarially, and venereally. Once infected, the virus can be maintained for years in individual ticks or tick colonies for months to years even without on-going exposure to viraemic pigs.
12. Commercial disinfectants are available that are effective against ASF virus. In general terms, disinfectants with activity against enveloped virus are effective. Cleaning and degreasing prior to disinfection are critical steps to ensuring a disinfectant will be efficacious against ASF virus. One Stroke Environ, Virkon S, and bleach appear in a number of publications as common and effective disinfectants against the virus. Weak acids and alcohol-based disinfectants (as might be used against foot and mouth disease virus) are not likely to be effective against ASF virus.
13. African swine fever virus can be transmitted by aerosol over short distances (several metres) but this route of transmission does not generally appear to be significant in spread between farms.
14. The evidence for ASF virus transmission by embryo transfer, semen, or venereally is not strong but has been demonstrated.
15. There are few endemic pig diseases in Australia with which ASF would be confused. However, some forms of *Actinobacillus pleuropneumonia*, vitamin E/selenium deficiency, and porcine circovirus associated disease (specifically porcine dermatitis and nephropathy syndrome) could potentially produce gross lesions or clinical signs consistent with ASF.

16. A number of diagnostic techniques are available to confirm ASF virus infection. For rule-out of ASF in exotic disease investigations, PCR is the most heavily relied upon technique. This test is online at AAHL.
17. If infected during gestation, ASF virus is capable of inducing abortion. In these cases, virus can be detected both in the sow and in the fetuses. If a sow is infected with a sub-lethal isolate of ASF virus prior to mating and she is given adequate time to recover, the virus is unlikely to be transmitted to the fetuses.
18. Recovery from a sub-lethal infection will provide protection from clinical signs due to re-infection with the same strain (though won't necessarily provide sterilizing immunity). Recovery is unlikely to provide strong protection from infection (or clinical signs) with a heterologous isolate.
19. There are currently no effective vaccines available to protect against ASF. No treatment aside from supportive care is available to assist in the recovery of infected pigs. Administration of hyperimmune serum prior to infection may assist in lessening the severity of clinical signs or improving the likelihood of survival but will not prevent infection.
20. Serological tests are available as an ASF surveillance tool but are generally not adequate for diagnostic purposes at the level of an individual animal. No serological 'typing' scheme has been developed to differentiate isolates. Because there can be a high degree of antigenic variability between isolates, laboratories need to ensure they have current knowledge about differences in test performance particularly for serological assays and PCR. Not all ASF viruses can be readily cultured. OIE does provide recommended testing protocols but these should not be accepted as 'current and adequate' for any single point in time.

1 Nature of the disease

African swine fever (ASF) is a highly contagious disease of wild and domestic pigs. The disease is highly contagious amongst domestic pigs and produces highly variable severe clinical signs including fever, hyperaemia of the skin, anorexia and loss of condition, pneumonia, and death. Infection with less virulent strains of ASF virus can produce disease that is clinically indistinguishable from classical swine fever (CSF) including the presence of similar lesions at post-mortem examination. Aside from its clinical significance, occurrence of the disease is accompanied by severe restrictions on trade in pigs, pork, and other pig products. The virus is not zoonotic though it can readily infect several species of soft ticks creating the potential for establishment of an important ecological reservoir in many countries around the world.

1.1 Aetiology and pathogenicity

For a complete and recent review of all virological aspects of African swine fever (ASF) virus including detailed descriptions of its genomic structure, virus-host cell interactions, gene expression and virus replication, and mechanisms of virus-induced apoptosis the reader is encouraged to read the 'African Swine Fever Virus' chapter in Lesser Known Large dsDNA viruses (Tulman et al 2009). A synopsis of the most relevant aspects of these topics from this book and other sources are discussed below.

African swine fever (ASF) is caused by infection with the only known member of the viral family Asfarviridae. The virus is a large, double-stranded DNA arbovirus with a genome of approximately 180 to 190 kilobase pairs encoding at least 165 genes. The virus is many respects shares features with members of other intracytoplasmically-replicating virus families including Poxviridae and Iridoviridae (Dixon et al 2004). The virus replicates in two phases, an early phase in the cell nucleus and a later more prominent phase within 'virus factories' in the cytoplasm (Moulton and Coggins 1968b; Tabares and Sanchez Botija 1979; Pan et al 1980).

While antigenic, genetic, and virulence differences are known to exist between ASF virus isolates, all are regarded as belonging to a single serotype since attempts to distinguish them by serotyping methods have proved unreliable (Vigario et al 1974).

1.2 Susceptible species

African swine fever is a viral infection of pigs that affects all members of the family Suidae. It is generally accepted that there are four wild suid genera: *Phacochoerus*, *Potamochoerus*, *Hylochoerus*, and *Sus*. All vary in their ecology and clinical expression of disease caused by infection with ASF virus (Jori and Bastos 2009).

Historically, the primary transmission cycle for the virus was related to circulation between inapparently infected warthogs (and other wild suids) and an argasid tick vector, only causing severe disease outbreaks when domestic pigs became infected. However, two additional transmission cycles are now recognized, a domestic pig-tick cycle in which warthogs play no apparent role, and a cycle in which the virus is transmitted directly amongst domestic pigs without need for either the wild suid or tick host (Jori and Bastos 2009).

Over time, the virus has changed such that sub-lethal strains of the virus now circulate amongst domestic pigs in Africa and parts of Europe and Asia. This change is in some part due to evolution of the virus but it also seems that populations of domestic pigs at least partially resistant to the pathogenic effects of the disease have evolved in Malawi (Haresnape et al 1985, 1987), Angola (Mendes 1994), Zambia (Wilkinson et al 1988), and Mozambique (Penrith et al 2004a). This phenomenon was investigated experimentally by capturing pigs from the resistant population in Mozambique and then allowing them to breed naturally to create a potentially resistant offspring population. These offspring

were challenged with either homologous ASF virus isolated from the parent generation or with a heterologous ASF virus. All the pigs developed acute ASF regardless of the virus isolate with which they were inoculated and eventually died. Thus it appeared that the resistance to disease exhibited by the parent population was not passed to their offspring.

Other mammals in Africa with geographical ranges overlapping the warthog including hippopotamus, buffalo, black rhinoceros, wildebeest, hyena, cheetah, leopard, lion, baboon, porcupine, jackal, and ant-bear were examined in a small study and all appeared to be free from infection or prior exposure to the virus (Stone and Heuschele 1965; Thomson 1985).

1.3 World distribution and occurrence in Australia

1.3.1 World distribution

The first epizootics of ASF in domestic swine were reported from Kenya between 1907 and 1915 after imported swine, raised on open ranges, came into contact with infected warthogs or infected ticks and became lethally affected by the disease now known to be ASF. Further outbreaks around this time were minimized by construction of pig-proof fencing that minimized contact between domestic and wild pigs. The common tick infecting warthog burrows in East Africa at the time (and remains so today) was *Ornithodoros moubata porcinus* (now referred to as *O. porcinus porcinus*) and the tick was later discovered to be a carrier of the virus. This tick is only rarely found outside the burrow of the warthog and therefore it was postulated that the segregation worked in preventing infection in the domestic pigs because it kept them from coming in contact with the tick, more so than preventing direct contact between the two species of pigs (Dorman 1965) and reviews by (Hess 1971), (Coggins 1974) and (Gibbs 1981b).

After more than 40 years of being confined to Africa, the disease first spread outside the continent between 1957 and 1960 to Portugal. The disease next spread to Spain where it became endemic for a number of years on the Iberian Peninsula. This incursion into Europe was likely the source of further outbreaks of ASF on the Mediterranean islands of Malta and Sardinia, several Caribbean islands (Dominican Republic, Haiti, and Cuba), and Brazil during the late 1970s and early 1980s (Gibbs 1981a). These series of outbreaks were important as they were some of the earliest occurrences in domestic pigs of a sub-lethal form of the disease that was generally indistinguishable from CSF.

The occurrence of ASF in Spain in 1960 and the subsequent efforts to eradicate the disease from the country have been described (Bech-Nielsen et al 1995). A formal programme to eradicate ASF from Spain was not initiated until 1985 and was founded on improvements to basic farm biosecurity and sanitation, official reporting and control of pig movements between all pig farms, serological testing, and destruction of positive pigs. Extensive pig husbandry systems on the south-western Iberian Peninsula (seasonal free-grazing of pigs in oak forests) hampered eradication efforts as the practice allowed for domestic pigs to have direct contact with infected wild boar and established a reservoir of infected local argasid ticks, *Ornithodorus erraticus* (now referred to as *O. marocanus*). However despite this challenge, by 1990 the country was essentially free of infection though sporadic infections were detected on small extensive farms for several more years probably related to persistent infections in the *O. marocanus* tick population and intermittent infection of wild boar. The Iberian Peninsula was declared free of the infection in 1995 though one further isolated epizootic occurred in Portugal in 1999 (Kleiboeker 2002).

African swine fever crossed the Atlantic ocean for the first time in 1971 producing an outbreak in Cuba (Wilkinson et al 1981). In order to control the outbreak and eventually eradicate the disease,

the government was forced to destroy all swine in the affected area around Havana (Reichard 1978). Though Cuba suffered a second outbreak of the disease in 1980, it was quickly confined and eradicated.

In 1977, there was a considerable increase in the scale and severity of ASF that had been occurring in Portugal and Spain. This increase likely contributed to the 1978 appearance of ASF on the islands of Malta and Sardinia. Malta moved relatively quickly to eradicate the infection by killing out the country's entire pig population. Sardinia, in response to concerns raised by local pig farmers, chose not to follow the lead of Malta. Reliant on open-grazing practices similar to that practiced by farmers on the Iberian Peninsula, Sardinia chose to manage rather than eradicate ASF and today remains infected. The country is presented with significant challenges in managing the disease in the disease in light of the resident populations of infected wild pigs and the likely presence of an infected tick population. Results of epidemiological studies of semi-free ranging pigs on Sardinia are consistent with observations from Spain suggesting that once a feral population of pigs becomes infected with ASF virus, that population is likely to remain infected and be a persistent threat to domestic pigs in the area unless the two populations can be reliably separated (Mannelli et al 1997; Mannelli et al 1998).

In 1978, the disease was identified in Rio de Janeiro, Brazil when pigs fed raw garbage from the city's international airport became acutely ill with high levels of mortality. The infection, which was likely introduced from Spain (Alexander 1992) soon spread to other Brazilian States. Most affected properties were feeding swill obtained from multiple local and regional sources. Officials later reported that the disease had likely been circulating for at least two months before being detected and diagnosed. Morbidity and mortality on affected properties varied widely but was generally less severe than what had been described for outbreaks of the disease in Portugal and Africa earlier in the century (Mebus et al 1978). Within six months of ASF being confirmed in Brazil, the disease had spread to all 15 States. Detailed reports of the eradication campaign in Brazil have been published (Moura et al 2010). The eradication programme in Brazil relied on controlled movement of swine in and around affected areas, destruction of all pigs within affected areas, improvements in biosecurity and sanitation on farms and trucks, a complete prohibition of animal exhibitions and fairs, prohibition of swill feeding, and community education about the disease.

Concurrent with Brazil becoming infected with ASF in 1978, the disease was also confirmed in the Dominican Republic. Similar to Brazil, the virus circulating in the Dominican Republic was of moderate virulence. During the first four months after appearance of widespread clinical disease (but with no confirmed diagnosis), farmers and disease officials were vaccinating extensively for classical swine fever (CSF) under the impression they were dealing with a severe outbreak of that disease. Eventually after less intensive interventions failed to control the outbreak, the entire population of more than one million pigs in the Dominican Republic was killed out in order to eradicate the disease.

Along with the Dominican Republic, the country of Haiti comprises the island of Hispaniola. Not surprisingly, once the Dominican Republic became infected in 1978 Haiti followed and within two years, the country was considered endemically infected. Ultimately, the two countries worked jointly to slaughter out infected herds and eradicate the disease from the island (Alexander 1992). The eradication programme was started in earnest in May 1982 and was completed by December 1983. A simple and effective programme was implemented to ensure compliance with the programme by the island's pig farmers:

- Local sites for animal destruction were identified and publicised. On the designated day a site was operationalized, each local pig owner presented his pig(s) to the programme official at the site and both ears of the pig were tagged with identical numbers.

- The pig was then immediately killed by the official, exsanguinated, and then one ear was removed and retained by the official.
- The owner then took the dead pig away to be processed for consumption, returning the same day to the official government paymaster at the site with the other tagged ear which was matched to the ear collected by the official at the time the pig was euthanized. The owner was then immediately compensated with a cash payment.
- In addition to cash compensation, the owner was also permitted to retain the processed carcass for sale or consumption. These killing sites often became lively trading posts for fresh pork.
- Once all local pigs had been destroyed, the sites were decontaminated with 1-Stroke Environ (O-phenylphenol) and any materials or carcass scraps were either burnt or buried.
- During the eradication programme, all importation of pork into the countries was stopped in order to force rapid consumption of the domestic pork being generated as a result of the forced destruction of local pigs.

It was determined that a competent tick vector (*Ornithodorus puertoricensis*) was present on the island. Ticks were collected from 90 sites in Haiti but none were found to be infected, increasing the likelihood that the eradication campaign would be successful. After the end of the eradication programme, a small re-break of the disease was detected in March 1984 based on detection of serum antibodies collected as part of an on-going surveillance programme but no clinical signs or secondary spread of the disease was identified; today Hispaniola is considered to be free of ASF.

Outbreaks of ASF in the Brazil, the Caribbean, the Iberian Peninsula, and the Mediterranean islands were controlled and eventually eradicated (with the exception of Sardinia). Periodic incursions of ASF have since occurred in other European countries but were quickly identified, contained and eradicated. In addition, the disease has continued to spread across many parts of south, west, and east Africa in both wild and domestic pigs. The next most epidemiologically significant outbreak of the disease occurred in 2007, when ASF was identified in the country of Georgia. The disease rapidly spread north and east through the Caucasus region (notably Armenia and Azerbaijan) and eventually to the Russian Federation. Local spread through the region was most likely a result of contact amongst semi-commercial free-ranging pigs and feral pigs, with longer distance spread a result of the sale and subsequent transport of infected pigs into previously uninfected areas. Additional long distance spread (greater than 1000 km) was likely due to trade in pork harvested from infected pigs that eventually found its way into uninfected farms through swill feeding or other anthropogenic factors (Gulenkin et al 2011; Oganesyan et al 2012). Additional significant risk factors that explained the direction and extent of the spread of the disease across the Russian Federation were density of main highways, density of secondary roads, density of rivers, and density of domestic swine populations. As part of an investigation of potential risk factors for spread of the disease, researchers have estimated the basic reproduction ratio for ASF both within farms (R_o between 8 and 11) and between farms (R_o between 2 and 3) during the outbreak. Genetic analysis of ASF virus isolates from different affected regions of the Russian Federation between 2007 and 2011 suggested the outbreak was most likely related to a single introduction of the virus, probably originating from the Caucasus region (Malogolovkin et al 2012). Despite circulating in the region since 2007, the virus remains highly virulent and capable of causing high death loss (Gogin et al 2013).

With the advent of recent incursions of ASF into Eastern Europe, the results of several studies have been published that have investigated the risk of the disease becoming widespread (and endemic) in central Europe. Authors have speculated based on disease outbreak models that there was a high risk of the disease becoming endemic in the Caucasus region and that continued spread into central Europe

was likely (Wieland et al 2011). While importation of live pigs from ASF infected countries is currently banned in Europe, the virus may be introduced through other routes including illegal movement of pigs (Mur et al 2012a), livestock trucks returning to Europe after travel into an infected country, swill feeding using contaminated food waste obtained from international flights (Mur et al 2012b), and illegal trade in pork or pork products (Costard et al 2013a). Poland and the Russian Federation were thought to contribute the most risk related to movement of live pigs while Poland and Lithuania contributed the most risk related to transport-associated routes.

On a relative scale, five European countries (France, Germany, Italy, Spain, and the United Kingdom) were thought to have either high or moderate risk of inadvertently releasing of ASF virus contaminated pork products if they were to become infected. Similarly, five countries (France, Italy, Poland, Romania, and Spain) were identified as having the highest risk of being exposed to the virus as a result of release of virus contaminated pork products. The free-trade policy amongst EU member countries creates a significant risk of regional spread of contagious animal diseases once introduced into the region. Another study modelled the spatio-temporal spread of ASF virus during the 'high-risk period' (time from first infection until first detection) if an outbreak were to occur in central Europe (Nigsch et al 2013). Based on historical records of pig movements within and among countries, the overall sizes of most simulated outbreaks were moderate but several countries were identified as having a key role in the inter-country trading network. In particular Denmark, the Netherlands, Lithuania, and Latvia were identified as potential 'super-spreaders' if they were to become infected with ASF while German and Poland were identified as 'super-receivers' likely to become infected by importation of contaminated pork products.

A list of OIE member countries that have been previously infected (Table 1) or are currently infected (Table 2) with ASF are shown below.

Table 1. List of OIE member countries that have previously reported the occurrence of ASF but for which the disease is currently absent or for which the current status is unspecified (as of 2014).

	Domestic		Wild	
Country	**Surveillance**	**Date of last occurrence**	**Surveillance**	**Date of last occurrence**
Andorra	No surveillance specified	1975	No surveillance specified	1975
Armenia	General Surveillance	2011	General Surveillance	Unspecified
Azerbaijan	General and targeted surveillance	2008	Targeted Surveillance	Unspecified
Belgium	General and targeted surveillance	1985	Targeted Surveillance	Unspecified
Brazil	General Surveillance	1981	General Surveillance	Unspecified
Central African Republic	No surveillance specified	2012	No surveillance specified	Unspecified
Chad	No surveillance specified	2012	No surveillance specified	2012
Cuba	No surveillance specified	1980	No surveillance specified	Unspecified
Dominican Republic	General Surveillance	1981	No surveillance specified	Unspecified
Eritrea	No surveillance specified	Unspecified	No surveillance specified	Unspecified
France	General Surveillance	1974	General Surveillance	Unspecified
Georgia	Targeted Surveillance	2007	Targeted Surveillance	2007
Kazakhstan	General Surveillance	Unspecified	General Surveillance	Unspecified
Kenya	General and targeted surveillance	2012	General Surveillance	Unspecified
Korea (Dem. People's Rep.)	No surveillance specified	Unspecified	No surveillance specified	Unspecified
Liechtenstein	General Surveillance	Unspecified	No surveillance specified	Unspecified
Malta	General Surveillance	1978	General Surveillance	1978
Mauritania	No surveillance specified	Unspecified	No surveillance specified	Unspecified
Mauritius	General and targeted surveillance	2008	General and targeted surveillance	Unspecified
Netherlands	No surveillance specified	1986	No surveillance specified	Unspecified
Niger	General Surveillance	Unspecified	No surveillance specified	Unspecified
Portugal	General and targeted surveillance	1999	General and targeted surveillance	1999
Sao Tome and Principe	No surveillance specified	1992	No surveillance specified	Unspecified

Senegal	General Surveillance	2009	General Surveillance	2007
Sierra Leone	General Surveillance	Unspecified	No surveillance specified	Unspecified
Somalia	No surveillance specified	Unspecified	No surveillance specified	Unspecified
Spain	No surveillance specified	1994	No surveillance specified	1994
Syria	No surveillance specified	Unspecified	No surveillance specified	Unspecified
Thailand	General Surveillance	Unspecified	General Surveillance	Unspecified
Zimbabwe	General Surveillance	1992	No surveillance specified	Unspecified

Anonymous. (2014). World Animal Health Information Database (WAHID) Interface, retrieved October 30, 2014 from http://www.oie.int/wahid-prod/public.php?page=home

Table 2. List of OIE member countries currently reporting the occurrence of ASF (as of 2014).

Country	Domestic	Wild
Angola	Y	N
Benin	N	N
Burkina Faso	Y	N
Burundi	N	N
Cameroon	Y	N
Cape Verde	Y	N
Congo (Dem. Rep. of the)	Y	N
Cote D'Ivoire	Y	Y
Estonia	Y	Y
Ghana	Y	N
Guinea-Bissau	Y	N
Italy	Y	N
Latvia	Y	Y
Lithuania	N	Y
Madagascar	Y	N
Malawi	Y	N
Mozambique	Y	N
Namibia	Y	Y
Nigeria	Y	N
Poland	Y	Y
Russia	Y	Y
Rwanda	Y	N
South Africa	N	Y
Tanzania	N	Y
Togo	Y	Y
Uganda	Y	N
Ukraine	Y	Y
Zambia	Y	N

Anonymous. (2014). World Animal Health Information Database (WAHID) Interface, retrieved October 30, 2014 from http://www.oie.int/wahid-prod/public.php?page=home

Several studies of the genomic evolution of ASF virus, based on RFLP patterns or differences in nucleotide sequence, have been published. In one study, 23 isolates were classified into five distinct groups based on analysis of their *Sal*I restriction pattern and it was determined that all the European and American isolates clustered together suggesting a common origin (Blasco et al 1989).

Using a different technique, approximately 22 major ASF virus genotypes have been identified based on sequencing of a capsid protein gene B646L (Costard et al 2013b). Using this grouping system, all ASF virus isolates from west Africa and from the early outbreaks in Europe, the Caribbean, and South America have been placed into genotype 1. Genotype 2 includes viruses from Mozambique and viruses from Madagascar, Mauritius, the Caucasus region, and the Russian Federation suggesting an epidemiologic link between the countries. Genotypes 3 through 22 include virus isolates restricted to areas of south or east Africa. More recent work on ASF virus phylogeny has further delineated the 22 genotypes that were initially proposed into 35 different clusters with emphasis on a more thorough understanding of minor differences amongst isolates in genotype 1 (Michaud et al 2013)

1.3.2 Occurrence in Australia

There have been no outbreaks of ASF in Australia.

1.4 Epidemiology

1.4.1 Incubation period

The incubation period for ASF is usually 5–15 days but may be as long as 20 days.

1.4.1.1 OIE incubation period

The OIE Terrestrial Animal Health Code 2014 describes the longest incubation period for ASF as 15 days.

1.4.2 Persistence of agent and modes of transmission

In general terms, three transmission cycles have been described to explain the transmission and maintenance of ASF virus in pigs. The first cycle describes a sylvatic relationship between sub-Saharan warthogs and the tick *O. porcinus porcinus*, in which neither species develops significant clinical diseases from the infection despite high levels of viremia during the acute stage of infection. The second cycle describes the relationship between domestic pigs (broadly including the Eurasian wild boar, 'feral pigs' or free-living pigs derived from escaped or released domestic pigs, and truly domestic pigs) and any of several argasid ticks including but not limited to *O. porcinus porcinus*. The third cycle describes those situations whereby the infection can persistent within populations of truly domestic pigs (direct pig-to-pig transmission) without any need for a tick vector. In this third cycle, ASF virus is thought to be primarily transmitted via direct contact with excreted virus in oro-nasal secretions, faeces, and perhaps short distance aerosols (Wardley et al 1983; Sanchez-Vizcaino et al 2012).

Transmission rates of ASF virus between infected and uninfected pigs have not been widely reported. However, recent experiments using two different isolates demonstrated that the transmission rate for both resulted in the occurrence of between 0.45 and 3.63 secondary infections each day during the infectious period (de Carvalho Ferreira et al 2013a). The minimum infectious period ranged from 6 to 7 days while the average maximum infectious period ranged from approximately 20 to nearly 40 days. Estimates of the reproduction ratio for the first generation of transmission events ranged from 5 to 24 when calculated based on the minimum infectious period and from 10 to 66 when calculated based on the maximum infectious period, depending on the isolate.

Study of ASF using experimental inoculation of pigs has been occurring over a number of decades. A number of studies have relied on intramuscular (IM) inoculation as it is easy to induce highly reproducible clinical disease though this method suffers from a significant drawback in that the method does not replicate the normal route of exposure in domestic pigs. To ascertain the significance of this issue and improve future studies of the disease, a study was conducted to compare viral dynamics and the resulting pathology when pigs were infected be either IM, intra-nasopharyngeal (INP), intra-oropharyngeal (IOP), or by direct contact (DC) with recently infected pigs (Howey et al 2013). All routes of exposure predictably induced clinical disease with approximately the same temporal features though INP inoculation resulted in the most consistent progression of disease across the widest range of doses while effectively simulating natural exposure and was therefore recommended as the preferred method for use in future experimental studies.

1.4.2.1 General properties

African swine fever virus is stable across a wide range of pH and temperatures, particularly when stored in the presence of protein. This characteristic of the virus was noted even by very early researchers who determined that infectivity remained for more than 400 days when the virus was

stored in filtered serum at room temperature, at least six years in a cold room, and for at least 16 days in putrefying blood (reviewed by Plowright and Parker 1967). The results of further controlled experiments by Plowright and Parker confirmed that the virus was very stable (no decline in titre) for periods up to 18 months when held at 4C and for two years when held at -70C (Plowright and Parker 1967). Interestingly, when held at a slightly warmer temperature (-20C) the virus had a predictable and progressive loss of infectivity. In serum-containing medium, the virus was shown to have a half-life of between 36 and 119 days when held at 4C but only 8 hours in the absence of serum. Virtually all ASF virus was inactivated within one hour when maintained at 60C though a small fraction of virus was determined to survive for up to 20 minutes at that temperature. In serum-free media, the virus was not rapidly inactivated until the pH fell below 3.9 with a very resistant fraction persisting down to pH 3.1 (but not pH 2.7). In an alkaline environment, inactivation of the virus occurred slowly at pH 10.8 but rapidly at pH 11.5. However, even at pH 13.4 in serum-free media there was a highly resistant fraction which persisted for at least 20-22 hours; with 25% serum some infectivity was still detected after seven days.

ASF virus remains viable for long periods of time in faeces, blood, and soil and on wooden surfaces (Kovalenko et al 1965). Virus suspended in a matrix rich in protein may withstand rather extensive variations in pH, and proteolytic enzymes have little or no effect on the virus (Hess 1971).

In order to assess the suitability of various disinfectants against ASF virus in a field setting, ten commercial disinfectants were tested in the presence of a high concentration of proteins (whole blood or filtered tissue homogenate) (Stone and Hess 1973). The efficacy of each product was initially screened using an *in vitro* virus isolation assay. Once initial product screening was completed, further efficacy testing was conducted using a more sensitive pig bioassay model to determine if virus was completely inactivated. Amongst the disinfectants that were evaluated (halogen derivatives, substituted phenols, polyphosphates, quaternary amines, non-ionic and anionic surface-active compounds, acetic acid, and sodium hydroxide), phenols appeared to be the most effective in inactivating the virus with one product in particular, One Stroke Environ (a combination of *o*-phenylphenol, *o*-benzyl-*p*-chlorphenol, and *p*-tert-amylphenol) determined to be the most effective. However, the pig bioassay confirmed the importance of having adequate contact time between the disinfectant and the agent as 60 minutes of contact time was required to maximize the effectiveness of One Stroke Environ.

In other studies, common disinfectants including chlorine at 0.0075 to 0.003% concentration, iodine at 0.0075 to 0.015% concentration, and quaternary ammonium 0.003% concentration have also been reported as being effective against ASF virus (Shirai et al 2000). The virus has also been shown to be inactivated by cresol, 2% sodium hydroxide, 1% formalin, 4% anhydrous, 10% crystalline sodium carbonate (with 0.1% detergent), strong iodophors (1%) in phosphoric acid, ionic and non-ionic detergents, and lipid solvents including chloroform (Plowright et al 1994).

Most standardized methods for assessing the efficacy of disinfectant solutions rely on use of experimentally contaminated non-porous surfaces. However, farm environments present a much more difficult situation as animal holding areas typically are contaminated with organic material and are often constructed from porous materials such as wood or cement. In an effort to determine the effectiveness of citric acid (acidic) and sodium hypochlorite (alkaline) on porous wood surfaces, researchers developed a model to test their efficacy using small wooden boards (Krug et al 2012). A solution of 2% citric acid or 2000 ppm of sodium hypochlorite was effective in inactivating ASF virus after 30 minutes of exposure at 22C.

1.4.2.2 Environment (including windborne spread)

The virus has been shown to be shed in blood, urine, and faeces and can remain stable in the environment for several days (Montgomery 1921). After experimental infection of 8 to 10 week old pigs, investigators measured the amount of virus that was excreted through several routes (McVicar 1984). During the first 11 days post-inoculation (PI), virus was detected in nasal mucus, conjunctival fluid, tonsil swabs, faeces, preputial fluid, and urine but not in saliva. The highest concentrations of virus over the most prolonged period were found in faeces and on the tonsillar swabs. In addition, blood was consistently found to contain infectious virus throughout the study period. Approximately half of the pigs remained viraemic as long as five weeks PI and in the surviving pigs, viraemia remained for at least two weeks after pigs became clinically normal. Blood and lymph nodes were harvested from three persistently infected pigs in the study 18 to 21 weeks PI and the tissues were confirmed to be infectious by exposure of naïve pigs to the harvested tissues.

1.4.2.3 Susceptible animals

As described above, virus can be transmitted between pigs by various routes (including insect vectors) but the relative importance of these different routes varies between domestic and wild pigs.

Live domestic animals

Once ASF becomes established in a population of domestic pigs, the most important route of transmission is direct contact between infected and uninfected individuals, usually through the oral or upper respiratory tract; no tick vector is required to maintain the infection in populations of infected domestic pigs (Heuschele 1967; Plowright et al 1968; Colgrove et al 1969; Wardley et al 1983).

Aerosol infection over short distances (2.3 m) was first demonstrated by successful infection of pigs housed on a platform above infected pigs and in pigs exposed to ducted air from a group of acutely affected pigs (Wilkinson et al 1977). When infection is due to aerosol exposure, it appears as though virus enters through the lower respiratory tract (rather than tonsils or the nasal cavity), where it replicates locally and is then disseminated to other tissues in the body via the blood (Wilkinson and Donaldson 1977). Since this early work, air sampling techniques have been optimized to detect and quantify the amount of virus excreted into the air after pigs become infected (de Carvalho Ferreira et al 2013b). In experiments using ASF virus isolates of varying pathogenicity, the half-life in air of most viruses tested was between 14 and 19 minutes. Infectious virus could be recovered from air samples of expired air from infected pigs from four days PI until at least 70 days PI.

A small number of studies have been published describing farm-level risk factors for infection with ASF virus. A case-control study to determine farm-level risk factors for AFS infection was conducted in Nigeria, a country endemically infected with the virus (Fasina et al 2012). Herds of varying levels of production intensity were included in the study. The presence of an abattoir or the presence of a known infected farm nearby placed a farm at significantly higher risk of becoming infected. Interestingly, among the 28 biosecurity measures that were investigated in the study, 'visit(s) by a veterinarian when animals were sick' put the farm at risk while good management of feeds, separation or isolation of sick pigs, and washing and disinfection of farm equipment and tools had a protective effect for a farm. Important risk factors for infection have also been identified in other studies including the practice of raising pigs in a free-range environment (Allaway et al 1995; Edelsten and Chinombo 1995; Mannelli et al 1997) and previous occurrence of the disease on the farm (Randriamparany et al 2005).

Live wild (including feral) animals

The historic reservoir for ASF virus is maintained in a sylvatic cycle primarily between the African warthog (*Phacochoerus aethiopicus*) and argasid ticks, primarily *O. porcinus porcinus* (Thomson 1985; Luther et al 2007). In addition, African bush pigs (*Potamochoerus spp.*) are also known to be endemically

infected with the virus in some regions though their relationship with argasid ticks is likely somewhat different than warthogs (Anderson et al 1998). As least one report exists of the giant forest pig (*Hylochoerus meinertzhageni*) being infected with the virus but its role in the overall epidemiology of the disease does not appear to be significant (Heuschele and Coggins 1965b). The collared peccary (*Tayassu tajacu*) and the white-lipped peccary (*T. albirostris*) have been shown to carry the virus in the Americas (Vinuela 1985) though information presented by other authors suggests the species is resistant to infection (Fowler 1996; Kleiboeker 2002).

As many as 90% of individuals in warthog populations in sub-Saharan Africa have experienced ASF virus infection though some other areas of the continent remain free of the disease (Thomas and Kolbe 1942; Plowright et al 1969; Taylor et al 1977). However, most adult warthogs in endemic areas of Africa are seropositive and likely to be persistently infected. There is little evidence that horizontal or vertical transmission of ASF virus occurs within warthog populations suggesting the critical role of ticks in maintaining the infection in the wild population (Plowright et al 1969). Despite the presence of maternally acquired anti-ASF virus antibody in neonatal warthogs, it appears that infection occurs in the first few weeks of life. Following this primary infection, high levels of viremia are common in the young pigs sufficient to ensure that at least some uninfected ticks that feed on the pigs become infected with the virus thus enabling the cycle to continue. When ASF first escaped from Africa and became established amongst free-ranging wild pigs on the Iberian Peninsula, a local argasid tick *Ornithodoros erraticus* (also known as *O. marocanus*) was found to act similarly to *O. porcinus porcinus* as a maintenance host in that region (Sanchez-Botija 1963).

As described above, the warthog and bush pig are susceptible to infection and have an important role in the epidemiology of the disease in Africa. However, there are also two wild suids of the genus *Sus* that also have a role in the epidemiology of ASF; the 'wild boar' and the 'feral pig' (Jori and Bastos 2009). In the context of this review, the wild boar (also referred to as the Eurasian wild boar) is widely distributed across Europe, Asia, and parts of Africa and is considered to be the ancestor of most common domestic pigs. The feral pig by contrast, is considered to be a domestic pig that is living in the wild either through escape or release from containment. Wild boar and feral pigs can be difficult to differentiate and in fact, hybridization of the two through inter-breeding is likely very common. Pigs in this genus are fully susceptible to infection by ticks or through contact with another infected member of the *Sus* family. While the clinical expression of ASF in *Sus* can vary dramatically, these pigs are much less likely to participate in a long-term sylvatic cycle as has been described for the warthog and bush pig.

Dead animal carcasses

Pigs that have died acutely from ASF, have died in a more chronic stage of the disease, or are a wild suid living in an area known to be endemically affected by ASF, all present a risk of virus transmission to other pigs. As omnivores, pigs that come across the carcass of a dead pig are likely to interact with if not actively feed on the carcass. Given the propensity of the virus to remain infectious for days to weeks in a protein rich substrate such as meat, tissue or blood, oral exposure to a contaminated carcass is likely to lead to transmission of the virus.

1.4.2.4 Animal products

Meat and meat products

ASF virus is very resistant to environmental inactivation remaining stable at a wide range of pH, particularly when held in a protein rich matrix such as meat, blood, or serum (Penrith and Vosloo 2009). The topic has been investigated thoroughly and review articles are available that cover the topic

in detail (Farez and Morley 1997). Of note, pork products such as cured ham can remain infectious for several months (McKercher et al 1987; Mebus et al 1993; Mebus et al 1997) and thus its improper disposal and use as a component of swill feeding is highly risky.

In an experiment using the highly virulent 1960 Portugal strain, virus was harvested from the spleen and blood of acutely infected pigs then used to inoculate two 125 kg pigs by IM injection (McKercher et al 1980). At 72 hours PI, the pigs were slaughtered and lymph nodes, blood, muscle, and long bone marrow were collected. In addition the intestines were removed, stripped, and held in 42C water for 30 minutes. The intestines were then flushed with cold water, inverted and scraped, then the resulting casings placed in brine at 4C for 24 hours when they were flushed with water and stored at 4C. To evaluate the effect of time and temperature on virus inactivation, one gram tissue samples were raised to 69C for 0, 30, or 60 minutes. Samples were then homogenized before being used as the source of an IM inoculum in a pig bioassay study; pigs were followed for 14 days PI. Only samples raised to 69C (for any length of time) did not induce disease in pigs. Further, casings prepared as described and then stored for up to 97 days were still able to cause lethal infection.

The virus has been recovered after 150 days from infected meat kept at 4C, after 104 days from meat kept at -4 °C, and after 188 days from bone marrow stored at -4C (as reviewed by MacDiarmid 1991). Other authors have reviewed the published literature and several studies are available documenting the viability of the virus in fresh meat and meat products for at least 150 at refrigerator temperatures and at least 1000 days when frozen (EFSA Panel on Animal Health and Welfare (AHAW) 2010, 2014). With the persistent occurrence of ASF in Sardinia and the importance of specialty dry-cured, aged salamis and hams as an export commodity from countries in southern Europe, survivability of the virus under processing conditions for Iberico, Parma, and Serrano hams has been studied in detail. Critically, reaching an internal temperature of 70C appears to be important for ASF virus inactivation as changes to pH as a result of aging and curing time are unreliable in inactivating the virus (Mebus et al 1997; EFSA Panel on Animal Health and Welfare (AHAW) 2010, 2014).

1.4.2.5 Animal by-products

Swill and meat meal

While both sylvatic and pig-to-pig transmission models can explain the persistence of ASF virus in populations of domestic or wild pigs, it appears that the most important means of transmitting the virus over long distances is through the movement of infected pigs and the feeding of pigs on swill containing infected pork products. Feeding of contaminated swill is particularly important as it is considered to be one of the most likely routes the virus could be introduce into ASF-free countries that do not share a contiguous border with countries having populations of wild pigs known to be infected.

1.4.2.6 Semen and embryos from live susceptible animals

African swine fever virus can likely be transmitted venereally or through use of contaminated semen given its ability to generate high levels of viraemia and for recovered pigs to remain infected for many months. Transmission by either route is not likely to feature significantly in the epidemiology of the disease though there is sufficient evidence to warrant controls on semen and embryos being exported from ASF infected countries.

1.4.2.7 Equipment, including personal items

Transmission of the virus through injection with needles that have been contaminated with blood from infected pigs has been demonstrated (Penrith et al 2004b). The virus has also been reported as being

able to survive for up to 60–100 days in faeces held at room temperature, and for at least 30 days in contaminated pig pens (Haas et al 1995; Anonymous 2006; Sanchez-Vizcaino et al 2012). Transfer of the virus by fomites, including bedding, feed, equipment, clothes and footwear, is a proven method for spread of ASF (Penrith and Vosloo 2009). People (especially veterinarians), veterinary instruments (especially hypodermic needles) and vehicles that have carried infected pigs have also been implicated in the spread of ASF (Wilkinson 1986).

1.4.2.8 Vectors

Ticks play an important role as both a transmission vector and reservoir for ASF virus. Argasid ticks (soft ticks) have been repeatedly shown to be important in both respects. Virus can be maintained through the moulting stages of nymphs (transtadial passage), be maintained transovarially between tick generations, and can be sexually transmitted between adult ticks (Plowright et al 1970; Plowright et al 1974) thus an infected population of ticks is able to maintain infection with ASF virus indefinitely without the need to be re-infected by feeding on viremic animals. The virus can be maintained in individual ticks for extended periods of time up to at least 15 months (Plowright et al 1970). The role of ixodid ticks (hard ticks) in the epidemiology of ASF is less clear. A number of ixodid ticks have been known to feed on pigs and have the potential to act as mechanical vectors of the virus though their ability to act as a long term reservoir appears to be unlikely. A summary of some of the key features associated with various tick species is shown in Table 3. The ability of a tick to maintain and transmit virus depends both upon the strain of the virus and the species of tick.

An extensive review of *O. porcinus porcinus* and its relationship to ASF has been published (Kleiboeker and Scoles 2001). The tick is considered to be nidicolous (burrow-dwelling) but significant populations can be carried transiently by the animal outside its burrow. The tick is a rapid feeder capable of completing a blood meal in less than an hour. Ticks moving between warthogs when in direct contact with domestic pigs are probably the main route of virus transmission between the species; construction of double-fences around domestic pigs raised in areas where warthogs are endemically infected is highly successful in eliminating transmission of the disease. Once infected, these ticks probably remain infected for life with no apparent harm to the tick.

The infection rate in tick populations can vary widely with reports of 0.028 to 1.7% (as reviewed by Wardley et al 1983 and Tulman et al 2009).

The only Ornithodorus ticks present in Australia are the inornate kangaroo tick (*O. gurneyi*) and the penguin tick (*O. capensis*), neither of which is known to feed on pigs. However, bloodsucking insects such as mosquitoes and biting flies (*Stomoxys spp.*) feeding on viraemic pigs can carry high levels of virus for 2 days and have been implicated in the mechanical spread of ASF within herds (as reviewed in Anonymous 2014a).

Table 3. The potential role of various species of arthropods in the epidemiology of ASF.

Species	Result	Representative citation
Ornithodoros erraticus (also known as *O. marocanus)*	Found to be associated with geographical foci of infection in Spain, capable of transtadial but likely not transovarial passage.	(Sanchez-Botija 1963; Endris and Hess 1992; Endris et al 1992b; Endris and Hess 1994; Basto et al 2006)
Ornithodoros moubata porcinus (also known as *O. porcinus porcinus)*	Positive, passage in ticks confirmed transtadially, transovarially, and venereally.	Numerous e.g. (Plowright et al 1969; Kleiboeker and Scoles 2001)
Amblyomma americanum	Maintain virus in adults and nymphs for 4-7 days after engorging, but not carried through the subsequent nymph moults and it was not transmitted vertically to the eggs and larvae of the infected females. When allowed to reefed, infection was not transferred to healthy swine.	(Groocock et al 1980)
Amblyomma cajennense	Same as *Amblyomma americanum* above.	(Groocock et al 1980)
Ornithodoros coriaceus	Adult females did not show transovarial passage but infected nymphs did maintain virus for 77-118 days through a moulting stage and did transfer the virus to healthy swine.	(Groocock et al 1980; Hess et al 1987)
Ornithodoros turicata	Adult stage able to become infected and transmit infection to other pigs.	(Hess et al 1987)
Ornithodoros parkeri	Adult stage able to become infected and transmit infection to other pigs.	(Hess et al 1987)
Ornithodoros puertoricensis	Adult and nymph stages able to transmit virus to pigs, virus is passed transtadially.	(Endris et al 1991, 1992a)
Haematopinus suis (the hog louse)	Variable, apparently able to transmit virus when pig is heavily infested.	(Montgomery 1921; Heuschele and Coggins 1965a; Sanchez and Badiola 1966)
Auchmeromyia spp. (flies, larvae, and pupae) Lice Phlebotomines *Culicoides spp.* *Simulium spp.* Fleas	Collected in or near warthog burrows known to be inhabited by infected warthogs. All were negative for ASF virus when tested by virus isolation.	(Plowright 1977, and reviewed by Thomson 1985)

1.5 Diagnostic criteria

1.5.1 Case definition

For the purposes of the AUSVETPLAN disease strategy manual for ASF, the case definition for ASF is (Anonymous 2014a):

- A confirmed laboratory diagnosis (for the index case), with or without clinical or pathological signs; or
- Clinical signs in a susceptible animal after an outbreak has been confirmed and for the duration of the outbreak.

1.5.2 Clinical signs

Until 1962, most infections of ASF virus in domestic swine were associated with severe disease and nearly 100% mortality. However, since that time increasing numbers of experimental and field infections resulted in less severe clinical signs including fever and persistent or recurring viraemia in surviving pigs, though death often followed within a few weeks (Detray 1957; Coggins et al 1968). Lesions in these cases included fibrinous pericarditis and arthritis, pleuropneumonia, hyperplastic and haemorrhagic lymph nodes, and cutaneous ulcerations (Moulton et al 1975).

In an early comparison of the clinical signs and pathology produced by different viral isolates, pigs were experimentally infected with either highly virulent (Lisbon 60) or less virulent (Brazil 1978) ASF virus isolates (Mebus et al 1978). After infection with the more severe isolate, pigs generally continued to eat but had high fever by two days PI and most pigs had died by seven days PI. On post-mortem examination, spleens were on average four-times their normal size and friable, internal lymph nodes (especially hepatogastric and renal nodes) were enlarged and dark reddish-black in colour, and peri-renal oedema was noted as were petechial haemorrhages on the kidneys and serosal surfaces of organs throughout the gastrointestinal tract. In contrast, pigs infected with the less virulent Brazilian 1978 isolate stayed in generally good health until between 8 and 12 days PI when they became progressively less active; most pigs continued to eat during this period. Despite a good clinical appearance most pigs had detectable fever by two days PI. Though the progression of the disease appeared slower and less severe, half of the pigs died between 14 and 24 days PI. Those pigs that did survive generally returned to normal beginning about 20 days PI. In the pigs that died, post-mortem lesions closely resembled those of the pigs that had been infected with the Lisbon strain. Viraemia was detected in both experiments by the second day PI and generally continued until at least 20 days PI. Authors specified raised concerns about disease caused by the low virulence strains because:

- Deaths were not tightly clustered in time as would be expected for a highly contagious exotic disease.
- The isolates caused lower mortality and therefore in a field situation, may not be recognized as an early ASF outbreak and thus prompt post-mortem examination.
- Pigs that died in the more chronic phase of infection (two to four weeks PI) had less indicative lesions (pleuritis, necrotic areas in lungs, pericarditis, enlarged lymph nodes, etc.) and therefore ASF may be incorrectly excluded.
- Tissue samples from chronic cases were less likely to have detectable levels of antigen.
- Survivors may harbour virus for extended period making them a risk for further direct or indirect (contaminated meat) transmission.

In a similar experiment using a moderate virulence ASF isolate from Malta in 1978, the clinical signs and gross pathology were indistinguishable from classical swine fever (CSF) (Wilkinson et al 1981).

When 20-30 kg pigs were infected by contact with donor pigs infected with this virus, fever was the earliest sign observed in all pigs (median time to onset of fever was 3.7 days PI). In one instance, a pig became infected after contact with a donor that had been infected 22 days previously. Temperatures peaked from 41-42C (mean time to peak fever was 3 days after the onset of fever) and high fevers tended to persist until death. The first occurrence of viraemia tended to precede pyrexia by approximately one day. Loss of appetite was variable but seemed to correlate with the occurrence of fever. Greater than 90% of pigs in the experiment died with most expiring between 9 and 11 days post-exposure (PE) which was approximately six to nine days after the onset of fever. Nasal discharge (sometimes with blood) was common though petechial or ecchymotic haemorrhage of skin was not a common finding. Diarrhoea was also an uncommon occurrence in this study. At post-mortem examination, though nearly all pigs had ascites, petechial haemorrhages on the surface of organs (except for the kidneys) tended to be uncommon. The spleen of most pigs was slightly enlarged and considerably darker than normal. Pneumonic lesions were inconsistent though many pigs had excess pericardial fluid and haemorrhages on the surface of the heart. The most frequently observed lesion was haemorrhagic lymph nodes, particularly those nodes in the abdominal cavity such as the gastro-hepatic nodes. There was substantial variation in the severity of lymph node lesions within a pig. The authors described the clinical picture as very similar to reports from the Brazilian outbreak in the same year and that neither the clinical signs nor gross lesions associated with infection by the Malta 1978 isolate could be easily differentiated from CSF.

Historically, ASF presented as a peracute infection of domestic pigs with mortality rates approaching 100%. (Montgomery 1921). However, over a number of decades of the virus being passaged through both feral and domestic pigs, the clinical picture has become more variable and is often described under four headings: peracute, acute, subacute ,and chronic (Wardley et al 1983).

Peracute form

In the peracute form, pigs may die abruptly with a near absence of clinical signs though severe lesions (notably haemorrhage) can often be seen on post-mortem examination.

Acute form

In the acute form of ASF, high fever is generally the first symptom noticed followed by anorexia, weakness, redness and/or haemorrhages of the skin, occasional diarrhoea or vomiting, increased respiratory rate, and eventual death in a few days to a week. Haemorrhage of various organs is again a common lesion, along with excess abdominal fluid in this acute form of the disease.

Subacute form

Subacute infections are characterised by fluctuating bouts of fever (sometimes high) accompanied by anorexia and loss of body condition. Though mortality rates may vary, death is often the final stage of the subacute form of ASF. Lesions are similar to those described for the acute form of the disease.

Chronic form

The clinical picture associated with chronic ASF can be quite variable. Over a course of weeks to months, affected pigs can show a range of symptoms including anorexia, loss of body condition, arthritis, skin lesions, and eventually death. In addition to lesions described above, pigs succumbing to the chronic form of ASF may include fibrinous pericarditis, pleuritis, and pneumonia. The clinical form of ASF that predominates in an outbreak is likely related to the viral strain, host factors (including genetics), and any prior exposure to the agent.

Abortion can occur during the acute stages of ASF (McDaniel 1979; Schlafer and Mebus 1984, 1987). Abortion can occur at any stage of gestation but tends to occur soon after the peak of viremia, one to two weeks post-infection (PI). Virus can be recovered from both maternal and fetal aborted tissues.

1.5.3 Pathology

1.5.3.1 Gross lesions

ASF virus replicates primarily in cells of the reticulo-endothelial system (Moulton and Coggins 1968b; Enjuanes et al 1977; Wardley and Wilkinson 1978; Wardley et al 1979) and therefore most pathology is associated with tissues rich in these cellular populations including lymph nodes, bone marrow, spleen, liver, and kidney. As the disease develops into more chronic stages, additional pathology due to the secondary effects from disseminated intravascular coagulopathy, immune dysfunction, and generalized loss of homeostasis, a more generalized form of the disease occurs.

Acute form (as reviewed in Anonymous 2014a)

Findings include:

- Enlarged and haemorrhagic lymph nodes, often resembling blood clots; the gastrohepatic, renal, mesenteric and submandibular lymph nodes are most often affected
- Enlarged spleen (2–3 times its normal size), which may be necrotic, dark, friable or pulpy
- Haemorrhages in almost any organ; they are most commonly seen on serosal membranes and in kidneys (as subcapsular petechiae), heart, urinary bladder, lung and gall bladder
- Septal oedema of lungs, resulting in prominent interlobular septa
- Fluid in body cavities.

Subacute form (as reviewed in Anonymous 2014a)

Findings are more variable than for the acute form and include:

- Lymph node and renal haemorrhage
- Enlarged but not congested spleen
- Lobular consolidation of cranial lung lobes
- Haemorrhage of the intestinal lining, lymph nodes and kidney.

Chronic form (as reviewed in Anonymous 2014a)

Findings include:

- Enlarged lymph nodes
- Fibrinous pericarditis and pleurisy
- Lobular consolidation of lungs, which may progress to lobular necrosis
- Small, hard, nodular white masses in lungs
- Arthritis
- Cutaneous ulcers
- Poor body condition.

1.5.3.2 Microscopic lesions (histopathology)

Infection with ASF virus results in dramatic changes to differential white blood cell counts and ratios (Wardley and Wilkinson 1977; and as reviewed by Tulman et al 2009). Studies have shown lymphocyte numbers may decrease by one-third to one-half by 2-4 days PI and neutrophil numbers increase by 2-4 times during the same period. The RBC count remains relatively unchanged during infection. While virus is associated with all cellular and non-cellular fractions of blood, there is strong evidence that the

virus is most strongly associated with erythrocytes. Approximately one to two viruses are associated with each RBC which is not thought to be a high enough concentration to cause complement-mediated RBC membrane rupture.

The virus replicates and causes cytolysis, primarily in the cells of the reticulo-endothelial system but it also appears to replicate in platelets or megakaryocytes. The haemorrhage associated with ASF is a result of the combined effect of thrombocytopenia, defective clot formation, and vascular leakage due to damaged endothelial cells (Neser et al 1986; Rodriguez et al 1996) and pigs that die acutely from the disease probably succumb to disseminated intravascular coagulopathy (Pan et al 1988). Virus isolates can be grouped into haemadsorbing and non-haemadsorbing types based on their ability to cause erythrocytes to adsorb to the surface of infected cells in porcine bone marrow or leucocyte cultures (Malmquist and Hay 1960; Coggins 1968; Pini and Wagenaar 1974; Vigario et al 1974; Pan and Hess 1985); this feature is not strictly correlated with virulence. Even amongst plaque-purified clones of ASF virus, epitopic diversity exists which likely contributes to some aspects of the immunopathology that are associated with the infection such as hypergammaglobulinaemia, persistent infection in the presence of ASF virus-specific antibodies, and an inefficient neutralizing antibody response (Pan et al 1988).

In the first one to two weeks PI, ASF virus is widely distributed amongst a number of tissues but particularly those containing high numbers of cells from the reticulo-endothelial system including lymph nodes, spleen, and liver (in their respective fixed and circulating macrophage populations), lung (alveolar, intravascular, and interstitial macrophages), and macrophages within the kidney interstitium (Fernandez et al 1992a; Fernandez et al 1992b). Some subpopulations of peripheral blood mononuclear leukocytes (macrophage/monocytes) have been shown by PCR to remain persistently infected for more than 500 days PI though this may not represent the presence of infectious virus (Carrillo et al 1994). Infection with ASF virus has also been shown to have dramatic effects on cells within the bone marrow (Gomez-Villamandos et al 1997). Viral replication has been detected in megakaryocytes, endothelial cells, and pericytes of the bone marrow by seven days PI which likely contribute to the lymphopaenia, neutrophilia (with left shift), increased erythropoiesis, and temporary thrombocytopenia that have been described with ASF (Detray and Scott 1957). In addition to direct cell death by necrosis as a result of infection with the virus, more recent studies have confirmed that a substantial amount of lymphocyte destruction is a result of apoptosis rather than the direct effects of infection (Oura et al 1998a). The apoptosis contributes not only to disruption of cell and organ function, it also has the effect of inducing a diminished immune response and enabling the virus sufficient time to produce a product infection more likely of being transmitted to a subsequent host. Recent detailed reviews of the pathogenesis of the virus are available (Blome et al 2013).

Given the variation in clinical signs produced by infection with different ASF virus isolates, several authors have attempted to develop a standardized method for measuring and expressing virulence in an effort to make comparisons between isolates and experimental studies. After controlled experiments with seventeen different isolates (13 haemadsorbing and four non-haemadsorbing), one author proposed a method for classifying the viruses into three groups based on the number of 50% haemadsorption units (HA_{50}) required to produce one LD_{50} in pigs, the number of HA_{50} required to produce one 50% pig infectious dose (PID_{50}), and the number of PID_{50} required for each LD_{50} (Pan and Hess 1984). Another author has recently proposed use of a more comprehensive scoring system using both clinical and histopathology severity scores (Galindo-Cardiel et al 2013).

1.5.4 Differential diagnosis

Differential diagnoses for ASF have been proposed (Kleiboeker 2002):

- Classical swine fever
- Aujeszky's disease
- Septicaemic salmonellosis
- Erysipelas
- Pasteurellosis
- Streptococcosis
- *Actinobacillus pleuropneumonia*
- *Haemophilus suis* infection
- Porcine reproductive and respiratory syndrome
- Porcine dermatitis and nephropathy syndrome
- Pseudorabies
- Mulberry heart disease (vitamin E and selenium deficiency)
- Eperythrozoonosis
- Any cause of generalized septicaemic or haemorrhagic condition
- Other causes of viral encephalomyelitis
- Coumarin poisoning
- Salt poisoning

Substantial delays have occurred in initial diagnoses of ASF in countries where CSF is endemic. ASF was not diagnosed until 4 weeks after the initial infection in both Belgium (1985) and the Netherlands (1986), which both have comprehensive, competent veterinary services.

1.5.5 Laboratory tests

1.5.5.1 Samples required

Polymerase chain reaction has become the standard test for detection of ASF virus antigen in suspect cases. Whole blood in EDTA, serum, faeces, and nasal swabs are all useful in establishing an ante-mortem diagnosis of ASF in pigs. When post-mortem samples are available, a full complement of fresh and formalin-fixed tissue samples (including tonsils) should be collected for submission to a laboratory.

1.5.5.2 Transport of specimens

Standard good practice for fresh, frozen, and formalin-fixed tissues are sufficient for transport of samples related to suspect ASF cases.

1.5.5.3 Laboratory diagnosis

A wide variety of antigen detection and serological (antibody-based) diagnostic tools have been developed for ASF. Because of their high numbers of infected cells, samples of blood, lymph node, spleen, liver, and tonsil are the preferred specimens for laboratory diagnosis of ASF. Many procedures have been described and reviewed (Oura et al 2013) for virus antigen detection including the traditional haemadsorption test (Malmquist and Hay 1960), radioimmunoassay (Crowther et al 1979; Wardley and Wilkinson 1980), direct immunofluorescence (Colgrove et al 1969), polymerase chain reaction (Aguero et al 2003; King et al 2003; Aguero et al 2004), isothermal amplification assays (Hjertner et al 2005; James et al 2010), antigen capture ELISA (Hutchings and Ferris 2006), and immunohistochemistry and *in situ* hybridisation (Oura et al 1998b).

ASF virus was first propagated in cell culture in 1960 and the topic has been previously reviewed (Malmquist and Hay 1960; Malmquist 1962; Wardley et al 1983). Some but not all strains of ASF virus can be grown in cell culture. The virus generally causes a cytopathic effect and induces haemadsorption.

Polymerase chain reaction (PCR) is probably the most widely used antigen detection method in use for ASF virus today. In addition to the protocol for PCR detection of ASF virus prescribed by the OIE (Anonymous 2014b), additional protocols with potentially improved performance for detection of the virus are available (Tignon et al 2011). These tests are highly sensitive and it appears that some PCR tests on tonsil samples can detect infection two to four days prior to the onset of clinical disease (Zsak et al 2005). A modification to the PCR technique has been validated for the extraction and detection of ASF virus antigen in blood that has been collected on filter paper (Michaud et al 2007). Using this technique, dried blood samples on filter paper can be stored between 22 and 37C for up to nine months and remain suitable for analysis by PCR or for phylogenetic sequencing.

The use of serological tests for diagnosis of ASF has recently been reviewed in detail (Cubillos et al 2013; Gallardo et al 2013). A number of testing platforms have been developed over the years but currently the OIE relies on ELISA as a screening test with confirmatory testing by either IFA or immunoblotting (Anonymous 2014b). However, given the extent of antigenic variation among isolates in particular amongst genotypes circulating in south and east Africa, and the current genotype 2 isolates circulating in central Asia and eastern Europe, investigators have proposed modifications to the OIE protocol that are likely to improve the sensitivity of serodiagnosis for ASF. Other investigators have expanded serodiagnosis to include development of ELISA and immunoperoxidase techniques for detection of antibodies against ASF virus present in oral fluids (Mur et al 2013)

1.6 Resistance and immunity

1.6.1 Innate and passive immunity

Hysterectomy-derived neonatal piglets have been used to determine the ability of passively acquired antibodies (colostrum or serum derived) to protect young piglets from infection with ASF virus (Schlafer et al 1984b). Neonatal piglets were fed a single dose of colostrum (or serum) from an ASF-recovered sow at two-hours of age. Compared to controls, these piglets had lower levels of viremia and substantially improved mortality when exposed to virulent virus though infection was not prevented. In related work, six young gilts (30 to 80 kg bodyweight) were inoculated with a moderate virulence ASF virus isolate (Schlafer et al 1984a). One of the gilts was bred 58 days later then euthanized on Day 90 of gestation. The remaining five gilts were mated 368 to 419 days PI and allowed to farrow normally. All sows became clinically ill after inoculation. Virus could be recovered from the blood of most sows for five to six weeks PI. When used to inoculate young naïve piglets, the fetal tissues collected from the sow euthanized on Day 90 of gestation did not cause infection nor were the tissues virus positive when tested by FA. However, one piglet inoculated with tissues harvested from this sow did become infected. Piglets born to the sows that were allowed to farrow normally were not infected at the time of birth and these sows remained free of virus during the peri-parturient period. Pigs in one of these litters were inoculated at seven weeks of age (after weaning) with a homologous strain of ASF virus. All but one of these piglets became infected after inoculation but with only mild clinical signs. There was no evidence in any of the sows that transplacental infection occurred.

In growing pigs, the efficacy of passive immunization to protect against infection with ASF virus has also been studied (Wardley et al 1985). Using heat-inactivated antiserum collected from pigs that had been hyperimmunized with ASF virus, pigs were passively immunised by intraperitoneal injection with 500 ml of antiserum. Similar to previous work, passive immunisation did not prevent infection but the treatment was able to reduce the level of pyrexia, severity of clinical signs and viremia, and increase survival time. These effects were thought to be mediated through an effect of antibody dependent cell-mediated cytotoxicity. Similar results have been shown by other authors (Onisk et al 1994).

1.6.2 Active immunity

Since the late 1960s, strains of ASF virus less virulent to domestic swine have appeared. With less virulent strains, the potential for long term carriers of the virus in recovered swine has increased; their occurrence and lesions have been previously described (Detray 1957; Moulton and Coggins 1968a; Moulton et al 1975). The characteristics of asymptomatic carrier pigs have been investigated using survivors from pigs that had been experimentally infected with either Brazilian or Dominican Republic low virulence strains of the virus (Mebus and Dardiri 1980). Specifically, a study was designed to determine their ability to transmit the virus to naïve contact pigs and to understand if they were resistant to infection after inoculation with homologous or heterologous strains of the virus. Naïve pigs placed in direct contact with the principal pigs (recovered) for 14 days did not become infected. However, naïve pigs fed or inoculated with tissues and serum from the principals did become infected. This is significant as at the time of tissue harvest, the principals were normal in all clinical respects and would have passed slaughter inspection. Additional principal pigs were inoculated with the highly virulent Lisbon 60 strain. Neither the Brazilian nor Dominican Republic principals became clinically ill though a transient viremia was detected in the Dominican Republic principals.

Recovered pigs commonly have high levels of complement-fixing antibody in serum, despite being chronically infected with the virus (De Boer et al 1972). Often, virus and antibodies are found concurrently in the blood of chronically infected pigs. Various classes of antibody are produced in response to infection and while they are specifically associated with the virus, their immunologic significance is unclear hence one of the difficulties in vaccine development. Even after repeated inoculation, guinea pigs, rabbits, and lambs also did not produce neutralizing antibodies. Cross-protection is not assured between isolates.

Though components of both humoral and cell-mediated immunity are activated after infection with ASF virus (Takamatsu et al 2013), neither type of response is completely understood and given the highly lethal nature of the disease tends not to be highly effective (Sanchez-Vizcaino et al 1981). Circulating lymphopaenia is a common finding as early as 7 days PI with T-lymphocytes being more severely affected than B-lymphocytes. Viraemia is often concurrent with high levels of circulating antibody; the level of antibody can be very high but is not necessarily correlated with protection or severity of the disease.

1.7 Vaccination and/or treatment of infected animals

As a viral disease, few options exist for direct treatment of the infection outside of supportive care and perhaps control of secondary bacterial infections with antibacterial compounds. However, based on earlier work with vaccinia virus (similar in some important respects to ASF virus), the effect of the antibiotic rifampin on ASF virus was investigated (Dardiri et al 1971). Rifampin was found to inhibit CPE and viral replication of ASF virus in culture suggesting it may be suitable for further experimental work as a direct therapy for ASF or as part of vaccine development studies.

Numerous efforts have been undertaken to develop an effective vaccine against ASF (Coggins 1974; Forman et al 1982; Kihm et al 1987; Mebus 1988) but to-date no effective experimental or commercial products are available. Some vaccination preparations (and recovery from natural infection) do provide partial protection from re-infection with a homologous strain but little protection against heterologous viruses has been demonstrated (Ruiz-Gonzalvo et al 1983; Hamdy and Dardiri 1984; Borca et al 1994; Gomez-Puertas et al 1996; King et al 2011).

References

Aguero M, Fernandez J, Romero L, Sanchez Mascaraque C, Arias M, Sanchez-Vizcaino JM. Highly sensitive PCR assay for routine diagnosis of African swine fever virus in clinical samples. *Journal of Clinical Microbiology* 41, 4431-4, 2003

Aguero M, Fernandez J, Romero LJ, Zamora MJ, Sanchez C, Belak S, Arias M, Sanchez-Vizcaino JM. A highly sensitive and specific gel-based multiplex RT-PCR assay for the simultaneous and differential diagnosis of African swine fever and Classical swine fever in clinical samples. *Veterinary Research* 35, 551-63, 2004

Alexander FC. Experiences with African swine fever in Haiti. *Annals of the New York Academy of Sciences* 653, 251-6, 1992

Allaway EC, Chinombo DO, Edelsten RM, Hutchings GH, Sumption KJ. Serological study of pigs for antibody against African swine fever virus in two areas of southern Malawi. *Revue Scientifique et Technique* 14, 667-76, 1995

Anderson EC, Hutchings GH, Mukarati N, Wilkinson PJ. African swine fever virus infection of the bushpig (Potamochoerus porcus) and its significance in the epidemiology of the disease. *Veterinary Microbiology* 62, 1-15, 1998

*__Anonymous.__ African swine fever, factsheet. www.cfsph.iastate.edu/Factsheets/pdfs/african_swine_fever.pdf (accessed October 31, 2014). Center for Food Security and Public Health, Ames, Iowa, USA, 2006

__Anonymous. Disease strategy: African swine fever (Version 4.0). *Primary Industries Ministerial Council*, Canberra, ACT, Australia, 2014a

*__Anonymous.** Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2014. Chapter 2.8.1 African swine fever (Version adopted by the World Assembly of Delegates of the OIE in May 2012). http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.01_ASF.pdf (accessed October 30, 2014). World Organisation for Animal Health, Paris, France, 2014b

Basto AP, Nix RJ, Boinas F, Mendes S, Silva MJ, Cartaxeiro C, Portugal RS, Leitao A, Dixon LK, Martins C. Kinetics of African swine fever virus infection in Ornithodoros erraticus ticks. *Journal of General Virology* 87, 1863-71, 2006

Bech-Nielsen S, Fernandez J, Martinez-Pereda F, Espinosa J, Perez Bonilla Q, Sanchez-Vizcaino JM. A case study of an outbreak of African swine fever in Spain. *British Veterinary Journal* 151, 203-14, 1995

Blasco R, Aguero M, Almendral JM, Vinuela E. Variable and constant regions in African swine fever virus DNA. *Virology* 168, 330-8, 1989

Blome S, Gabriel C, Beer M. Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Research* 173, 122-30, 2013

Borca MV, Irusta P, Carrillo C, Afonso CL, Burrage T, Rock DL. African swine fever virus structural protein p72 contains a conformational neutralizing epitope. *Virology* 201, 413-8, 1994

Carrillo C, Borca MV, Afonso CL, Onisk DV, Rock DL. Long-term persistent infection of swine monocytes/macrophages with African swine fever virus. *Journal of Virology* 68, 580-3, 1994

Coggins L. Segregation of a nonhemadsorbing African swine fever virus in tissue culture. *Cornell Veterinarian* 58, 12-20, 1968

Coggins L, Moulton JE, Colgrove GS. Studies with hinde attenuated African swine fever virus. *Cornell Veterinarian* 48, 525-40, 1968

Coggins L. African swine fever virus. Pathogenesis. *Progress in Medical Virology* 18, 48-63, 1974

Colgrove GS, Haelterman EO, Coggins L. Pathogenesis of African swine fever in young pigs. *American Journal of Veterinary Research* 30, 1343-59, 1969

Costard S, Jones BA, Martinez-Lopez B, Mur L, de la Torre A, Martinez M, Sanchez-Vizcaino F, Sanchez-Vizcaino JM, Pfeiffer DU, Wieland B. Introduction of African swine fever into the European Union through illegal importation of pork and pork products. *PLoS ONE* 8, e61104, 2013a

Costard S, Mur L, Lubroth J, Sanchez-Vizcaino JM, Pfeiffer DU. Epidemiology of African swine fever virus. *Virus Research* 173, 191-7, 2013b

Crowther JR, Wardley RC, Wilkinson PJ. Solid-phase radioimmunoassay techniques for the detection of African swine fever antigen and antibody. *Journal of Hygiene* 83, 353-61, 1979

Cubillos C, Gómez-Sebastian S, Moreno N, Nuñez MC, Mulumba-Mfumu LK, Quembo CJ, Heath L, Etter EMC, Jori F, Escribano JM, Blanco E. African swine fever virus serodiagnosis: A general review with a focus on the analyses of African serum samples. *Virus Research* 173, 159-67, 2013

Dardiri AH, Bachrach HL, Heller E. Inhibition by rifampin of African swine fever virus replication in tissue culture. *Infection and Immunity* 4, 34-6, 1971

De Boer CJ, Pan IC, Hess WR. Immunology of African swine fever. *Journal of the American Veterinary Medical Association* 160, 528-32, 1972

de Carvalho Ferreira HC, Backer JA, Weesendorp E, Klinkenberg D, Stegeman JA, Loeffen WL. Transmission rate of African swine fever virus under experimental conditions. *Veterinary Microbiology* 165, 296-304, 2013a

de Carvalho Ferreira HC, Weesendorp E, Quak S, Stegeman JA, Loeffen WL. Quantification of airborne African swine fever virus after experimental infection. *Veterinary Microbiology* 165, 243-51, 2013b

Detray DE. Persistence of viremia and immunity in African swine fever. *American Journal of Veterinary Research* 18, 811-6, 1957

Detray DE, Scott GR. Blood changes in swine with African swine fever. *American Journal of Veterinary Research* 18, 484-90, 1957

Dixon LK, Abrams CC, Bowick G, Goatley LC, Kay-Jackson PC, Chapman D, Liverani E, Nix R, Silk R, Zhang F. African swine fever virus proteins involved in evading host defence systems. *Veterinary Immunology and Immunopathology* 100, 117-34, 2004

Dorman AE. The control of African swine fever in Kenya. *Bulletin de l Office International des Epizooties* 63, 807-23, 1965

Edelsten RM, Chinombo DO. An outbreak of African swine fever in the southern region of Malawi. *Revue Scientifique et Technique* 14, 655-66, 1995

EFSA Panel on Animal Health and Welfare (AHAW). Scientific Opinion on African Swine Fever. *EFSA Journal* 8, 149, 2010

EFSA Panel on Animal Health and Welfare (AHAW). Scientific Opinion on African Swine Fever. *EFSA Journal* 12, 77, 2014

Endris RG, Haslett TM, Hess WR. Experimental transmission of African swine fever virus by the tick Ornithodoros (Alectorobius) puertoricensis (Acari: Argasidae). *Journal of Medical Entomology* 28, 854-8, 1991

Endris RG, Haslett TM, Hess WR. African swine fever virus infection in the soft tick, Ornithodoros (Alectorobius) puertoricensis (Acari: Argasidae). *Journal of Medical Entomology* 29, 990-4, 1992a

Endris RG, Hess WR. Experimental transmission of African swine fever virus by the soft tick Ornithodoros (Pavlovskyella) marocanus (Acari: Ixodoidea: Argasidae). *Journal of Medical Entomology* 29, 652-6, 1992

Endris RG, Hess WR, Caiado JM. African swine fever virus infection in the Iberian soft tick, Ornithodoros (Pavlovskyella) marocanus (Acari: Argasidae). *Journal of Medical Entomology* 29, 874-8, 1992b

Endris RG, Hess WR. Attempted transovarial and venereal transmission of African swine fever virus by the Iberian soft tick Ornithodoros (Pavlovskyella) marocanus (Acari: Ixodoidea: Argasidae). *Journal of Medical Entomology* 31, 373-81, 1994

Enjuanes L, Cubero I, Vinuela E. Sensitivity of macrophages from different species to African swine fever (ASF) virus. *Journal of General Virology* 34, 455-63, 1977

Farez S, Morley RS. Potential animal health hazards of pork and pork products. *Revue Scientifique et Technique* 16, 65-78, 1997

Fasina FO, Agbaje M, Ajani FL, Talabi OA, Lazarus DD, Gallardo C, Thompson PN, Bastos AD. Risk factors for farm-level African swine fever infection in major pig-producing areas in Nigeria, 1997-2011. *Preventive Veterinary Medicine* 107, 65-75, 2012

Fernandez A, Perez J, Carrasco L, Bautista MJ, Sanchez-Vizcaino JM, Sierra MA. Distribution of ASFV antigens in pig tissues experimentally infected with two different Spanish virus isolates. *Zentralbl Veterinarmed B* 39, 393-402, 1992a

Fernandez A, Perez J, Carrasco L, Sierra MA, Sanchez-Vizcaino M, Jover A. Detection of African swine fever viral antigens in paraffin-embedded tissues by use of immunohistologic methods and polyclonal antibodies. *American Journal of Veterinary Research* 53, 1462-7, 1992b

Forman AJ, Wardley RC, Wilkinson PJ. The immunological response of pigs and guinea pigs to antigens of African swine fever virus. *Archives of Virology* 74, 91-100, 1982

Fowler ME. Husbandry and diseases of captive wild swine and peccaries. *Revue Scientifique et Technique* 15, 141-54, 1996

Galindo-Cardiel I, Ballester M, Solanes D, Nofrarias M, Lopez-Soria S, Argilaguet JM, Lacasta A, Accensi F, Rodriguez F, Segales J. Standardization of pathological investigations in the framework of experimental ASFV infections. *Virus Research* 173, 180-90, 2013

Gallardo C, Soler A, Nieto R, Carrascosa AL, De Mia GM, Bishop RP, Martins C, Fasina FO, Couacy-Hymman E, Heath L, Pelayo V, Martin E, Simon A, Martin R,

Okurut AR, Lekolol I, Okoth E, Arias M. Comparative evaluation of novel African swine fever virus (ASF) antibody detection techniques derived from specific ASF viral genotypes with the OIE internationally prescribed serological tests. *Veterinary Microbiology* 162, 32-43, 2013

Gibbs EP. Persistent viral infections of food animals: their relevance to the international movement of livestock and germ plasm. *Advances in Veterinary Science and Comparative Medicine* 25, 71-100, 1981a

Gibbs EPJ. Virus diseases of food animals : a world geography of epidemiology and control, Academic Press, London ; New York, 1981b

Gogin A, Gerasimov V, Malogolovkin A, Kolbasov D. African swine fever in the North Caucasus region and the Russian Federation in years 2007-2012. *Virus Research* 173, 198-203, 2013

Gomez-Puertas P, Rodriguez F, Oviedo JM, Ramiro-Ibanez F, Ruiz-Gonzalvo F, Alonso C, Escribano JM. Neutralizing antibodies to different proteins of African swine fever virus inhibit both virus attachment and internalization. *Journal of Virology* 70, 5689-94, 1996

Gomez-Villamandos JC, Bautista MJ, Carrasco L, Caballero MJ, Hervas J, Villeda CJ, Wilkinson PJ, Sierra MA. African swine fever virus infection of bone marrow: lesions and pathogenesis. *Veterinary Pathology* 34, 97-107, 1997

Groocock CM, Hess WR, Gladney WJ. Experimental transmission of African swine fever virus by Ornithodoros coriaceus, an argasid tick indigenous to the United States. *American Journal of Veterinary Research* 41, 591-4, 1980

Gulenkin VM, Korennoy FI, Karaulov AK, Dudnikov SA. Cartographical analysis of African swine fever outbreaks in the territory of the Russian Federation and computer modeling of the basic reproduction ratio. *Preventive Veterinary Medicine* 102, 167-74, 2011

Haas B, Ahl R, Bohm R, Strauch D. Inactivation of viruses in liquid manure. *Revue Scientifique et Technique* 14, 435-45, 1995

Hamdy FM, Dardiri AH. Clinical and immunologic responses of pigs to African swine fever virus isolated from the Western Hemisphere. *American Journal of Veterinary Research* 45, 711-4, 1984

Haresnape JM, Lungu SA, Mamu FD. A four-year survey of African swine fever in Malawi. *Journal of Hygiene* 95, 309-23, 1985

Haresnape JM, Lungu SA, Mamu FD. An updated survey of African swine fever in Malawi. *Epidemiology and Infection* 99, 723-32, 1987

Hess WR. African swine fever virus. *Virology Monographs* 9, 1-33, 1971

Hess WR, Endris RG, Haslett TM, Monahan MJ, McCoy JP. Potential arthropod vectors of African swine fever virus in North America and the Caribbean basin. *Veterinary Parasitology* 26, 145-55, 1987

Heuschele W, Coggins L. Studies on the transmission of African swine fever virus by arthropods. *Proc. US Livestock Sanit. Ass* 69, 94-100, 1965a

Heuschele WP, Coggins L. Isolation of African swine fever virus from a giant forest hog. *Bulletin of Epizootic Diseases of Africa* 13, 255–6, 1965b

Heuschele WP. Studies on the pathogenesis of African swine fever. I. Quantitative studies on the sequential development of virus in pig tissues. *Archiv fur Die Gesamte Virusforschung* 21, 349-56, 1967

Hjertner B, Meehan B, McKillen J, McNeilly F, Belak S. Adaptation of an Invader assay for the detection of African swine fever virus DNA. *Journal of Virological Methods* 124, 1-10, 2005

Howey EB, O'Donnell V, de Carvalho Ferreira HC, Borca MV, 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 Research* 178, 328-39, 2013

Hutchings GH, Ferris NP. Indirect sandwich ELISA for antigen detection of African swine fever virus: comparison of polyclonal and monoclonal antibodies. *Journal of Virological Methods* 131, 213-7, 2006

James HE, Ebert K, McGonigle R, Reid SM, Boonham N, Tomlinson JA, Hutchings GH, Denyer M, Oura CA, Dukes JP, King DP. Detection of African swine fever virus by loop-mediated isothermal amplification. *Journal of Virological Methods* 164, 68-74, 2010

Jori F, Bastos AD. Role of wild suids in the epidemiology of African swine fever. *EcoHealth* 6, 296-310, 2009

Kihm U, Ackerman M, Mueller H, Pool R. Approaches to vaccination. In: Becker Y (ed) *African swine fever*. Pp 127-44. Kluwer Academic Publishers, Boston, MA, USA, 1987

King DP, Reid SM, Hutchings GH, Grierson SS, Wilkinson PJ, Dixon LK, Bastos AD, Drew TW. Development of a TaqMan PCR assay with internal amplification control for the detection of African swine fever virus. *Journal of Virological Methods* 107, 53-61, 2003

King K, Chapman D, Argilaguet JM, Fishbourne E, Hutet E, Cariolet R, Hutchings G, Oura CA, Netherton CL, Moffat K, Taylor G, Le Potier MF, Dixon LK, Takamatsu HH. Protection of European domestic pigs from virulent African isolates of African swine fever virus by experimental immunisation. *Vaccine* 29, 4593-600, 2011

Kleiboeker SB, Scoles GA. Pathogenesis of African swine fever virus in Ornithodoros ticks. *Anim Health Res Rev* 2, 121-8, 2001

Kleiboeker SB. Swine fever: classical swine fever and African swine fever. *Veterinary Clinics of North America. Food Animal Practice* 18, 431-51, 2002

Kovalenko J, Sidorov M, Burba L. Experimental investigations on African swine fever. *Bull. Off. Int. Epizoot* 63, 169-89, 1965

Krug PW, Larson CR, Eslami AC, Rodriguez LL. Disinfection of foot-and-mouth disease and African swine fever viruses with citric acid and sodium hypochlorite on birch wood carriers. *Veterinary Microbiology* 156, 96-101, 2012

Luther N, Majiyagbe K, Shamaki D, Lombin L, Antiagbong J, Bitrus Y, Owolodun O. Detection of African swine fever virus genomic DNA in a Nigerian red river hog (Potamochoerus porcus). *The Veterinary record* 160, 58, 2007

***MacDiarmid SC.** Importation into New Zealand of meat and meat products: A review of the risks to animal health. *New Zealand Ministry of Agriculture and Forestry*, Wellington, New Zealand, 1991

Malmquist WA, Hay D. Hemadsorption and cytopathic effect produced by African Swine Fever virus in swine bone marrow and buffy coat cultures. *American Journal of Veterinary Research* 21, 104-8, 1960

Malmquist WA. Propagation, modification, and hemadsorption of African swine fever virus in cell cultures. *American Journal of Veterinary Research* 23, 241-7, 1962

Malogolovkin A, Yelsukova A, Gallardo C, Tsybanov S, Kolbasov D. Molecular characterization of African swine fever virus isolates originating from outbreaks in the Russian Federation between 2007 and 2011. *Veterinary Microbiology* 158, 415-9, 2012

Mannelli A, Sotgia S, Patta C, Sarria A, Madrau P, Sanna L, Firinu A, Laddomada A. Effect of husbandry methods on seropositivity to African swine fever virus in Sardinian swine herds. *Preventive Veterinary Medicine* 32, 235-41, 1997

Mannelli A, Sotgia S, Patta C, Oggiano A, Carboni A, Cossu P, Laddomada A. Temporal and spatial patterns of African swine fever in Sardinia. *Preventive Veterinary Medicine* 35, 297-306, 1998

***McDaniel HA.** African swine fever. *Proceedings of 21st Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians* Pp 391-7, 1979

McKercher PD, Morgan DO, McVicar JW, Shuot NJ. Thermal processing to inactivate viruses in meat products. *Proceedings, Annual Meeting of the United States Animal Health Association* 84, 320-8, 1980

McKercher PD, Yedloutschnig RJ, Callis JJ, Murphy R, Panina GF, Civardi A, Bugnetti M, Foni E, Laddomada A, Scarano C, Scatozza F. Survival of Viruses in "Prosciutto di Parma" (Parma Ham). *Canadian Institute of Food Science and Technology Journal* 20, 267-72, 1987

McVicar JW. Quantitative aspects of the transmission of African swine fever. *American Journal of Veterinary Research* 45, 1535-41, 1984

Mebus C, Arias M, Pineda JM, Tapiador J, House C, Sánchez-Vizcaíno JM. Survival of several porcine viruses in different Spanish dry-cured meat products. *Food Chemistry* 59, 555-9, 1997

Mebus CA, Dardiri AH, Hamdy FM, Ferris DH, Hess WR, Callis JJ. Some characteristics of african swine fever viruses isolated from Brazil and the Dominican Republic. *Proceedings, Annual Meeting of the United States Animal Health Association*, 232-6, 1978

Mebus CA, Dardiri AH. Western hemisphere isolates of African swine fever virus: asymptomatic carriers and resistance to challenge inoculation. *American Journal of Veterinary Research* 41, 1867-9, 1980

Mebus CA. African swine fever. *Advances in Virus Research* 35, 251-69, 1988

Mebus CA, House C, Gonzalvo FR, Pineda JM, Tapiador J, Pire JJ, Bergada J, Yedloutschnig RJ, Sahu S, Becerra V, Sanchez-Vizcaino JM. Survival of foot-and-mouth disease, African swine fever, and hog cholera viruses in Spanish serrano cured hams and Iberian cured hams, shoulders and loins. *Food Microbiology* 10, 133-43, 1993

Mendes AM. A história de peste suína africana em Angola. *Revista Portuguesa das Ciencias Veterinarias* 89, 110–20, 1994

Michaud V, Gil P, Kwiatek O, Prome S, Dixon L, Romero L, Le Potier MF, Arias M, Couacy-Hymann E, Roger F, Libeau G, Albina E. Long-term storage at tropical temperature of dried-blood filter papers for detection and genotyping of RNA and DNA viruses by direct PCR. *Journal of Virological Methods* 146, 257-65, 2007

Michaud V, Randriamparany T, Albina E. Comprehensive phylogenetic reconstructions of African swine fever virus: proposal for a new classification and molecular dating of the virus. *PLoS ONE* 8, e69662, 2013

Montgomery RE. On a form of swine fever occurring in British East Africa (Kenya Colony). *Journal of Comparative Pathology and Therapeutics* 34, 159-91, 1921

Moulton J, Coggins L. Comparison of lesions in acute and chronic African swine fever. *Cornell Veterinarian* 58, 364-88, 1968a

Moulton J, Coggins L. Synthesis and cytopathogenesis of African swine fever virus in porcine cell cultures. *American Journal of Veterinary Research* 29, 219-32, 1968b

Moulton JE, Pan IC, Hess WR, DeBoer CJ, Tessler J. Pathologic features of chronic pneumonia in pigs with experimentally induced African swine fever. *American Journal of Veterinary Research* 36, 27-32, 1975

Moura JA, McManus CM, Bernal FE, de Melo CB. An analysis of the 1978 African swine fever outbreak in Brazil and its eradication. *Revue Scientifique et Technique* 29, 549-63, 2010

Mur L, Martinez-Lopez B, Martinez-Aviles M, Costard S, Wieland B, Pfeiffer DU, Sanchez-Vizcaino JM. Quantitative risk assessment for the introduction of African swine fever virus into the European Union by legal import of live pigs. *Transbound Emerg Dis* 59, 134-44, 2012a

Mur L, Martinez-Lopez B, Sanchez-Vizcaino JM. Risk of African swine fever introduction into the European Union through transport-associated routes: returning trucks and waste from international ships and planes. *BMC Vet Res* 8, 149, 2012b

Mur L, Gallardo C, Soler A, Zimmermman J, Pelayo V, Nieto R, Sanchez-Vizcaino JM, Arias M. Potential use of oral fluid samples for serological diagnosis of African swine fever. *Veterinary Microbiology* 165, 135-9, 2013

Neser JA, Phillips T, Thomson GR, Gainaru MD, Coetzee T. African swine fever. I. Morphological changes and virus replication in blood platelets of pigs infected with virulent

haemadsorbing and non-haemadsorbing isolates. *Onderstepoort Journal of Veterinary Research* 53, 133-41, 1986

Nigsch A, Costard S, Jones BA, Pfeiffer DU, Wieland B. Stochastic spatio-temporal modelling of African swine fever spread in the European Union during the high risk period. *Preventive Veterinary Medicine* 108, 262-75, 2013

Oganesyan AS, Petrova ON, Korennoy FI, Bardina NS, Gogin AE, Dudnikov SA. African swine fever in the Russian Federation: Spatio-temporal analysis and epidemiological overview. *Virus Research*, 2012

Onisk DV, Borca MV, Kutish G, Kramer E, Irusta P, Rock DL. Passively transferred African swine fever virus antibodies protect swine against lethal infection. *Virology* 198, 350-4, 1994

Oura CA, Powell PP, Parkhouse RM. African swine fever: a disease characterized by apoptosis. *Journal of General Virology* 79 (Pt 6), 1427-38, 1998a

Oura CA, Powell PP, Parkhouse RM. Detection of African swine fever virus in infected pig tissues by immunocytochemistry and in sity hybridisation. *Journal of Virological Methods* 72, 205-17, 1998b

Oura CA, Edwards L, Batten CA. Virological diagnosis of African swine fever--comparative study of available tests. *Virus Research* 173, 150-8, 2013

Pan IC, Shimizu M, Hess WR. Replication of African swine fever virus in cell cultures. *American Journal of Veterinary Research* 41, 1357-67, 1980

Pan IC, Hess WR. Virulence in African swine fever: its measurement and implications. *American Journal of Veterinary Research* 45, 361-6, 1984

Pan IC, Hess WR. Diversity of African swine fever virus. *American Journal of Veterinary Research* 46, 314-20, 1985

Pan IC, Whyard TC, Hess WR, Yuasa N, Shimizu M. Epitopic diversity of African swine fever virus. *Virus Research* 9, 93-106, 1988

Penrith ML, Thomson GR, Bastos AD, Phiri OC, Lubisi BA, Du Plessis EC, Macome F, Pinto F, Botha B, Esterhuysen J. An investigation into natural resistance to African swine fever in domestic pigs from an endemic area in southern Africa. *Revue Scientifique et Technique* 23, 965-77, 2004a

Penrith ML, Thomson GR, Bastos ADS. African swine fever. In: Coetzer JAW, Tustin RC (eds). *Infectious diseases of livestock*. Oxford University Press, Oxford, 2004b

Penrith ML, Vosloo W. Review of African swine fever: transmission, spread and control. *Journal of the South African Veterinary Association* 80, 58-62, 2009

Pini A, Wagenaar G. Isolation of a non-haemadsorbing strain of African swine fever (ASF) virus from a natural outbreak of the disease. *Veterinary Record* 94, 2-, 1974

Plowright W, Parker J. The stability of African swine fever virus with particular reference to heat and pH inactivation. *Archiv fur Die Gesamte Virusforschung* 21, 383-402, 1967

Plowright W, Parker J, Staple RF. The growth of a virulent strain of African swine fever virus in domestic pigs. *Journal of Hygiene* 66, 117-34, 1968

Plowright W, Parker J, Peirce MA. African Swine Fever Virus in Ticks (Ornithodoros moubata, Murray) collected from Animal Burrows in Tanzania. *Nature* 221, 1071-3, 1969

Plowright W, Perry CT, Peirce MA, Parker J. Experimental infection of the argasid tick, ornithodoros moubata porcinus, with African swine fever virus. *Archiv fur Die Gesamte Virusforschung* 31, 33-50, 1970

Plowright W, Perry C, Greig A. Sexual transmission of African swine fever virus in the tick, Ornithodoros moubata porcinus, Walton. *Research in Veterinary Science* 17, 106, 1974

***Plowright W.** Vector transmission of African swine fever virus. *Proceedings of Seminar on Hog cholera, Classical swine fever, and African swine fever: EUR 5904EN*. Hannover, Germany. (ed) Liess B) Pp 575-87. (Commission of the European Communities), 1977

Plowright W, Thomson GR, Neser JA. African swine fever. In: Coetzer JAW, Thomson GR, Tustin RC (eds). *Infectious diseases of livestock with special reference to Southern Africa*. Pp 568–99. Oxford University Press, Cape Town, South Africa, 1994

Randriamparany T, Grenier A, Tourette I, Maharavo Rahantamalala C, Rousset D, Lancelot R. Epidemiological Situation of African Swine Fever in Lake Alaotra Region (Madagascar) and Possible Consequences on the Organization of Disease Control and Surveillance. *Revue d'Elevage et de Medecine Veterinaire des Pays Tropicaux* 58, 15-20, 2005

Reichard RE. African swine fever in the Americas. *Proceedings, Annual Meeting of the United States Animal Health Association*, 226-31, 1978

Rodriguez F, Fernandez A, Martin de las Mulas JP, Sierra MA, Jover A. African swine fever: morphopathology of a viral haemorrhagic disease. *Veterinary Record* 139, 249-54, 1996

***Ruiz-Gonzalvo F, Carnero ME, Bruyel V.** Immunological responses of pigs to partially attenuated ASF and their resistance to virulent homologous and heterologous viruses. *Proceedings of FAO/CEC research seminar on African swine fever: EUR 8466EN*. Sassari, Sardinia, Italy. (ed) Wilkinson J) Pp 206-16. (Commission of the European Communities), 1983

Sanchez-Botija C. Reservorios del virus de la peste porcina africana. Investigacion del virus de la PPA en los artropodos mediante la prueba de la hemoadsorcion. *Bull. Off. Int. Epizoot* 60, 895-9, 1963

Sanchez-Vizcaino JM, Slauson DO, Ruiz-Gonzalvo F, Valero F. Lymphocyte function and cell-mediated immunity in pigs with experimentally induced African swine fever. *American Journal of Veterinary Research* 42, 1335-41, 1981

Sanchez-Vizcaino JM, Mur L, Martinez-Lopez B. African Swine Fever: An Epidemiological Update. *Transbound Emerg Dis*, 2012

Sanchez BC, Badiola C. Presence of the African swine pest virus in Haematopinus suis. *Bulletin-Office international des épizooties* 66, 699, 1966

Schlafer DH, McVicar JW, Mebus CA. African swine fever convalescent sows: subsequent pregnancy and the effect of colostral antibody on challenge inoculation of their pigs. *American Journal of Veterinary Research* 45, 1361-6, 1984a

Schlafer DH, Mebus CA. Abortion in sows experimentally infected with African swine fever virus: clinical features. *American Journal of Veterinary Research* 45, 1353-60, 1984

Schlafer DH, Mebus CA, McVicar JW. African swine fever in neonatal pigs: passively acquired protection from colostrum or serum of recovered pigs. *American Journal of Veterinary Research* 45, 1367-72, 1984b

Schlafer DH, Mebus CA. Abortion in sows experimentally infected with African swine fever virus: pathogenesis studies. *American Journal of Veterinary Research* 48, 246-54, 1987

Shirai J, Kanno T, Tsuchiya Y, Mitsubayashi S, Seki R. Effects of chlorine, iodine, and quaternary ammonium compound disinfectants on several exotic disease viruses. *Journal of Veterinary Medical Science* 62, 85-92, 2000

Stone SS, Heuschele WP. The role of the hippopotamus in the epizootiology of African swine fever (a survey of the incidence of African swine fever virus in hippopotami in Queen Elizabeth National Park, Uganda). *Bulletin of Epizootic Diseases of Africa* 13, 23-8, 1965

Stone SS, Hess WR. Effects of some disinfectants on African swine fever virus. *Applied Microbiology* 25, 115-22, 1973

Tabares E, Sanchez Botija C. Synthesis of DNA in cells infected with African swine fever virus. *Archives of Virology* 61, 49-59, 1979

Takamatsu HH, Denyer MS, Lacasta A, Stirling CM, Argilaguet JM, Netherton CL, Oura CA, Martins C, Rodriguez F. Cellular immunity in ASFV responses. *Virus Research* 173, 110-21, 2013

Taylor W, Best J, Couquhoun I. Absence of African swine fever from Nigerian warthogs. *Bulletin of animal health and production in Africa*, 1977

Thomas AD, Kolbe FF. The wild pigs of South Africa: their distribution and habits and their signficance as agricultural pest and carriers of disease. *Journal of South African Veterinary Medicine Association* 13, 1-11, 1942

Thomson GR. The epidemiology of African swine fever: the role of free-living hosts in Africa. *Onderstepoort Journal of Veterinary Research* 52, 201-9, 1985

Tignon M, Gallardo C, Iscaro C, Hutet E, Van der Stede Y, Kolbasov D, De Mia GM, Le Potier MF, Bishop RP, Arias M, Koenen F. Development and inter-laboratory validation study of an improved new real-time PCR assay with internal control for detection and laboratory diagnosis of African swine fever virus. *Journal of Virological Methods* 178, 161-70, 2011

Tulman ER, Delhon GA, Ku BK, Rock DL. African swine fever virus. *Current Topics in Microbiology and Immunology* 328, 43-87, 2009

Vigario JD, Terrinha AM, Moura Nunes JF. Antigenic relationships among strains of African swine fever virus. *Archiv fur Die Gesamte Virusforschung* 45, 272-7, 1974

Vinuela E. African swine fever virus. *Current Topics in Microbiology and Immunology* 116, 151-70, 1985

Wardley RC, Wilkinson PJ. The association of African swine fever virus with blood components of infected pigs. *Archives of Virology* 55, 327-34, 1977

Wardley RC, Wilkinson PJ. The growth of virulent African swine fever virus in pig monocytes and macrophages. *Journal of General Virology* 38, 183-6, 1978

Wardley RC, Hamilton F, Wilkinson PJ. The replication of virulent and attenuated strains of African swine fever virus in porcine macrophages. *Archives of Virology* 61, 217-25, 1979

Wardley RC, Wilkinson PJ. Detection of African swine fever virus antigen and antibody by radioimmunoassay. *Veterinary Microbiology* 5, 169-76, 1980

Wardley RC, de MAC, Black DN, de Castro Portugal FL, Enjuanes L, Hess WR, Mebus C, Ordas A, Rutili D, Sanchez Vizcaino J, Vigario JD, Wilkinson PJ, Moura Nunes JF, Thomson G. African Swine Fever virus. Brief review. *Archives of Virology* 76, 73-90, 1983

Wardley RC, Norley SG, Wilkinson PJ, Williams S. The role of antibody in protection against African swine fever virus. *Veterinary Immunology and Immunopathology* 9, 201-12, 1985

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. *Preventive Veterinary Medicine* 99, 4-14, 2011

Wilkinson PJ, Donaldson AI. Transmission studies with African swine fever virus. The early distribution of virus in pigs infected by airborne virus. *Journal of Comparative Pathology* 87, 497-501, 1977

Wilkinson PJ, Donaldson AI, Greig A, Bruce W. Transmission studies with African swine fever virus. Infections of pigs by airborne virus. *Journal of Comparative Pathology* 87, 487-95, 1977

Wilkinson PJ, Wardley RC, Williams SM. African swine fever virus (Malta/78) in pigs. *Journal of Comparative Pathology* 91, 277-84, 1981

Wilkinson PJ. Epidemiology of African swine fever. *Revue Scientifique et Technique* 5, 487-93, 1986

Wilkinson PJ, Pegram RG, Perry BD, Lemche J, Schels HF. The distribution of African swine fever virus isolated from Ornithodoros moubata in Zambia. *Epidemiology and Infection* 101, 547-64, 1988

Zsak L, Borca MV, Risatti GR, Zsak A, French RA, Lu Z, Kutish GF, Neilan JG, Callahan JD, Nelson WM, Rock DL. Preclinical diagnosis of African swine fever in contact-exposed swine by a real-time PCR assay. *Journal of Clinical Microbiology* 43, 112-9, 2005