African Swine Fever

Gap Analysis Report

November 2018

Global African Swine Fever
Research Alliance

The Global African Swine Fever Research Alliance (GARA) aims to expand ASF research collaborations worldwide and maximize the use of resources and expertise to achieve its five strategic goals:
1. To facilitate research collaborations and serve as a communication gateway for the global ASF research community.
2. To conduct strategic research to increase our understanding of ASF.
3. To develop the next generation of control measures and strategies for their application.
4. To determine social and economic impacts of the new generation of improved ASF control
5. To provide evidence to inform development of policies for safe trade of animals and animal products in ASF-endemic areas.

Additional information on the GARA and the work of the alliance can be found on the following website: http://www.ars.usda.gov/GARA

The purpose of this ASF Gap Analysis Report is to assess current scientific knowledge and the available countermeasures to effectively control and mitigate the impact of an ASF outbreak in countries experiencing outbreaks, and also support global control and eradication initiatives in ASF-endemic countries.

This ASF Gap Analysis Report is the compilation of four workshops organized by the GARA with the support of its partners.

To cite this report:
Global African Swine Fever Research Alliance (GARA) Gap Analysis Report. 2018.
(https://go.usa.gov/xPfWr**)**

CONTENTS

EXECUTIVE SUMMARY 5

GROUP PICTURES 8

GLOSSARY 11

INTRODUCTION 13

BACKGROUND 15
- GARA Gap Analysis Workshops 15
- Reference Material 15

DEFINITION OF THE THREAT 16
- Economic Impact 16
- Epidemiology 16
- Surveillance 16
- Biosecurity 17
- Depopulation 17
- Vaccines 17
- Diagnosis 18

GAP ANALYSIS 19
- Virology 19
- Pathogenesis 25
- Immunology 29
- Vaccines 33
- Diagnosis 36
- Epidemiology 44
- Surveillance 46
- Feral Swine and wild Suidae 47
- Tick Vector 47

COUNTERMEASURES ASSESSMENT 48
- ASSUMPTIONS 48
- DECISION MODEL 49
- Vaccines 51
- Diagnostics 52
- Disinfectants/Inactivation 55
- Acaricides 56
- Drugs 56
- Personal Protective Equipment (PPE) 56

RECOMMENDATIONS 57
- RESEARCH 57
- PREPAREDNESS 60

CONCLUSION 61

FIGURE 1: ASFV PHYLOGENETIC ANALYSIS 62
TABLE I: ASF OUTBREAKS 2007-2018 63
TABLE II: SUMMARY OF COMPLETE GENOMES AVAILABLE ON NCBI 65
TABLE III: ASF EXPERIMENTAL VACCINES 66
TABLE IV: DISINFECTANTS FOR INACTIVATING AFRICAN SWINE FEVER VIRUS 67
APPENDIX I: COUNTERMEASURES WORKING GROUP INSTRUCTIONS 69
APPENDIX II: EXPERIMENTAL VACCINES 70
APPENDIX III: DIAGNOSTICS FOR SURVEILLANCE 71
APPENDIX IV: DIAGNOSTICS FOR RESPONDING TO A DISEASE OUTBREAK 72
APPENDIX V: EXPERIMENTAL DIAGNOSTICS FOR SURVEILLANCE 73
APPENDIX VI: EXPERIMENTAL DIAGNOSTICS FOR RESPONDING TO A DISEASE OUTBREAK . . 74
APPENDIX VII: REPORT CONTRIBUTORS 75
REFERENCES 86

EXECUTIVE SUMMARY

The Global African Swine Fever Research Alliance (GARA) organized four scientific conferences from 2013 to 2016 to conduct gap analyses of our current knowledge and the available veterinary medical countermeasures to effectively control and mitigate the impact of a disease outbreak cause by African swine fever (ASF).

The key and consistent conclusion from these workshops is that although ASF has historically been confined to the continent of Africa, the risk of an introduction of ASF in Europe, North America, South America, Eurasia, or Asia is significant and would be economically devastating. African swine fever is one of the most complex viral disease affecting domestic pigs, wild boars, and wild suids. Soft ticks are considered a biological reservoir and transmission vector. African swine fever virus (ASFV) usually induces an unapparent infection in a variety of African warthog and bush pig populations.

Currently ASF is endemic in more than twenty sub-Saharan African countries and 16 new introductions have been declared since the report of an outbreak in the Caucasus region in 2007, affecting Georgia, Armenia, Azerbaijan, Russia, Baltic Sea countries, and Eastern and Central Europe. In 2018, ASF continued to spread towards Western Europe. And 2018, China reported its first ASF outbreak. The disease situation in these countries has not improved, thus increasing the ASF risk of spread towards other countries.

The initial expression of ASF in swine is variable and unpredictable due to the myriad of host factors and the broad diversity of virulence among ASF virus isolates. Viral mechanisms involved in induction of disease, tissue tropism, host range, and induction of immune responses are still not well understood. The disease occurs in several forms, ranging from acute lethal to chronic clinical disease. Thus, clinical diagnosis is almost impossible and has to be supplemented with reliable laboratory tools. Antibody response elicited by infection with highly virulent strains of the virus does not begin to appear to detectable levels until at least 7-14 days post infection, which can make early detection difficult and a challenge for surveillance programs. However, combining viral genome and antibody detection provides reliable diagnosis from the end of the incubation period, or even a little earlier.

The GARA determined that the following countermeasures were important but several weaknesses were identified.

Surveillance

Routine surveillance for early detection is the first line of defense against a disease outbreak. Rapid and accurate detection affects the time when control measures can be implemented and affects the extent of the disease outbreak. Viral strains of ASF can vary from low to highly virulent; and clinical signs range from persistent infections with no apparent signs in endemic countries to outbreaks of acute infection and severe disease in previously ASF-free countries. The initial expression of ASFV in swine in an ASF-free country like the U.S could be variable and unpredictable due to the myriad of factors including the epidemiology of ASF and the broad diversity of virulence among ASF virus isolates. Historically there have been at least

two surveillance programs in place: 1) a 'syndromic' surveillance program based on reporting of clinical signs and 2) a laboratory-based surveillance program that includes diagnostic testing of populations at risk. Recent experience with ASF outbreaks due to the smuggling of contaminated feed strongly warrants the onset of a new branch of testing and validating methods to detect ASFV in foods, food scraps, and agricultural processed products as part of the laboratory-based surveillance programs. However, caution with this kind of testing needs to be considered due to vulnerabilities associated with sampling size and the fact that a negative result does not tell you anything, while conversely a viral genome positive result does not provide sufficient information to judge the true risk of a possible introduction.

Depopulation

Depopulation is the primary countermeasure to reduce virus shedding and stop the spread of ASF virus. Minimum control measures will include depopulation of infected herds, surveillance and movement restriction within established control zones together with surveillance in herds that have been in contact with infected herds. Depopulation of contact herds and neighboring herds might be established. However, this method of control results in significant financial implications and the culling of thousands of animals has also become ethically debatable. The effectiveness of stamping out in the absence of a fair and timely compensation scheme is highly dubious; i.e., in the absence of compensation there is no incentive for pig owners to report, who will instead sell or slaughter their pigs further spreading the disease. There is a need to come up with sustainable and effective alternatives to stamping out for countries that cannot afford compensation.

Biosecurity

On-farm biosecurity is a critical countermeasure for preventing the introduction and spread of ASF. Optimal biosecurity is effective by controlling the movement of pigs, people, equipment and supplies, and the potential biological or mechanical carriers of ASF. Priority biosecurity measures include the banning of swill feeding and the containment of scavenging pigs, which may be a challenge in developing countries. The identification of the source of transmission and entry into a target herd is a critical step in the implementation of an effective biosecurity program. However, after measures to curtail the spread of the disease are implemented, the most likely routes of transmission of ASFV may change. Since ASFV is an Arbovirus, a biosecurity plan should address procedures for cleaning and disinfecting facilities when *Ornithodoros* ticks are present; although a comprehensive biosecurity plan should include the control of insects and pests. Animal contacts as source of the virus may decrease, and transport trucks, people contacts, and pick-up for rendering services may contribute equally in the spread of the disease between premises. In endemic areas like Africa, the ideal solution for commercial pig farms has been the use of compartmentalization and has been used successfully in ASF control areas.

Vaccines

There is currently no commercial vaccine available for ASFV. In fact, an effective commercial vaccine for ASF has never been successfully developed. Although not formally classified, ASF scientists know there is a lack of cross protection among animals becoming immune to a certain virus isolate and subsequently exposed to another heterologous strain. This constitutes an important issue that will need to be addressed by both the ASF research community and veterinary authorities when considering vaccination strategies for the control and eradication of

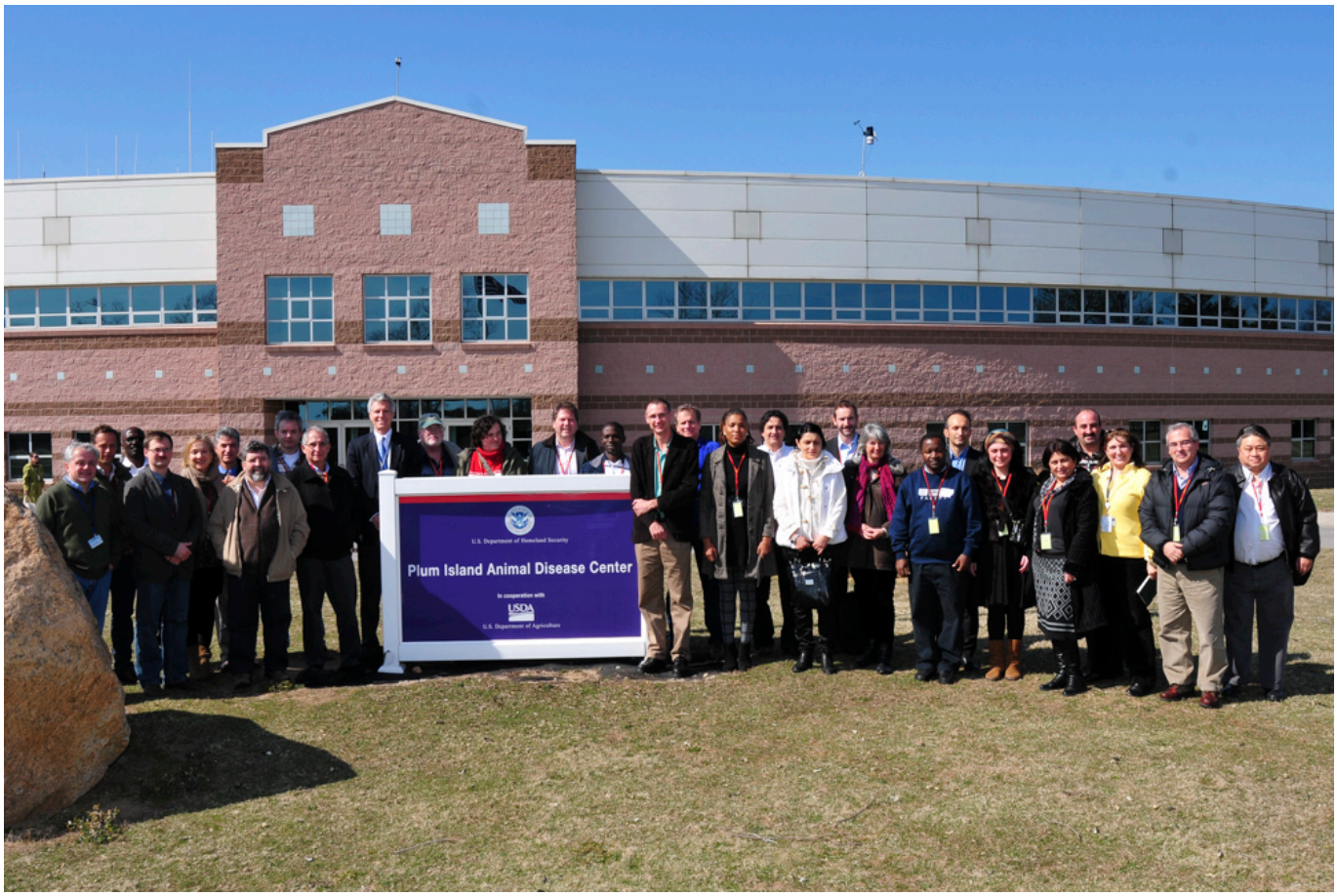
ASF. If vaccination of wild boar populations is considered, only safe, live-attenuated vaccines may be feasible.

Diagnostics

African swine fever is usually suspected based on clinical signs, but clinical evidence may be nonspecific and difficult to differentiate from other infectious diseases of swine; e.g., Classical Swine Fever. Diagnosis is based on a combination of a pathogen detection test such as PCR and a serological test for specific antibodies, usually performed by ELISA. In industrialized countries, both detection tools are commercially available and allow disease detection from three to four days post infection by PCR, and roughly day 7 to 14 by ELISA. Confirmatory tests are so far not commercially available.

GROUP PICTURES

1st GARA Gap Analysis Workshop
Plum Island Animal Disease Center
Orient Point, New York
April 6-8, 2013



2nd GARA Gap Analysis Workshop
ARC - Onderstepoort Veterinary Institute
Pretoria, South Africa
November 11-14, 2014

No group picture taken

3nd GARA Gap Analysis Workshop
ANSES - Laboratoire de Ploufragan-Plouzané
Ploufragan, France
September 6-8 2016

4th GARA Gap Analysis Workshop
Istituto Zooprofilattico Sperimentale
Cagliari, Sardinia, Italy
April 11-13, 2018

GLOSSARY

AHT: Animal Health Technician

APHIS: Animal and Plant Health Inspection Service

ARS: Agricultural Research Service

BSL: BioSafety Level

ELISA: Enzyme-linked immunosorbent assay

ASF: African swine fever

ASFV: African swine fever virus

DIVA: Differentiating between infected and vaccinated animals

FADDL: Foreign Animal Disease Diagnostic Laboratory

GMP: good manufacturing practice

HSPD-9: Homeland Security Presidential Directive Nine

Ig: Immunoglobulin

MLV: Modified live virus vaccine

NAHLN: National Animal Health Laboratory Network

NVS: National Veterinary Stockpile

OIE: World Organization for Animal Health

PCR: Polymerase Chain Reaction.

qPCR: Real-time PCR

cPCR: Conventional PCR

PPE: Personal Protective Equipment

INTRODUCTION

African swine fever (ASF) is a contagious viral disease of domestic pigs with significant economic consequence. In Africa, ASF virus (ASFV) produces unapparent infections in wild suids: wart hog (*Phacochoerus africanus*), bush pigs (*Potamochoerus larvatus, P. porcus*) and the giant forest hog (*Hylochoerus meinertzhageni*) (a single report). The reservoir of ASFV is considered the soft tick *Ornithodoros moubata* (Dixon *et al*., 2005).

African swine fever virus is a large enveloped virus containing a double stranded (ds) DNA of approximately 190 kilobase pairs. African swine fever virus shares aspects of genome structure and replication strategy with other large dsDNA viruses, including the *Poxviridae, Iridoviridae,* and *Phycodnaviridae*. Although initially classified as an iridovirus, based largely on virion morphology, increasing knowledge of ASFV molecular biology led to its reclassification as the sole member of a new DNA virus family, *Asfarviridae* (*Asfar*, African swine fever and related viruses) (Costard *et al*. 2009).

African swine fever virus infections in domestic pigs are often fatal and are characterized by fever, hemorrhages, ataxia and severe depression. However, the course of infection varies depending on host characteristics and the particular virus strain. African swine fever occurs in several forms, ranging from highly lethal to sub-clinical. Acute forms of ASF, associated with highly virulent ASF strains, are characterized by high fever, purple discoloration of the skin, multiple hemorrhages, respiratory distress, incoordination and death 3 to 7 days post-infection. Only a low percentage of animals will survive. Sub-acute and chronic forms of the disease are characterized by high fever, staggering gait, cough, diarrhea, purple discoloration of the skin, and death in 20 to 45 days post infection. These forms are accompanied by a higher proportion of surviving animals, and can be associated with ASFV strains of moderate and low virulence, respectively (Sánchez-Vizcaíno *et al.* 2015a).

African swine fever was considered a disease of sub-Saharan Africa. However, in 1957, ASF was introduced into Portugal and later on to other European countries and some States of Central and South America. From Europe, ASF was eradicated at the end of the 1990s with the exception of the Italian island of Sardinia. Eradication was also achieved in the other affected countries outside Europe. However, in 2007, a very virulent viral strain of ASF was introduced into the Republic of Georgia, probably through untreated food waste from international ships in the harbor of Poti. Subsequently, the virus (Georgia 2007) started to spread in the Trans-Caucasian region and reached the Russian Federation. From the beginning, this new introduction affected both domestic pigs and European wild boar. The latter proved to be as susceptible as domestic pigs and the disease established self-sustaining cycles within the wild boar population. This was unprecedented as so far, any previous introduction into the European wild boar population had been self-limiting, unless sustained by co-infection and spillover from domestic pigs. From Russia, the virus moved further and reached the European Union in 2014. At present, all Baltic EU Member States, Poland, Romania, Bulgaria, Hungary, and Belgium are affected. Moreover, a limited outbreak occurred among wild boar in Czech Republic. The latter was declared as resolved very recently. In August 2018, the disease also reached the world's largest pig producer, China. Thus, the disease has conquered three continents over the last decade. In consequence, the transboundary disease threat has now an unprecedented

geographical scope and its multi-sectoral nature necessitates inclusion of all stakeholders into the design of control measures (OIE, ASF Handbook).

Countries free of ASF currently employ isolation and preemptive slaughter of animals in outbreak areas. Although effective, isolation and preemptive slaughter result in huge economic losses (Arias and Sanchez-Vizcaino 2002). There is no available vaccine against ASF. Consequently, detection and elimination of infected animals is so far the only methodology to control and eradicate ASF (Costard *et al*. 2009).

BACKGROUND

GARA Gap Analysis Workshops

This gap analysis report is the compilation of gap analyses conducted during scientific conference organized by the GARA 2013-2018:

1st GARA Scientific Conference, Plum Island Animal Disease Center, Orient Point, New York, United States of America, April 6-8, 2013

2nd GARA Scientific Conference, Agricultural Research Council, Pretoria, South Africa, November 10-14, 2014

3rd GARA Scientific Conference, ANSES, Ploufragan, France, September 6-8, 2016

4th GARA Scientific Conference, Istituto Zooprofilattico Sperimentale, Cagliari, Sardinia, Italy, April 11-13, 2018

The gap analyses conducted by ASF experts were determined both by presented research updates reported from 44 research institutes from 34 different countries from across the world, coupled with scientific literature reviews. Using this information, priority areas for ASFV research were determined.

Reference Material

The GARA recommends the following websites and reports as background information on the biology, epidemiology, and control of ASF:

1. https://www.ars.usda.gov/GARA/ (GARA official website)
2. http://www.fao.org/docrep/004/y0510e/y0510e00.HTM (FAO: ASF Contingency Plans)
3. http://www.oie.int/wahis/public.php?page=disease (WAHIS Interface)
4. http://athena.bioc.uvic.ca/organisms/Asfarviridae (Viral Bioinformatics: Asfarviridae)
5. http://www.fao.org/3/a-i7228e.pdf (FAO Manual: Detection and Diagnosis)
6. http://www.fao.org/ag/againfo/programmes/en/empres/ASF/index.html (ASF Resources)
7. http://web.oie.int/RR-Europe/eng/eng/Regprog/docs/docs/GF-TADs%20Handbook_ASF_WILDBOAR%20version%202018-09-25.pdf ((Handbook on ASF in wild boar and biosecurity during hunting)

DEFINITION OF THE THREAT

The current threat for an introduction of African swine fever (ASF) into new geographical locations has never been higher. Since the introduction of ASF into the Republic of Georgia in 2007, 16 countries outside Africa have reported ASF outbreaks (Table I). With the continued spread of ASF in Africa, Russia, Europe, and now China, there is an unbroken perpetual threat of introducing ASF in previously ASF-free countries. Potential routes of infection include 1) the importation of infected pig products fed as contaminated swill to domestic pigs, 2) the spread of the virus to new geographical areas from infected pigs and wild boars, and 3) accidental or intentional nefarious events.

ECONOMIC IMPACT

The introduction of ASF into countries outside Africa has had important economic consequences for swine industries. A significant consequence of the introduction of ASF is the loss of status for international trade and the implementation of drastic and costly control strategies to eradicate the disease (Costard et al., 2009). In Cuba, the introduction of the disease in 1980s led to a total cost of U.S $9.4 million (Simeon-Negrin and Frias-Lepoureau 2002). In Spain, the final 5 years of the eradication program alone were estimated to have cost $92 million (Arias and Sanchez-Vizcaino 2002). Given the effect on pork production and trade as well as the costs of eradication, Rendleman and Spinelli estimated in 1994 that the net benefit of preventing ASF introduction in the United States would amount to almost $450 million, nearly 5 per cent of the value of total sales of pork products. In endemic countries, ASF has huge economic implications both for the individual farmers, especially the smallholder producers and at national scales (Fasina et al., 2012). In Africa the impact is also considerable, particularly on numerous poor households that depend on pigs to pay for many of the necessities of life.

EPIDEMIOLOGY

African swine fever may show unique regional patterns of presentation, associated with unique set of risk factors that should be assessed to establish proper surveillance and control strategies. The epidemiology of ASF varies depending on two types of transmission cycles among swine populations, which can be defined as a domestic pig cycle and sylvatic-wild pig cycle. To this, an additional cycle has been added: the environmental cycle, which includes the issue of wild boar and persistence in the environment as observed in the Baltics and Central Europe. The initial expression of ASF in a previously ASF-free country could be variable and unpredictable due to the myriad of host factors and the broad diversity of virulence among strains of ASF viruses. Additionally, the presence of competent arthropod vectors may also impact the maintenance of the virus in the environment.

SURVEILLANCE

Surveillance is the most important countermeasure to be able to eliminate the disease at the source through early detection and containment of a disease outbreak. However, different surveillance strategies are required to detect the different clinical manifestations resulting from ASFV infections. For acute infection, surveillance activities can be based on clinical signs; however, for mild cases or chronic infections, where recognition of ASF symptoms is less likely, surveillance activities must be based on diagnostic testing to supplement surveillance based on

clinical signs. Passive surveillance is often the only economically viable solution for many countries but has weaknesses due to the difficulty of differentiating ASF from many common endemic infectious diseases. Passive surveillance can be greatly improved by awareness campaigns targeting all stakeholder along the pig value chain, as well as the establishment of fair compensation plans to incentivize reporting. Active surveillance programs are expensive and currently must rely on direct diagnostic tests such as viral isolation and nucleic acid-based assays because of challenges and weaknesses of antibody-based assays.

BIOSECURITY

Implementing biosecurity measures on the farm is one of the most important countermeasures to prevent and protect commercial swine operations, but specific measures need also to be included and integrated in an eradication campaign to prevent further transmission and geographical spread through transport and person-to-person contacts. The main goal of a biosecurity plan is to decrease the probability of infection and significantly reduce the cost associated with losses (Fasina et al., 2012). A set of zoo-sanitary measures should be put in place to accomplish the goals set by the biosecurity plan. The more measures are implemented, the higher the cost, but warranted in a disease outbreak situation (Fasina et al, 2012). The OIE *Terrestrial Animal Health Code* (Chapter 15.1 and Chapters 4.3 and 4.4) provides guidelines for the establishment of compartments free of ASF.

DEPOPULATION

Depopulation is the primary countermeasure to reduce virus shedding and stop the spread of ASF virus. Minimum control measures will include depopulation of infected herds, surveillance and movement restriction within established control zones together with surveillance in herds that have been in contact with infected herds. Depopulation of contact herds and neighboring herds might be established. Thus, this method of control has resulted in significant financial implications and the culling of thousands of animals has also become ethically debatable. The effectiveness of stamping out in the absence of a fair and timely compensation scheme is highly dubious; i.e., in the absence of compensation there is no incentive for pig owners to report, who will instead sell or slaughter their pigs further spreading the disease. Lack of funds to compensate owners, particularly for culling of healthy pigs on neighboring premises, also constitutes a challenge in less wealthy countries. There is a need to come up with sustainable and effective alternatives to stamping out for countries that cannot afford compensation. When implemented, the speed of depopulation of infected herds including disposal of carcasses, and disinfection of premises may have an effect on disease spreading, duration of the outbreak, and overall effectiveness of the control measure (Boklund *et al*., 2009). This control measure is effective in countries or geographic areas where pigs are housed in well-defined premises or pig farms. In areas where domestic pigs are kept on free-ranging scavenging systems, depopulation might be difficult.

VACCINES

There is no vaccine available for ASF and the control of the disease is strictly dependent on animal quarantine, biosecurity measures, and slaughter. This presents a major gap in the availability of veterinary medical countermeasures to effectively prevent, control, or eradicate an ASF outbreak. Some of the challenges in developing a vaccine include the following technical hurdles: 1) ASFV is one of the largest DNA virus known with the large majority of the viral

genes uncharacterized or known functions; 2) there are no cell lines readily available to grow ASFV for vaccine production; 3) there are several ASFV genotypes with different phenotypic characteristics, with little or no cross-protection demonstrated amongst experimental vaccines tested to date; 4) vaccine are needed for parenteral administration for domestic pigs, and oral administration to vaccinate feral swine and wild boars, and potentially other wild suids where a sylvatic cycle exists, although wild suids in Africa are known to be fairly resistant to ASF viruses.

DIAGNOSIS

ASF is usually suspected based on clinical signs, but clinical evidence may be nonspecific and difficult to differentiate from other endemic infectious diseases and transboundary diseases such as classical swine fever. Real time and conventional PCR used simultaneously with ELISA antibody testing is an important tool for this purpose. Serological and virological differentiation of other etiological agents producing ASF-like diseases is critical. Available ELISA tests are particularly useful if a large number of samples have to be examined. There is a need for useful pen side tests that can be used in an outbreak situation to make rapid decisions in the field about the status of a test herd.

GAP ANALYSIS

The following section summarizes what we know about African swine fever virus, gaps in our knowledge, and research needs.

VIROLOGY

African swine fever virus (ASFV) is a large, enveloped virus containing a double stranded (ds) DNA of approximately 190 kilobase (Kb) pairs. ASFV encodes novel genes involved in host immune response modulation, viral virulence for domestic swine, and in the ability of ASFV to replicate and spread in its tick vector. ASFV shares aspects of genome structure and replication strategy with other large dsDNA viruses, including the *Poxviridae, Iridoviridae*, and *Phycodnaviridae* (Dixon *et al*., 2000 and 2008). ASFV and poxviruses replicate in the cytoplasm of the infected cell, primarily in discrete perinuclear assembly sites referred to as virus factories. They also exhibit temporal regulation of gene expression and have similar genome structures, including terminal inverted repeats, terminal crosslinks, a central conserved region and variable regions at each end of the genome (Dixon *et al.* 2008). Although initially classified as an iridovirus based largely on virion morphology, increasing knowledge of ASFV molecular biology led to its reclassification as the sole member of a new DNA virus family, *Asfarviridae* (*Asfar*, African swine fever and related viruses) (Dixon *et al*. 2000).

The ASFV virion is comprised of more than 50 polypeptides and has a complex but regular structure by electron microscopy, icosahedral in symmetry and containing several concentric layers for an overall diameter of approximately 200 nm (Breese and DeBoer 1966; Carrascosa *et al*., 1984, 1985; Estevez *et al*., 1986 and 1987; Schloer, GM, 1985). The 80-nm virion core is composed of a nucleoid, (Andres *et al*., 1997 and 2002). Surrounding the nucleoid are two lipid bilayers, (Andres *et al*., 1997 and 1998; Rouiller *et al*., 1998). External to the inner membrane is the capsid, composed of the structural protein p72 (also referred to as p73), which comprises approximately one-third the protein content of the virion, and providing the icosahedral structure to the virion (Andres *et al*., 1997; Carrascosa *et al*., 1986; Garcia-Escudero *et al*., 1998; Tabares *et al*., 1980a). Covering the capsid is a loose external membrane obtained by virion budding through the plasma membrane, which is not required for virus infection (Andres *et al*., 2001; Breese and DeBoer 1966; Carrascosa *et al*., 1984; Moura Nunes *et al*., 1975).

Similar to what has been found in poxvirus virions, ASFV virions contain enzymatic activities that contribute to early events in, and activities critical for, viral replication in the cell cytoplasm, including RNA polymerase, nucleoside triphosphate phosphohydrolase, topoisomerase, mRNA capping, and protein kinase activity (Kuznar *et al*., 1980 and1981; Polatnick 1974; Salas *et al*., 1981 and1983).

Genomic heterogeneity among African ASFV isolates associated with disease outbreaks in domestic swine relative to isolates isolated from ticks has been reported (Dixon and Wilkinson, 1988; Sumption *et al*., 1990). Subsequent molecular phylogenetic studies utilizing part of the p72 gene support some of these findings, including relative homogeneity among West African, European, and American isolates, homogeneity among certain African lineages associated with

outbreaks in domestic swine, and relative heterogeneity among isolates from southern and East Africa (Bastos *et al*., 2003; Lubisi *et al*., 2003; Wambura *et al*., 2006).

Nevertheless, the ASFV proteins are quite conserved across the different isolates. The central genomic core is identified as relatively conserved among different virus isolates. These include membrane and other structural proteins known to be present in the virus particle, and those that more recently have been shown to affect different stages of virion morphogenesis in the infected cell (Afonso *et al*., 1992; Alcami *et al*., 1992 and 1993; Brookes *et al*., 1998b; Camacho and Viñuela 1991; Lopez-Otin *et al*., 1988 and1990; Munoz *et al*., 1993; Rodriguez *et al*., 1994; Simon-Mateo *et al*., 1995; Sun *et al*., 1995 and 1996). Other ASFV proteins share sequence similarity to cellular proteins or enzymes, including those involved in aspects of nucleotide metabolism, DNA replication and repair, transcription, and protein modification, and those that likely account for enzymatic activities present in ASFV virions or induced in infected cells (Baylis *et al*., 1992, 1993a; Blasco *et al*., 1990; Boursnell *et al*., 1991; Freije *et al*., 1993; Hammond *et al*., 1992; Lu *et al*., 1993; Martin Hernandez and Tabares 1991; Martins *et al*., 1994; Rodriguez *et al*., 1993b; Yanez 1993; Yanez *et al*., 1993a, 1993b and1993c). Several of these proteins appear to be distantly related to homologs identified in poxviruses (Baylis *et al*., 1993b; Blasco *et al*., 1990; Boursnell *et al*., 1991; Freije *et al*., 1993; Martin Hernandez and Tabares 1991; Roberts *et al*., 1993; Yanez *et al*., 1993b). Additional enzymatic components encoded in the ASFV genome include homologs of cellular ubiquitin conjugating enzyme, trans-prenyltransferase, NifS-like protein, and components of a base-excision repair pathway (Hingamp *et al*., 1992; Rodriguez *et al*., 1992). ASFV also encodes proteins predicted to mediate virus–host interaction, virulence, and mechanisms that enhance the ability of the virus to successfully replicate within the host, including homologs of cellular inhibitor of apoptosis (IAP), Bcl-2, I Kappa B (IKB) myeloid differentiation primary response antigen MyD116, lectin-like, and CD2 proteins (Borca *et al*., 1994b; Neilan *et al*., et al. 1993a; Rodriguez *et al*., 1993a; Sussman *et al*., 1992). Notably, several of these putative virulence/host range proteins, along with certain multigene family (MGF) proteins, the central variable region protein 9-RL (pB602L as annotated in BA71V), and the variable tandem repeat-containing structural protein p54 (pE183L) (Irusta *et al*., 1996; Rodriguez *et al*., 1994; Sun *et al*., 1995), are among the most variable among multiple field isolates.

Recently, several reports have contributed to understanding the role of several viral proteins that were previously uncharacterized or partially characterized. Some of these proteins are critical for virus replication as the E2 ubiquitin conjugation enzyme I215L (Freitas *et al*., 2018), the viral decapping enzyme D250R or g5R (Quintas *et al*., 2017), a virus histone like protein pA104R (Frouco *et al*., 2017), the apoptosis inducing protein A179L (Banjara *et al*., 2017), the virus topoisomerase II protein (Freitas *et al*., 2016), and the viral protein Ep152R of unknown function, except for its specific binding to host protein BAG6 (Borca *et al*., 2017), which has been previously described to be essential for virus replication.

The process of virus replication has also received attention in the last few years. Besides the essentiality of virus proteins described above, the critical importance of the putative cell receptor CD163 in mediating virus infection in swine macrophage cultures and in *in vivo* replication in swine has been addressed. Genetically edited pigs lacking CD163 did not differ from their normal counterparts in terms of susceptibility to virus infection and are fully susceptible to

ASFV (Popescu *et al*., 2017) clearly indicating at least an alternative pathway for virus entry to susceptible cells. In addition, the role of cellular vesicular system has been shown to be critical during virus replication (Cuesta Geijo *et al*., 2017), as well as the ubiquitine proteasome system (Barrado Gil *et al*., 2017).

Gaps

Although there is only a single virus species, currently 24 genotypes have been described, with Genotype 23 and 24 just described in 2017 and 2018, respectively (Achenbach *et al.* 2017; Quembo *et al.* 2018); however, this designation is based on the sequencing of a single gene. Full genome sequence of the *p54*-gene has been confirmed as a valuable additional genotyping method for molecular epidemiological studies. Enhanced discrimination is obtained by analysis of the central variable region (CVR) within the B602L-gene, described as the most variable locus to distinguish between closely related isolates and identify virus subgroups within several of the 24 genotypes (Gallardo et al, 2009). Clearly, there are significant differences in genome size, virulence and immunogenicity (no cross-protection), but little is known about the genes responsible for virulence, host range, and viral-vector-host interactions.

<u>Lack of available sequences for ASFV genomes and hosts genomes of ASFV susceptible species</u>

Another important gap is the lack of available sequences for ASFV genomes and host genomes of ASFV susceptible species. Currently there are very few full length genome sequences of ASFV isolates deposited into the common databases, with only nineteen available at NCBI (National Center for Biotechnology Information). These strains are summarized in Table II, including their collection date, country of origin and the host they were isolated from. The very limited sequence data was obtained from six isolates that originated from ticks, ten from domestic swine and one from warthogs, and one from wild boar. The lack of diverse sequence information allows for only very limited interpretation of the differences between different isolates. The major gaps identified in the available full length genomic sequence of ASFV isolates are as follows:

1) Isolates from different origins and hosts including domestic pigs, wild boar, warthog and ticks.
2) Naturally occurring or lab derived attenuated isolates
3) Evolution of isolates from endemic areas in regard to both time and distance.
4) Field strains from current outbreaks in Africa

Interesting information was presented in Ploufragan related to the attenuated phenotype of genotype II ASFV found in Estonia (Zani et al., 2016). This virus was re-isolated from an experimentally infected wild boar in the clinical phase of infection. This animal recovered later and was found virus negative and antibody positive by the end of the trial. Commingling of this survivor with sentinel pigs did not lead to disease transmission despite the fact that the wild boar was still positive for viral genome at the time of commingling. The virus isolate was further tested in mini-pigs, domestic pigs and European wild boar. While very mild disease was produced in the former, all wild boar died showing an acute-lethal disease course. This viral strain showed a large deletion in its 5'-end of the genome and a reorganization. Further studies are under way, but awareness should be raised to the fact that mild disease courses can occur.

Interestingly, the variant virus was found in the field only for a very short period, suggesting a disadvantage in the disease cycle. Similar attenuated viral strains have been reported from other Baltic States, warranting further studies.

The genomes of susceptible host species is an additional a major gap in ASFV research. While there are fourteen individual genomes from breeds of *Sus scrofa* on NCBI, the genome sequences of the wild and domestic pigs, boar and warthogs in outbreak areas of Africa and Europe remains largely unknown. In order to conduct genomic studies as to what factors contribute to resistance of some of these breeds, a large scale sequencing effort would have to be conducted. The major GAPs would include the species and subspecies of both domestic and wild pigs in endemic or outbreak areas, and the genomic sequences of animals that are able to survive an outbreak.

Although recent advances in next-generation sequencing have proven to be of value for both the sequencing of ASFV and host genomes, the problem still exists, largely due to the extensive cost and amount of work involved not only in sampling but also in sequencing and building these large genomes. In the case of ASFV genome sequencing, better protocols to separate the viral DNA away from the host DNA could make the sequencing effort easier and more cost effective. Without this information functional genomic studies are limited only to a particular strain being used by an individual laboratory. As this information becomes more readily available it will allow for a better prediction of the potential cross protection between isolates and virus evolution, both over time and during individual outbreaks.

Transcriptomics of ASFV and of the host during various stages of infection

ASFV has 150-170 ORF (open reading frames), however the majority of these ORFs are only predictions and very few have any experimental evidence on either the RNA or protein level. While it is likely that the majority of these ORFs do produce a protein product, it is possible that the expression profiles of these viral genes could vary between isolate and could differ depending on which host is infected, which could explain the varying outcome of ASFV varying from 100% mortality to sub-clinical infections. However to date there is no published information on the RNA or protein profiles of ASFV expressed genes, even at the experimental or in vitro level.

In Ploufragan (Jaing et al., 2016) presented the gene expression profile analysis of whole blood RNA from pigs infected with low and high pathogenic ASFV. RNAseq analysis identified 395 genes most differently expressed at euthanasia day in the highly pathogenic Georgia 2007 strain and 181 genes modified at 7 days post infection in the attenuated OURT88/3 group. The top 20 common genes that had the highest differential expression between both groups were genes related with macrophage markers, natural killer cell markers, chemokines and other important immune response markers.

There is a gap in knowledge for which are the receptor(s) that the virus uses to infect swine macrophages. It is possible that a number of molecules act as receptors and co-receptors at infection. One of the candidate genes CD163, a scavenger receptor which is expressed by mature macrophages and correlates with permissiveness to ASFV infection. However, using

CRISPR/Cas9 gene-edited pigs with a mutated CD163 demonstrated that the recombinant pigs lacking CD163 were still susceptible to infection with no changes observed in viral virulence, suggesting that CD163 is not the receptor for ASFV (Popescu et al., 2017). The use of a wild boar isolated stable cell line (WSL) to grow adapted ASFV from several different isolates was reported as an useful tool for virus growth in cell culture as no observed large deletions in the genome were detected with full length sequencing of these viral genomes after successive growth passages of several ASFV virus isolates. (Keil et al., 2016).

These new reports are increasing knowledge of mechanisms of infection and will help in understanding virus-induced immunity and the identification of new gene candidates for vaccination. However, new experimental data will be necessary to fully elucidate and understand how the virus interacts with the host on a cellular level.

One of the major gaps identified was that the global expression profiles of host cells infected with ASFV has not been fully determined. This information could be used to determine how the host responds to viral infection, and the molecular pathways that are both turned on or off during viral infection. These types of experiments could be used to compare the host cell response to different strains, for example to compare an attenuated ASFV strain with that of the parental virulent virus. This information could lead to a better knowledge of both naturally occurring or lab generated attenuated isolates of ASFV. Understanding this information could help in the understanding of how these viruses are attenuated. Once the host cell response is understood at a cellular level, this information could also be gathered and applied to the response in the host.

Increasing the knowledge on the transcriptomics of both ASFV and the host infected with ASFV will bring a better understanding to both strain variability in ASFV and in the varying host immune response to the virus between both virus isolates and host species.

Functional Genomics of ASFV proteins

The majority of ASFV proteins, have limited experimentally proven functions; functional genomics has been limited to mostly prediction, either by conserved protein sequences or domains in other virus families or host proteins. The lack of experimentally proven functions is a major gap in ASF research. Understanding the role for ASFV proteins during infection is critical to understanding both the pathogenesis of ASF, but also understanding how ASFV is able to avoid detection by the host immune system and cause disease. Understanding the functionality of any particular ASFV protein shouldn't stop at functional prediction, and identification of the protein partners both viral and host for a particular protein is a major gap in ASFV research. To date there has been very little information reported for host-viral protein interactions.

Some relevant recent information was released at the GARA Gap Analysis Workshop in Ploufragan, France, referring to the previously uncharacterized ASFV Ep152R gene functionality and its interaction with cellular protein Bag6 (Borca et al., 2016). It was also reported the identification the mechanism of virus uncoating at viral entry by endocytosis and some cellular molecules that are relevant to this process (Cuesta-Geijo et al., 2016). Also, they reported the previously unidentified role of natural innate immunity mechanisms related to interferon induced proteins that are able to inhibit virus entry to the cytoplasm from the endosome called IFITM

proteins (Muñoz-Moreno et al., 2016). In addition the purification of ASFV particles and characterization of the proteome of mature extracellular ASF virions using a mass spectrometry approach was reported, and the identification of new viral and host-derived ASFV structural proteins was also presented (Kessler et al, 2016).

A clear understanding of the functionality of ASFV proteins and the role they play during infection, in particular how ASFV evades the immune response, is critical for the development of rationally designed live-attenuated vaccines. Large scale functional genomic studies represent a significant part of this gap that could be accomplished either by direct protein-protein identification methods such as yeast two-hybrid or Co-immunoprecipitation (Co-IP) followed by mass spectroscopy. Purification of viral proteins and in vitro assays to confirm their functional prediction functions would be of additional value.

Host Genomic Screens to determine virulence factors for ASFV

In light of today's technology, in which large scale genomic screens have been accomplished for many other viruses, to date no such screens have been reported for ASFV, representing a large Gap in ASFV research. These genomic screens need swine specific reagents, for example loss of gene function screens both either by siRNA screening or CRISPR/Cas9 screening would require a library that is targeted against the swine genome. Development of these swine specific libraries would be necessary for reliable large scale *in vitro* genomic screens for ASFV. These screens would help gain an insight into the host pathways that are critical for ASFV replication, and could lead to the discovery of the virus cellular receptors, immune markers of infection, and the pathways involved in virus replication and virus virulence. This information would contribute to understanding virus cell tropism and thus to development of cell lines that support virus replication as well as improving understanding of virus pathogenesis.

Research needs

1) ASFV genomic sequences:
With current DNA sequencing technologies it would be relatively easy and cheap to sequence the complete genomes from 1) 1-3 isolates from each genotype, 2) a series of viruses (>10) with different virulence and 3) a series of viruses (>5) that have replicated exclusively in domestic pigs, wild pigs and ticks.

2) ASFV bioinformatics resource:
There is a need to continue the annotation and analysis of ASFV genomes. The size range of ASFV is difficult and requires specialized tools. The acquisition of more genome sequences will make the management and comparison of the gene complement even more complicated. Although there is a good amount of sequencing data available for ASFV, using current, very robust technologies, it has been possible to develop a comprehensive database, which includes full length genome sequence of large number of isolates to replace the current less meaningful genotype based classification: https://virology.uvic.ca/organisms/dsdna-viruses/asfarviridae/

3) CRISPR/Cas9 or siRNA libraries targeting swine genomes.
Development of a swine specific knockout libraries are critical for *in vitro* genomic screens for ASFV. Libraries targeting the swine genome would be highly valuable to perform host genomic

screens for ASFV for a wide range of experimental avenues that could lead to the discovery of potential receptors, pathways modulated to avoid immune detection or for increased virus virulence.

4) Viral Transcriptomic Studies
Genomic wide either on the RNA or Protein level data for ASFV gene expression would be relatively easy with current technologies and could provide data to determine differences in ASFV gene expression *in vitro* and *in vivo* in different hosts.

PATHOGENESIS

African swine fever virus infection of domestic swine results in several forms of the disease, ranging from highly lethal acute manifestations to subclinical depending on contributing viral and host factors (Tulman *et al*., 2009). In Africa, highly virulent viruses produce a broad range of responses in populations of pigs in endemic areas. At the herd or population level, infections may result in 50-100 percent of the pigs seroconverting, but showing no signs of disease, with variable proportion of the pigs dying of acute ASF. Unlike domestic swine, wild African suids infected with ASFV are generally asymptomatic with low viremia titers (Heuschele and Coggins 1969; Montgomery 1921; Plowright 1981; Thomson 1985). These features of ASF presentation and the resemblance of the clinical manifestation to other diseases in swine such as Erysipelas and Classical Swine Fever hamper syndromic surveillance in domestic swine based exclusively on clinical signs.

Infection usually occurs through the oronasal route with primary virus replication in tonsils followed by a viremia with further secondary replication of all organs of the hemolymphatic system. In the acute form of the disease, the incubation period ranges from 5 to 15 days. Affected animals exhibit fever and anorexia followed by congestion and cyanosis of the skin, increased respiratory and heart rates, nasal discharge, incoordination, vomiting and, finally, coma and death. Hemorrhage may be observed clinically in multiple forms and secretions including epistaxis, melena, hematochezia, and hematemesis. Survival times for animals infected with African ASFV strains range from 2 to 9 days (Conceicao 1949; Creig and Plowright 1970; Haresnape *et al*., 1988; Mendes 1961; Thomson *et al*., 1979; Howey et al, 2013). Typical clinical pathological findings in acute ASF include leukopenia (Detray and Scott 1957; Edwards *et al*., 1985; Wardley and Wilkinson 1977), B and T cell lymphopenia (Sánchez Vizcaino *et al*., 1981; Wardley and Wilkinson 1980), thrombocytopenia (Anderson *et al*., 1987; Edwards 1983; Edwards *et al*., 1985), lymphocyte and mononuclear cell apoptosis (Carrasco *et al*., 1996; Gomez-Villamandos *et al*., 1995; Oura *et al*., 1998c; Ramiro-Ibañez *et al*., 1996; Salguero *et al*., 2004). Morphologic lesions may include hemorrhage in lymph nodes, spleen, kidneys, and respiratory and gastrointestinal tracts, congestion of skin and serosae, severe interlobular lung edema, and cavitary effusions which may range from serofibrinous to hemorrhagic. (DeKock *et al*., 1994; Detray 1963; Konno *et al*., 1972; Manso Ribeiro and Rosa Azevedo 1961; Maurer *et al*., 1958; Montgomery 1921; Nunes Petisca 1965; Steyn 1928 and 1932; Howey et al, 2013). The extensive necrosis in affected tissues and severe hemostatic and hemodynamic changes are likely important factors leading to death. Acute ASF also induces significant changes in acute-phase proteins (Carpintero *et al*., 2007; Sanchez-Cordon *et al*., 2007). Subacute cases last 3–4 weeks and the most prominent signs include remittent fever, loss of condition, pneumonia, dyspnea, cardiac insufficiency and swelling of the joints. While hemorrhage of lymph nodes and

other tissues may be found, it is not as prominent as in acute ASF (Moulton and Coggins 1968a). The primary cell types infected by ASFV are those belonging to the mononuclear- phagocytic system, including fixed tissue macrophages and specific lineages of reticular cells (Colgrove *et al*., 1969; Konno *et al*., 1971a and 1971b; Mebus 1988; Moulton and Coggins 1968a). Affected tissues show extensive damage after infection with highly virulent viral strains. Moderately virulent ASFV strains also appear to infect these cell types, but the degree of tissue involvement and the resulting tissue damage are much less severe. The ability of ASFV to replicate and efficiently induce marked cytopathology in macrophages *in vivo* in numerous porcine tissues (Howey et al, 2013) appears to be a critical factor in ASFV virulence.

Long term persistence following infection of pigs with genotype I isolates of reduced virulence has been demonstrated (Wilkinson 1984; Carrillo et al., 1994). These persistent infections have been demonstrated to be transmissible from pigs persistently infected with the low virulence genotype I NH/P68 isolate to contact pigs (Gallardo et al. 2015). Low virulence isolates can cause chronic forms of the disease, which are characterized by the absence of typical acute-phase lesions and low mortality rates, but distinct clinical signs including delayed growth, emaciation, joint swelling, skin ulcers and secondary bacterial infections are common (Sanchez-Vizcano 2015). Pigs that survive infection have been shown to carry virus in tissues or blood for long periods of time, which may contribute to virus transmission, disease persistence, sporadic outbreaks and sudden reactivation of the disease (Costard et al. 2013; Gallardo et al. 2015). Some studies in Africa have identified ASFV nucleic acid in apparently healthy pigs (Kalenzi Atuhaire et al. 2013; Thomas et al, 2016) that were positive for ASFV by PCR in tissues but negative in blood by PCR and serology (Okoth et al. 2013; Abworo et al. 2017). There is limited experimental evidence for transmission from persistently infected to naïve animals, and the relevance of persistently infected animals as carriers of ASF in the field is not clear, but data on healthy infected animals keeps accumulating (Titov et al. 2017, Abworo et al., 2017, Thomas et al., 2016, Muhangi et al., 2015, Braae et al., 2015, Athuaire et al., 2013), suggesting that the virulent virus could survive for long periods of time in the recovered pigs and a recrudescence of virulence may occur at later times (Titov et al., 2017).

Persistent infection with ASFV has been reported to occur in warthogs and in domestic pigs surviving acute viral infection [DeKock *et al*., 1940; Detray 1957; Plowright et al., 1969). Under experimental conditions, long-term persistent infection is the sequel to infection with ASFV (E75-L7 administered at low dose intramuscularly (im), E75-CV1 on and challenged twice with E75-L7, and E75-CV1 challenge once with E75-L7] in domestic pigs (Carrillo *et al*., 1994). In these animals, viral DNA was detected in the peripheral blood monocyte fraction more than 500 days post inoculation (p.i.) by PCR in intermittent periods; however, infectious virus could not be isolated from these samples and transmission was never demonstrated. Recent data using a moderately virulent isolate of different genotype during a shorter period of time (Petrov *et al*., 2018), do not support the establishment of a carrier status in animals surviving infection, though long term detection of viral genome in blood (for at least 90 pi) is consistent with many other reports (McVicar 1984; Mebus and Dardiri, 1980; Carrillo et al. 1994; Gallardo et al. 2015; Carvalho Ferreira et al. 2012).

In sub-Saharan Africa, ASFV is maintained in a sylvatic cycle between wild suids (warthogs) and argasid ticks of the genus Ornithodoros (Plowright *et al*., 1969a and 1969b; Thomson *et al*.,

1983; Wilkinson 1989). However, other wild pigs such as bush pigs do not inhabit burrows, and therefore would most likely spread ASFV via direct transmission, although evidence for such occurrences is limited (Jori and Bastos 2009; Jori *et al.* 2013). Unlike domestic swine, wild suids infected with ASFV are generally asymptomatic with low viremia titers (Heuschele and Coggins 1969; Montgomery 1921; Plowright 1981; Thomson 1985). Most adult warthogs in ASFV enzootic areas are seropositive and are likely to be persistently infected. Like warthogs, bushpigs develop subclinical infection and are more resistant to direct-contact transmission than are domestic species; however, the duration of ASFV viremia may be extended (Anderson *et al*., 1998). Although ASFV replication in blood leukocytes of domestic swine, warthogs, and bushpigs *in vitro* is similar, ASFV replication, spread, and induction of lymphocyte apoptosis *in vivo* is reduced in bushpigs when compared to domestic swine (Anderson *et al*., 1998; Oura *et al*., 1998a and 1998b).

There has been some characterization of the role of some of the ASFV genes in virus virulence. It is increasingly apparent that the terminal genomic regions and Multigene Family (MGF) genes play a significant role in ASFV host range. ASFV proteins involved in nucleotide and nucleic acid metabolism have been implicated in macrophage host range and similar to those in other large DNA viruses, may provide the deoxynucleotide pools favorable for efficient virus replication in specific cell types. Deletion of the dUTPase (E165R gene) and thymidine kinase (K196R gene) genes from ASFV reduces its ability to replicate in macrophages and attenuated the virus for swine, similarly correlating macrophage host range with virulence in swine (Moore *et al*., 1998).

Alternatively, several ASFV genes or gene regions are associated with viral pathogenesis and virulence in domestic swine but do not affect viral replication in macrophages *in vitro*. Two of these, UK (DP96R) and 23-NL (DP71L or l14L), are adjacently located in the genome. UK, an early protein, is quite variable between virus isolates, lacks similarity to other known proteins and deletion of UK from pathogenic ASFV, although it does not affect viral growth in macrophages *in vitro*, markedly attenuates the virus in swine (Zsak *et al*., 1998). The other gene, 23-NL, encodes NL a protein with similarity to cellular MyD116 and to the herpes simplex virus neurovirulence factor ICP34.5 (Sussman *et al*., 1992; Zsak *et al*., 1996). Deletion of NL from the ASFV E70 strain attenuates the virus in swine without affecting viral replication in macrophages *in vitro*.

Large deletion of six MGF360 genes and two MGF530 genes significantly reduces viral replication in macrophages (Neilan *et al*., 2002). Recently, it has been shown that these deletions, either naturally developed during the process of adaptation to grow in established cell lines (Krug et al., 2015), or introduced by genetic manipulations (O'Donnell *et al*., 2015b; Reis *et al*., 2016; Sanchez-Cordon *et al*., 2016), closely associate with decrease virus virulence in experimental infection studies in swine. Virulent isolates Georgia2007 (O'Donnell *et al*., 2015b) and Benin (Reis *et al*., 2016; Sanchez-Cordon *et al*., 2016) became completely attenuated and were able to induce solid protection against the challenge with the homologous virus.

In addition, a novel gene, DP148R, has been recently characterized (Reis *et al*., 2017). Deletion of this gene, which is clearly involved, as MGF360/530 genes, in interferon response

modulation, reduces virulence of the Benin isolate in pigs and induces protection against challenge with the homologous virus.

These studies have made significant contributions in increasing our understanding of the molecular basis of ASFV pathogenesis and the particular role of viral proteins in the outcome of the disease.

Gaps

In the course of chronic forms, the morbidity and mortality rates are lower than in acute and subacute forms of ASF, where severe inflammatory changes responsible for intense tissue injury and lymphoid depletion have been observed, inducing the death of the animals. Research to understand pathogenic mechanisms of chronic forms induced by low virulence ASFV isolates could provide useful information. The mechanisms responsible for animal survival to infection are not well understood, including the mechanisms involved in the protective immune response responsible for the appearance of carrier animals.

The use of attenuated strains obtained by genetic manipulation or by adaptation to different cell substrates provides a valuable tool to study mechanisms of attenuation. Comparative analysis of host and virus behaviour using parental virulent versus their derived attenuated strains, particularly focusing the early stages of the infection, would provide critical data regarding the host and virus mechanisms causing virus attenuation. Of particular interest are the studies that may be performed using pairs of virulent/attenuated strains differing only in a single gene, eliminating noise created by different genetic backgrounds among different virus strains. Differences in patterns of virus replication, kinetics and severity in presentation of micro and macro pathology and patterns of host gene activation should be analysed in swine infected with each of the paired virus.

Significant gaps in the basic knowledge of ASFV pathology include the identification of:

1) Basic mechanisms governing animal to animal infection.
2) The analysis of different aspects in the process of host-virus interaction, particularly during early (primary) infection.
3) Molecular differences in the pathogenesis process induced by virus with different degree of virulence
4) The role of specific genomic determinant(s) in disease outcome.

Research needs

1) Basic parameters governing host to host infection, including domestic and wild swine as well as the insect host.
2) Study the pathogenesis of ASFV isolates with different virulence in diverse susceptible host in order to identify, and ultimately subvert the early events of infection.
3) Assess the rate of transmission of strains of ASFV of different virulence in infected-contact animal experiments.
4) Determine patterns of activation of immunologically relevant host genes particularly at early stages after infection.
5) Identify ASFV genes and genetic determinants (group of genes like multigene families) involved in host range, virulence and pathogenicity.

IMMUNOLOGY

A key hurdle in developing a safe and effective ASF vaccine has been the lack immunological information. Attempts to vaccinate animals using infected cell extracts, supernatants of infected pig peripheral blood leukocytes, purified and inactivated virions, infected glutaraldehyde-fixed macrophages, or detergent-treated infected alveolar macrophages failed to induce protective immunity (Coggins 1974; Forman *et al*., 1982; Kihm *et al*., 1987; Mebus 1988). Homologous protective immunity does develop in pigs surviving viral infection. Pigs surviving acute infection with moderately virulent or attenuated variants of ASFV develop long-term resistance to homologous, but rarely to heterologous, virus challenge (Hamdy and Dardiri 1984; Ruiz-Gonzalvo *et al*., 1981). Pigs immunized with live attenuated ASF viruses containing engineered deletions of specific ASFV virulence/host range genes were protected when challenged with homologous parental virus (Lewis *et al*., 2000; Moore *et al*., 1998; Zsak *et al*., 1996 and 1998). Humoral and cellular immunity are significant components of the protective immune response to ASF. Antibodies to ASFV are sufficient to protect pigs from lethal ASFV infection (Hamdy and Dardiri 1984; Onisk *et al*., 1994; Ruiz-Gonzalvo *et al*., 1981). Although ASFV neutralizing antibodies directed against virion proteins p30, p54, and p72 have been described (Borca *et al*., 1994a; Gomez-Puertas *et al*., 1996; Zsak *et al*., 1993), they are not sufficient for antibody-mediated protection (Neilan *et al*., 2004). CD8 + lymphocytes also appear to have a role in the protective immune response to ASFV infection (Oura *et al*., 2005).

ASFV, similar to other large DNA viruses, affects and modulates host immune responses. ASFV-infected macrophages mediate changes in cellular immune function, and they likely play a role in the severe apoptosis observed in lymphoid tissue (Childerstone *et al*., 1998; Oura *et al*., 1998c; Ramiro-Ibañez *et al*., 1996; Takamatsu *et al*., 1999). ASFV inhibits phorbol myristic acid-induced expression of proinflammatory cytokines such as TNF-α, IFN-α, and IL-8 while inducing production of TGF-β from infected macrophages (Powell *et al*., 1996). Conversely, increased TNF-α expression has been reported after ASFV infection *in vitro* and *in vivo* and TNF-α may play a key role in ASFV pathogenesis, including changes in vascular permeability, coagulation, and induction of apoptosis in uninfected lymphocytes (Gomez del Moral *et al*., 1999; Salguero *et al*., 2002 and 2005). Notably, ASFV strains with different virulence phenotypes differ in their ability to induce expression of proinflammatory cytokine or IFN-related genes in macrophages early in infection (Afonso *et al*., 2004; Gil *et al*., 2003; Zhang *et al*., 2006). The ASFV ankyrin repeat-containing protein pA238L (5EL) is the only known viral homolog of cellular IκB proteins, the cytoplasmic inhibitors of the NFκB/Rel family of cellular transcription factors, and it is thought to be important in evading host immune responses (Miskin *et al*., 1998; Powell *et al*., 1996). The activity of pA238L provides a novel mechanism for ASFV to modulate the response of host cells to infection, especially considering the role of NFκB transcriptional pathways in inducing expression of a wide range of proinflammatory and antiviral mediators and cytokines. Consistent with this role, pA238L is able to regulate expression of cyclooxygenase-2 (COX-2), TNF-α, and inducible nitric-oxide synthase (iNOS). COX-2 downregulation occurs in an NFκB-independent, but NFAT-dependent, manner (Granja *et al*., 2004b). Similarly, pA238L inhibits expression of iNOS, and ultimately production of nitric oxide, by a mechanism likely involving p300 transactivation. Interestingly, deletion of A238L from pathogenic ASFV does not affect viral growth in macrophages *in vitro* or viral pathogenesis and virulence in domestic swine (Neilan *et al*., 1997b). Additional ASFV-encoded proteins

modulate or interfere with host immune responses. The ASFV 8DR protein (pEP402R) is the only known viral homolog of cellular CD2, a T cell protein involved in co-regulation of cell activation (Borca *et al*., 1994b; Rodriguez *et al*., 1993a). 8DR is necessary and sufficient for mediating hemoadsorption by ASFV-infected cells (Borca *et al*., 1994b; Rodriguez *et al*., 1993a). Deletion of the 8DR gene from the ASFV genome led to decreased early virus replication and generalization of infection in swine, and 8DR suppressed cellular immune responses *in vitro* (Borca *et al*., 1998). The ASFV pEP153R (8CR) protein is similar to cellular and poxviral proteins resembling C-type lectin-like proteins, including membrane-bound immunoactivation and immunoregulatory proteins CD69 and NKG2 (Neilan *et al*., 1999; Yanez *et al*., 1995). A potential role for pEP153R in immunomodulation may be subtle, however, since pEP153R does not affect viral pathogenesis or virulence in domestic swine (Neilan *et al*., 1999). Evidence also suggests that ASFV dramatically affects Th2/B cell responses, including upregulation of Th2 cytokines by a soluble virulence factor (p36) released from ASFV-infected monocytes and the nonspecific activation and apoptosis seen in B cell populations from ASFV-infected animals (Takamatsu et al. 1999; Vilanova *et al*., 1999). ASFV multigene family 360 and 530 genes play a role in modulating host innate responses. Unlike wild type virus, infection of macrophages with Pr4Δ35, a mutant virus lacking MGF360/530 genes, resulted in increased mRNA levels for several type I interferon early-response genes (Afonso *et al*., 2004). Analysis of IFN-α mRNA and secreted IFN-α levels at 3, 8, and 24 hours post infection (p.i.) revealed undetectable IFN-α in mock and wild type-infected macrophages but significantly increased IFN-α levels at 24 hours p.i. in Pr4Δ35-infected macrophages, indicating that MGF360/530 genes either directly or indirectly suppress a type I IFN response. This effect may account for the growth defect of Pr4Δ35 in macrophages and its attenuation in swine (Zsak *et al*., 2001).

In this regard, several reports support the importance of the function of MGF genes in the modulation of IFN responses, and their importance in virus virulence. The original experiments from Neilan *et al*., 2002, showing that deletion of MGF360/MGF530 genes reduce viral replication in macrophages, have recently been extended by others to show that these deletions also closely associate with decrease virus virulence in experimental infection studies in pigs (Krug et al., 2015; O'Donnell *et al*., 2015b; Reis *et al*., 2016; Sanchez-Cordon *et al*., 2016). Similarly, deletion of a recently characterized gene, DP148R, also involved in interferon response modulation, was also shown to reduce virulence of the Benin isolate in pigs (Reis *et al*., 2017).

Important progress has also been made in the characterization of immunogenicity of ASFV proteins in pigs. For example, several reports are now available describing cellular and antibody responses elicited by ASFV antigens individually expressed using a diversity of expressing vectors: vaccinia virus (Lopera-Madrid *et al*., 2017); DNA immunization (Argilaguet *et al*., 2012; Argilaguet *et al*., 2013; Lacasta *el al*., 2014); adenovirus (Lokhandwala *et al*., 2016; Lokhandwala *et al*., 2017); or the combination of these expression vectors (Jancovich *et al*., 2018). Some of these reports have gone further and also evaluated the ability of these viral proteins to induce a protective immune response in pigs (Argilaguet *et al*., 2012; Argilaguet *et al*., 2013; Lacasta *el al*., 2014; Jancovich *et al*., 2018). However, to date, most of these studies have only shown protection that does not reach values higher than 50% of the individuals under study, indicating the need of further work in order to expand the identification of virus antigens involved in the induction of protective immune responses.

In conclusion, a significant amount of data has now been accumulated toward the immunogenic characterization of several ASFV proteins. Importantly, in some cases, these virus proteins have also been evaluated for their ability to induce a protective immune response in pigs, which is the first step on the road towards the potential development of subunit vaccines.

Gaps

Attempts to induce protective immunity using different vaccine platforms have to date failed. Homologous protective immunity does develop in pigs surviving acute infection with moderately virulent or experimentally attenuated variants of ASFV. These animals develop long-term resistance to homologous, but rarely to heterologous, virus challenge. Humoral and cellular immunity have been shown to be significant components of the protective immune response to ASF. However, antibodies to ASFV are insufficient in protecting pigs from lethal ASFV infection. Although ASFV neutralizing antibodies have been described to be directed against particular virus proteins, they are not sufficient for antibody-mediated protection. Additionally, CD8+ lymphocytes also appear to have a role in the protective immune response to ASFV infection. Thus, although humoral and cellular immune response are involved in contributing to the protection against the infection, the actual immune mechanism(s) mediating that protection is still unclear. Additionally, the viral protein\proteins inducing the protective immune mechanism are still largely unknown. On the other hand, ASFV proteins have been shown to affect and modulate host immune responses *in vitro*.

As described above, advances have been achieved in identifying and understanding the function of virus genes modulating the host response and its direct effect during the process of infection in the natural host. Additionally, important progress has been achieved in the study of the immunogenicity of many previously uncharacterized viral proteins when administered to the natural host. Some of the key gaps that remain include:

1) The identification of immune mechanism(s) mediating protection against infection in swine remains one of the major questions to be answered.
2) Advances in the identification of the virus protein(s) responsible for the induction of protective immune mechanism.
2) Understanding the actual role of virus driven host immunomodulation in the process of virus infection in swine.
3) Correlation of protection between heterologous viral strains remains unclear.

<u>Mechanisms of protection</u>

Currently there is a large gap in understanding the mechanisms of protection induced by experimental vaccines for ASFV. Currently several reported experimental single or double gene deletions in the ASFV genome have resulted in live attenuated vaccine candidates, however how these vaccines protect against ASF is largely unknown. There is a lack of knowledge of the specific role for individual ASFV proteins in induction of protection, beyond a basic protein functional prediction. The cellular and antibody mediated mechanisms involved in protection are also largely uncharacterized. In addition the mechanisms of virus (or experimental vaccine) persistence is unknown. This is important to understand in order to avoid the persistence of live

attenuated vaccines in the field and to understand and predict the persistence of filed isolates of reduce virulence.

The cellular immune response to ASFV or to any experimental vaccine is largely unknown, in part due to the lack of available knowledge on swine immunology. Currently it is largely unknown what specific cell types are involved in inducing an immune response or what cell types are involved in inducing long term protection to ASFV. To date there has been very little discovery of neutralizing or T-cell epitopes for ASFV. Recently p30, p54, p72 when expressed could produce neutralizing antibodies, but these antibodies did not confer protection to ASFV (Neilan et al., 2004). This suggests that we need to understand more broadly the role of antibodies in protection or disease enhancement, as there is potential for antibodies produced that are not neutralizing to be involved in disease, perhaps inhibiting the spread of the virus. It is also possible that antibody mediated enhancement of disease could occur as virus uptake into macrophages could be mediated by Fc receptors. Understanding the role for both antibodies to ASFV and the immune response to ASFV could allow us to understand the differences in both virulence between different ASFV isolates, and potentially an understanding why some wild African pigs become infected, but clinically do not exhibit any clinical signs of ASF. Understanding these mechanisms of protection will allow the generation of safer vaccines, including the possibility of creating subunit vaccines.

Correlation of protection between heterologous viral strains

The understanding of cross protection between heterologous viral strains is largely unknown, in part due to the lack of available genomic sequences of the different ASFV strains; thus, the diversity of ASFV is not clear. Largely, there has been very little work involving field strains in Africa, especially in current endemic areas. In Mozambique, serologically positive pigs were resistant to at least two highly virulent viruses, a genotype II and a genotype VIII virus, which are not closely related to one another, suggesting cross protection events under field conditions (Penrith et al., 2004). Possible countries identified for conducting cross protection studies include Uganda, Tanzania, Mozambique, and Kenya. In these countries, using endemic strains for experimental vaccines could allow the possibility of conducting long term vaccine studies involving a large number of animals.

Understanding the mechanisms of cross protection will allow better cross protective next generation vaccines, and the ability to predict which vaccine to use if a new emerging ASFV strain were to cause an outbreak. Understanding the specific viral proteins involved in cross protection would involve a large effort in sequencing current circulating ASFV strains, and large cross protection studies with divergent strains.

Research needs

1) Discovery of the immune mechanism mediating effective homologous and heterologous protection against virus infection.
2) Identification of viral genetic patterns that correlate with presence/absence of homologous versus heterologous protection.
3) Identification of virus protein\s involved in the induction of protective immune response.

4) Identify regulatory genes involved in pro-inflammatory cytokines and antibodies production and the assessment of their actual roe in the process of virus infection\virulence in swine.
5) Explore the development of new assays based on cellular immunity for the early detection of the disease.
6) Improve our understanding of the role of multigene families in antigenic variability and evasion of immune response.

7) Identify and characterize genes related to host protection.

VACCINES

There is currently no commercial vaccine available for ASFV, nor has an effective commercial vaccine for ASF ever been available. A summary of experimental ASF vaccines reported in peer-reviewed scientific publications 2012-2018 is provided in Table III. Experimentally, homologous protection can be achieved by inoculation of pigs with low-virulence isolates obtained by passage in tissue culture or by deletion of genes involved in virulence, as well as low-virulence isolates from the field (Lewis *et al*., 2000; Leitao *et al*., 2001; Boinas *et al*., 2004). Usually these animals develop long-term resistance to homologous, but rarely to heterologous, virus challenge (Hamdy and Dardiri 1984; Ruiz-Gonzalvo *et al*., 1981). This lack of cross protection among different isolates constitutes an important issue to be considered in the development of ASF vaccine candidates.

The mechanism of protection involves cell-mediated immunity, since depletion of CD8+ T cells abrogates protection (Oura *et al*., 2005; Denyer *et al*., 2006). A role for antibodies in protection had been shown since passive transfer of antibodies from immune pigs conferred partial protection to lethal challenge (Onisk*et al*., 1994). In experiments using recombinant proteins, partial protection was achieved using a combination of two proteins, p54 and p30, as well as with recombinant CD2-like protein (Ruiz-Gonzalvo *et al*., 1996; Gomez-Puertas *et al*., 1998). However, some of these results could not be repeated by others using highly virulent ASFV isolates (Neilan *et al*., 2004). The failure to achieve complete protection in these experiments may be because of the delivery method of the antigens and/or because more or different antigens are required to confer protection. Alternatively, it is possible that full protection can only be achieved by using live-attenuated replication competent ASF viruses as vaccines.

Pigs immunized with live attenuated ASF viruses containing engineered deletions of specific ASFV virulence/host range genes (for review see Dixon *et al*., 2008 and Tulman *et al*., 2009) were protected when challenged with homologous parental virus (Lewis *et al*., 2000; Moore *et al*., 1998; Zsak *et al*., 1996 and 1998). This preliminary work, mostly produced using historical virus strains, has been recently extended to virus isolates with current epidemiological significance. In addition, novel genes have now been identified as additional targets for deletions leading to virus attenuation. For example, deletion of 9GL, previously described with the Malawi isolate (Lewis *et al*., 2000), was deleted in the Georgia 2007 isolate (O'Donnell *et al*., 2015a), resulting in virus attenuation and demonstrated use as an experimental vaccine to protect against homologous challenge. Other genetic manipulations including deletion of a group of MGF genes either in the Georgia 2007 isolate (O'Donnell *et al*., 2015b), or Benin (Sanchez-Cordon *et al*., 2018; Reis *et al*., 2016), or the previously uncharacterized DP148R gene

(Reis *et al*., 2017) attenuated the parental virus and protected against challenge with the virulent homologous virus.

Interestingly, for the first time, complex genetic manipulations with multiple gene deletions in the same virus were introduced in the Georgia 2007 isolate. Some of these attempts were initially unsuccessful, resulting in profound attenuation but failing in inducing a protective immune response (O'Donnell *et al*., 2016a; Abrams *et al*., 2013). Conversely, in other cases, combinations of gene deletions successfully increased vaccine safety and immunogenicity, as in the case of a Georgia 2007 mutant harboring deletions of both the 9GL and UK genes (O'Donnell *et al*., 2016b).

One concern about the use of ASFV vaccines is the potential genetic diversity of strains circulating within the same geographical area. Although anecdotal information exist suggesting the existence of cross-protection between viruses belonging to different genotypes, it was not until recently that this has been experimentally demonstrated. Deletion of CD2-like gene from the virulent Badajoz71 isolate produced an attenuated virus that induced protection to the homologous parental virus but also to the heterologous Spain75 and Armenia2010 isolates (Monteagudo *et al*., 2017). Interestingly, this particular virus possesses the unique characteristic of growing in an established cell line, which is paramount for a potential vaccine strain that will need to grow at high titers for vaccine production. Therefore, it may be possible to develop vaccines that can cross-protect against infection with several genotypes.

Identification and characterization of novel ASFV genes involved in virulence and in evasion of the host's immune response is still needed to facilitate and improve the development of rationally attenuated vaccines through sequential deletion/modifications of these genes. Although further research is required, it appears that to development of effective vaccines is now more realistic than just a few years ago.

Alternative approaches investigating subunit vaccines based on the expression of protective antigens have not significantly progressed due to the lack of investigative work to identify viral antigens inducing protection. The recent development of high-throughput methods for constructing recombinant viral vectors opens a route for global analysis of the protective potential of all ASFV-expressed genes. In this regard, several reports studied the cellular and antibody response elicited by ASFV antigens individually expressed using a diversity of expressing vectors. Thus, vaccinia (Lopera-Madrid *et al*., 2017), DNA immunization (Argilaguet *et al*., 2012; Argilaguet *et al*., 2013; Lacasta *el al*., 2014), adenovirus (Lokhandwala *et al*., 2016; Lokhandwala *et al*., 2017) or their combination (Jancovich *et al*., 2018) have been used to immunize pigs with several different viral proteins that were selected using different criteria. Some of the reports are restricted to the characterization of the immune response elicited by each of the virus antigens expressed in the different vectors (Lokhandwala *et al*., 2016; Lopera-Madrid *et al*., 2017; Lokhandwala *et al*., 2017), without assessing the protective effect of immunization against challenge with virulent virus in pigs. In those reports where challenge studies were included (Argilaguet *et al*., 2012; Argilaguet *et al*., 2013; Lacasta *el al*., 2014; Jancovich *et al*., 2018), protection values have not reach higher than 50% of the individual animals under study. These results indicate that further work is needed in order to expand the identification of virus antigens involved in the induction of protective immune response. Future success using subunit vaccine platforms may require optimization of the immunization protocols,

including the selection of an effective vaccine vector. Importantly, it should be mentioned that it has been reported that successive immunization using vaccinia and adenovirus as vectors expressing eight undisclosed specific virus proteins protected 100% of immunized pigs from the challenge with the virulent Benin isolate (Netherton *et al*., 2018). This constitutes so far the only report presenting an ASFV subunit vaccine with total protective efficacy.

Gaps

Although live attenuated viral vaccines appear to be the most promising, one major concern is their safety. What is considered a safe vaccine could depend on the area it is going to be deployed; for example, deploying a vaccine with an identical backbone to ASFV that is circulating in an endemic area, or during an outbreak situation, is very different than deploying a vaccine in an uninfected area. Currently there has been no vaccine with a marker for differentiation of infected from vaccinated animals (DIVA).

Currently there is no validated cell line to support vaccine production, leading to another significant gap in ASFV vaccine research. Without a validated cell line, commercialization of an ASFV vaccine will not be possible. The only reported cells that supports a live attenuated vaccine are primary swine macrophages, which are commercially unfeasible to use for large scale vaccine production.

Some of the key research gaps that impede the discovery of safe and effective ASF vaccines, and in particular, vaccines that have been engineered for the purpose of control and eradication, include:

1) Determinants of ASFV virulence have yet to be identified and fully characterized, thereby limiting the development of rationally attenuated vaccine strains.
2) An immortalized cell line for ASFV vaccine production.
3) ASFV gene products that can induce a protective immune response to engineer subunit vaccines
4) Mechanisms of protective immunity.
5) Antigenic diversity between different ASFV strains and its impact on vaccine cross protection against heterologous strains.
6) Identification of antigenic markers to engineer a vaccine with negative markers to differentiate infected from vaccinated animals DIVA).

Research needs

1) ASFV virology and functional genomics studies to identify targets that will inform vaccine discovery research, including novel determinants of virulence in the ASFV genome, antigenic targets required for immunity, and mechanisms of immune evasion
2) Determine safety characteristics associated with experimental live attenuated vaccines.
3) Development of a stable cell line for vaccine production that is capable of robust ASFV growth and scalable to commercialized vaccine production.
4) Engineer additional gene-deleted recombinant ASFV as potential vaccine candidates, including addition of DIVA markers.

DIAGNOSIS

A wide variety of laboratory techniques are available either for ASF virus and antibody detection, and a combination of both is the recommended approach for detecting ASFV. It is important to point out that ASF presents three significant advantages for detection: i) viremia begins usually at 2-3 dpi, and it is maintained for several weeks; ii) specific antibodies appear detectable in blood from the 8-15th day post infection at high levels and persist for long periods of time, even years; iii) since there is not a vaccine available, specific antibodies (if they appear before the animal dies) are a very good marker of infection. However, oral and intramuscular experimental infections of piglets, juveniles and adult wild boar with moderately virulent isolates resulted in acute forms of ASF with 100% lethality within less than 12 days, with no detectable antibody response in serum or effective transmission to domestic contact pigs and wild boars (Gabriel et al. 2011; Blome et al. 2012). Under experimental conditions, older pigs appear to have better survival than younger pigs after infection with a moderately virulent isolate of ASFV, regardless of the inoculation dose (Post et al., 2017).

The persistence the specific ASF-IgG antibodies for long periods of time in infected pigs provide the primary strategy to detect the sub-acute and chronic forms of ASF, which is essential for ASF eradication programs. Several techniques have been adapted to ASF antibody detection, but the most common, practical and inexpensive test normally used are enzyme-linked-imunosorbent assay (ELISA), and as confirmatory tests: Immunoblotting assay (IB); Indirect immunofluorescence antibody test (IFA); and the Immunoperoxidase Test (IPT). The samples that should be collected for ASF laboratory diagnosis are: whole blood, tonsil scrapings and/or tonsil swabs from suspect live animals alive, and lymph nodes, kidney, spleen, lung, blood and serum from dead pigs. Tissues are used for virus isolation (HA test), viral antigen detection (DIF test), and DNA viral detection (PCR test), while blood, tonsil scrapings and tonsil swabs are only used for virus isolation and DNA viral detection. Serum is used for antibody detection by IFA, ELISA or IB. Tissue exudates can be used for viral detection by PCR and for antibody detection by the serological tests listed above.

The most commonly used techniques for virus detection and identification are haemadsorption (HA), direct immunofluorescence (DIF), and since 2000, the molecular detection of ASF virus by PCR. While HA and DIF tests are not commercially available, several qPCRs are now on the market and licensed in European countries (e.g., there are five licensed kits for ASFV detection in Germany from international companies and two further tests are currently in the process of being validated). It has been shown that the tests are very robust, specific and sensitive. An example is a PCR that includes all reagents dried down, as well as a rehydration buffer and a positive control (Zsak et al. 2005). An internal control is an important prerequisite for reliable testing.

Virus Detection Techniques

Virus detection and isolation.

The hemadsorption test (HA) is definitive for ASF virus identification because of its sensitivity and specificity. HA is based on the hemadsorption characteristics that most of the ASF virus isolates induce when pig macrophages are infected in the presence of the porcine erythrocytes. A

characteristic rosette around the infected macrophages develops before the cytopathic effect appears. It is important to point out that it has been observed that a small number of field strains show only cytopathic effect without producing the hemadsorption phenomenon. These strains are identified using PCR and/or DIF test on the sediments of these cell cultures.

ASF-DNA detection.

Since 2000, laboratory PCR tests, based on conventional and real-time procedures, have been developed and some of them have already been validated (OIE, 2000; Agüero et al, 2003; King et al, 2003). These techniques use primer pairs selected from a highly conserved region of the viral DNA, within the VP72 genome region, detecting a wide range of ASF isolates belonging to all the known virus genotypes. It is an excellent and relatively rapid technique to be included in epidemiology surveillance and diagnosis of ASF. There are also fully validated commercial kits and in-house protocols targeting alternative genome regions (useful to exclude amplicon contaminations). Among the commercially available kits licensed in at least one country are: INgene q PPA (Ingenasa), virotype ASFV (Indikal Bioscience), ID Gene ASF Duplex (IDvet), and RealPCR ASFV (IDEXX). Comparative tests have been performed at the level of the EU Reference Laboratory as well as some National Reference Laboratories and results should be published soon.

Direct immunofluorescence (DIF).

The DIF assay is based on the demonstration of viral antigen on impression smears or frozen tissues section with an immunoglobulin conjugated against ASF virus. It is a very fast (one hour) and economic test with high sensitivity to the acute ASF form. For subacute or chronic forms, DIF test presents a sensitivity of only 40 %. This decrease in sensitivity seems to be related to the formation of antigen-antibody complexes, which do not allow the reaction with the ASF conjugate.

Additionally, there is a unique commercial ELISA viral antigen detection test, the Ag-ELISA. Both antigen detection techniques DIF and Ag-ELISA exhibit a very low sensitivity in case of chronic forms of the disease, while antigen-antibody complex are present. These techniques are only recommended for the diagnosis of acute forms of the disease. The antigen detection techniques are not recommended in case of chronic forms of the disease, in endemic areas, or for an individual diagnosis of the disease.

Additional Tests.

In recent years a number of diagnostic platforms have been adapted to ASF diagnosis, most of them based on DNA detection, either as part of multiplexed techniques (Lung et al., 2018, Xiao et al., 2018, Erickson et al., 2018, Hu et al., 2015, Shi et al., 2016, Sastre et al. 2016) or single detection, which includes portable PCR systems (Liu et al., 2017), lateral flow devices for antigen detection (Sastre et al. 2016), and new platforms such as use of biosensors (Mujibi et al. 2018), the droplet digital PCR ddPCR (Wu et al., 2018), the recombinase polymerase amplification, RPA (Wang et al. 2017), the Polymerase cross-linking spiral reaction, PCLSR (Wozniakowski et al., 2017), the isothermal cross priming amplification CPA (Fraczyk et al.

2016), some of them with times as short as 10 minutes for the result, and sensitivity and specificity as high as the OIE recommended ULP-PCR.

Antibody Detection Techniques

Antibody ELISA.

This is the most useful method for large-scale serological studies. At present, at least three commercial ELISAs are in routine use (from Ingenasa, Svanova, and IDVet). Recently, another competitive ELISA was validated and found to give reliable results (IDVet, based on the p32 protein). Other ELISAs are in house or in development and validation process, and all have their strengths and weaknesses, which should be considered when testing samples of doubtful or bad quality. These tests are based on the detection of ASF antibodies bound to the viral proteins, which are attached to a solid phase by addition of protein A-conjugated with an enzyme that produces a visible colour reaction when it reacts with the appropriate substrate. A commercial Antibody ELISA is available (INgezim PPA Compact Ingenasa), and it has been validated by the Central Reference Laboratory (CRL), Spain. Also, an indirect ELISA (ID Screen® ASF indirect) and P32 based competitive ELISA (ID Screen® ASF competition) were both validated by the European Reference Laboratory (EURL, CISIA-INIA, Spain). The procedure of an "in house" OIE ELISA as well as a standardized/validated soluble antigen for the OIE ELISA test can also be provided by CRL upon request.

Immunoblotting assay (IB).

This is a highly specific, sensitive and easy to interpret technique which is successfully used as an alternative method to IFA recommended as a confirmatory test of the positive or doubtful results by ELISA. There is no commercial IB Kit available, and standardized/validated IB antigen strips should be prepared by the own laboratory. It could be provided by CRL previous a request. However, due to the complexity of the IB antigen-strip production, the annual amount of it is limited.

Indirect immunofluorescence antibody test (IFA).

The IFA test is a fast technique with high sensitivity and specificity for the detection of ASF antibodies from either sera or tissue exudates. It is based on the detection of ASF antibodies that bind to a monolayer of cell lines (MS) infected with an adapted ASF virus. The antibody-antigen reaction is detected by a labelled fluorescein A-protein.

Immuno Peroxidase Test (IPT). The IPT is an immune-cytochemistry technique on fixed cells to determine the antibody-antigen complex formation through the action of the peroxidase enzyme. In this procedure, Vero or MS cells are infected with ASFV adapted isolates to these cell cultures. The infected cells are fixed and are used as antigens to determine the presence of the specific antibodies against ASF in serum samples (Gallardo et al., 2015).

Pen-side Tests. Recently, Cappai et al. (2017) reported on the use and validation of a commercial serological pen-side test in Sardinia, Italy. The implemented test is a lateral flow

device (LFD) that is produced by Ingenasa (INgezim PPA CROM). The study on hunted wild boar showed a sensitivity of 82 % and a specificity of 96 % under field conditions (better performance under laboratory conditions). It was demonstrated that the use of pen-side tests was less expensive and laborious, while still providing expedited results. Thus, these tests could be considered under certain conditions.

The use of a combination of virological detection techniques (PCR test is recommended since Ag detection techniques such as DIF and antigen ELISA show very limited sensitivity in chronic cases) simultaneously with the use of serological test (ELISA, and confirmation of positives and doubtful results by IPT/IFA or IB), makes possible to detect all ASF epidemiology situations (acute, subacute and chronic) in very short time with accuracy and confidence.

The characterisation of ASFV isolates is performed by standardised protocol established at the international level and by the EU Regional Laboratory by genotyping. The genotyping strategy involve sequencing of three independent regions on ASFV genome; i) the C-terminal end of the gene encoding the VP72; ii) the full-gene sequencing of the VP54; and iii) the variable region within ASFV genome named CVR (central variable region) marked by the presence of tandem repeat sequences (TRS). The partial VP72 and full-length sequencing of VP54 places ASFV isolates into major subgroups prior to CVR analysis to resolve the intra-genotypic relationships of viruses causing ASF outbreaks. This method has provided additional information about strains of viruses circulating in Europe, America and Africa over a 45 year period. Furthermore, these methods have allowed determination of the genetic relationships and origin of viruses responsible for disease outbreaks occurred in the last years in Europe (Italy and Caucasus countries) and Africa.

Recent Advances in the Development of ASF Diagnosis

Advances in pathogen detection.

Recently, a study was undertaken to compare the available diagnostic test systems in the framework of the current outbreak in Eastern Europe (Gallardo et al., 2015). In this study, the Universal Probe Library (UPL) PCR (Fernandez-Pinero et al., 2013; Gallardo et al., 2015) was the most sensitive method followed by the OIE prescribed real-time PCR (King et al., 2003) and the conventional PCR. In general, agreement among the methods was fair to good (94% between UPL and real-time PCR; 88% between UPL and conventional PCR).The commercial antigen ELISA (INgezim PPA DAS K2; Ingenasa) showed a sensitivity of roughly 77%.

Isothermal amplification methods can aid diagnosis under basic laboratory or even field settings and have been investigated over the last years. Among the isothermal methods that have been tried for ASFV is cross-priming amplification (targeting the p72 gene). Fraczyk et al. (2016) could demonstrate that the method was as sensitive as the UPL PCR, at least under the chosen conditions. Quite similar results were obtained for a loop-mediated isothermal amplification (LAMP) assay targeting the topoisomerase II gene. Apart from standard settings, visualization through the use of dual-labelled biotin and fluorescein amplicons on lateral flow devices was demonstrated (James et al., 2010). Apart from other isothermal methods such as RPA, different

field-applicable PCR machines have been tested over the last years. However, these methods need further assessment.

Advances in antibody detection.

For ASF antibody detection, five serological methods were recently tested, including three commercial ELISAs (from Ingenasa, IDVet, and Boehringer Ingelheim Svanova), the OIE-ELISA, and the confirmatory immunoperoxidase test (IPT). The IPT was shown to be the most sensitive assay that also allowed testing of tissue exudates (Gallardo et al., 2015). The IPT was able to detect ASF antibodies at an earlier point in the serological response, when few antibodies were present.

Generally, knowledge was gained and collated on suitable antigens and their expression for serodiagnosis of ASF. Evaluation of the available data also revealed strengths and weaknesses in terms of broad range of detection (Perez-Filgueira et al., 2006; Cubillos et al., 2013). For the African setting, it was demonstrated that recombinant p30 of Morara/Georgia was able to mirror the overall situation. These data fed (see Svanovir ELISA and IDScreen) and will feed into new test developments towards early and reliable detection of ASF antibodies. At present at least three commercial ELISAs are in routine use (from Ingenasa, Svanova, and IDVet), and others are under development and validation. All these tests have different strengths and weaknesses, especially when it comes to testing of bad quality wild boar sera. The results were shown to vary among laboratories.

Recent developments in pen-side tests and alternative sampling and testing schemes.

Apart from the single LFD for antibody detection to ASF, multiplexing has been reported with CSF, which would facilitate surveillance for both diseases (INgezim ASFV-CSFV-CROMAb) (Sastre et al. 2016a).

Data were presented on the last ASF-STOP meeting in Pulawy, that lateral flow devices could also be used in combination with blood swabs (Carlson et al., submitted).

A LFD for the detection of viral antigen has been described (INgezim ASFV-CROM) (Sastre et al., 2016b). The assay showed a sensitivity that is comparable with antigen ELISA. Generally, a viral load of $>10^4$ HAU would be detectable. Thus, sick animals or animals that died from ASF should be positive (in the majority of cases). In combination with other methods, this tool could add to a swift detection in remote or otherwise problematic areas (e.g. carcasses of wild boar that cannot be transported) but has, as detailed above, very limited sensitivity.

Non-invasive sampling strategies could mean an optimization for wildlife surveillance by circumventing the necessity of fitness-biased hunting/capture sampling schemes that can in the worst case even further disperse the virus. Recently, different approaches for the in-life sampling have been evaluated both under experimental and field conditions.

One option for a non-invasive approach could be the collection of faeces from the wild boar habitat. Along these lines, de Carvalho Ferreira et al. (2014) tested the suitability of fecal

samples. They demonstrated that, in comparison with virus detection in blood, virus can be detected in faeces 50-80% of the time. For the subacute/chronic phase, this percentage decreases below 10%. Despite this rather variable detection in the course of the infection, ASFV DNA was proven to be rather stable in faeces (half-life of more than two years at 12°C or ~15 days at 30°C, respectively) and thus, testing of faeces could supplement the tool box of monitoring methods. Apart from genome detection, it was recently shown that faeces could also be suitable for ASFV specific antibody detection (Nieto-Pelegrín et al., 2015).

Another option is the use of (bait) ropes for the collection of oral fluid. Oral fluids were shown to be suitable for antibody detection (Mur et al., 2013; Giménez-Lirola et al., 2016) and genome detection (Grau et al., 2015).

With regard to bait ropes, published studies cover mainly CSF (Mouchantat et al., 2014; Dietze et al., 2017) and FMD (Mouchantat et al., 2014), but similar approaches have been followed for ASF under both field and laboratory conditions. In Russia, ropes were left at feeding places and wild boar were shown to chew on the ropes.

Braae et al. (2013) investigated the use of FTA cards for blood collection and subsequent testing by qPCR under field conditions in Tanzania. Detection of viral DNA was demonstrated in a subset of clinically healthy animals and the principle was confirmed under laboratory settings.

These results are in line with the work published by Randriamparany et al. (2016) and Michaud et al., (2007). Here, ASFV diagnosis (and characterization) was successfully performed from dried-blood filter papers (experimental and field samples) over extended periods of time. Especially under tropical conditions, these approaches ensure suitability and stability for downstream applications without a cold chain and sophisticated transport. Randriamparany et al. could additionally demonstrate the suitability for antibody detection. In detail, the study showed that real-time UPL PCR from filter papers is as sensitive as conventional testing by virus isolation and conventional PCR, and ELISA from filter papers was comparable with the same assay from serum. No problems with specificity were encountered.

Along the same lines as the above mentioned FTA cards and filter papers, Petrov et al. (2014) could show that dry blood swabs (in general using different cotton, foam or tissue swabs) can be a valuable, stable and easy-to-handle method to test carcasses for ASFV (and CSFV) genomes. The advantage is that the swab is already combined with a shipment-suitable receptacle, and no direct contact or further equipment is needed. In the reported studies, so-called Genotubes (Thermofisher) were the optimum in terms of handling and stability. In subsequent studies, the suitability of these swabs for ASFV antibody detection by ELISA (in the protocol meant for filter paper punches) was demonstrated in a proof-of-concept study (Blome et al., 2014).

Recently, these initial data were supplemented by a broader validation study that also included the combination with antibody lateral flow devices (Carlson et al., submitted). The mentioned validation study showed the following performance characteristics for the Genotube based samples (when compared to routine diagnostic sample matrices and tests): qPCR 98.8% sensitivity [CI 93.4, 100.0] and 98.1% specificity [CI 90.1, 100.0] under laboratory conditions (85.7% [CI 71.5, 99.6] with stored field samples), and for serology by ELISA 93.1% sensitivity

[CI 83.3, 98.1] and 100% specificity [CI 95.9, 100.0]. Good agreement was found when using the above mentioned antibody LFDs from Ingenasa. This concept is particularly interesting as it was shown that it had almost no problems with bad sample quality.

For pathogenesis and immune response studies (correlates of protection and virus distribution in vaccination/challenge trial) but also diagnosis from necropsies, an optimized in situ hybridization protocol for the detection of African swine fever virus (ASFV) DNA in formalin-fixed, paraffin-embedded tissues using digoxigenin-labeled probes as been described by Ballester et al. (2015).

Genotyping

To better understand the molecular epidemiology of the recent outbreaks, additional genome markers are under investigation. Among them are different intergenetic regions.
Next-generation sequencing could be aided by enrichment through targeted sequence capture technology (Fernández Pinero, unpublished).

Conclusion

In conclusion, our tool box of diagnostic tests has grown considerably over the past years but there is still a need for harmonization, situation-adapted diagnostic workflows, and general knowledge of disease biology that helps us in further adjusting our methodologies. Optimized and harmonized workflows are needed for next-generation sequencing. However, it is important to note that in the last years the field of diagnostics has expanded into the search for new models of detection. Not only for the use of current techniques in new types of samples like feces (de Carvalho Ferreira et al. 2014; Nieto-Pelegrin et al., 2015), FTA cards (Braae et al. 2013), dry blood swabs (Petrov et al. 2014: Blome et al., 2014), and oral fluids (Mur et al., 2013; Grau et al., 2015; Gimenez-Lirola et al., 2016), but also searching for new models as air samples (de Carvalho Ferreira et al. 2013) and feed (Dee et al., 2018). This line of expansion will help to reach a more environmental and less invasive diagnostic as the industry desires.

Gaps

ASF is usually suspected based on clinical signs, but clinical evidence is usually nonspecific and would be difficult to differentiate from other diseases of swine, including Classical Swine Fever, Erysipelas, Salmonellosis, Eperythrozoonosis, Pasteurellosis, Pseudorabies, thrombocytopenic purpura, warfarin poisoning, and heavy metal toxicity. Regional labs in endemic countries lack the infrastructure and/or expertise for reliable diagnostic services. Some of the existing regional laboratories in Africa have limited capacity and most of them use the fluorescent tests and not real time RT-PCR.

The overarching gaps for diagnostics are:

1) There is a lack of commercial tests for large scale and confirmatory diagnostics
2) Validation of serological and virological tests for different epidemiological situations (e.g., low versus virulent ASFV strains).
3) Need to perform biological characterization and determine serotypes and pathotypes of current ASF strains that will provide the knowledge to extrapolate such characteristics using in vitro tests in the laboratory
4) In order to replace laborious and homogeneous primary cultures for virus isolation, cell cultures need to be found that support ASFV replication
5) The global ASF situation has to be taken into account when developing diagnostic assays. Tailored approaches could be an option for some scenarios.
6) For an early detection of the disease by serological methods, ELISA systems should be improved including the possible use of alternative sample matrices.
7) To understand the genetic diversity, studies targeting the sylvatic cycle hosts in Africa should be undertaken.
8) Pen-side tests and other field-deployable diagnostic tests need further investigation
9) There is an urgent need to increase the knowledge of the survivor pigs from the clinical and ASF diagnosis point of view.
10) New phylogenetic markers associated with pathogenicity should be looked at.
11) Field validation of new assays is needed taking into account fitness-for-purpose and the overall situation
12) There is a need to intensify training and follow-up activities for international harmonization of diagnostic tests.

Research needs

1) Identify/develop cell lines that replace primary cultures for improved virus isolation techniques.
2) Full validation of novel or modified ELISA tests for detection of antibodies in alternative sample types (e.g., blood, exudate´s tissues, oral fluids, meat juice, filter papers, etc.).
3) Improved stability of reagents in commercial diagnostic kits (molecular virological and serological assays) regarding shipment and expiration issues. This could be overcome by exploring different strategies such as gelification lyophilization and others.
4) Automation and standardization of viral genome sequencing for subtyping ASFV strains.
5) Expanded field validation of novel assays, taking into consideration the worldwide scenarios.
6) Development and evaluation of non-invasive sampling methodologies in wild suids.
7) Validate available penside diagnostic tools to enhance detection and improve surveillance in wild life in Africa.
8) Development, evaluation and field validation of commercial confirmatory serological tests.
9) Standardization and validation of ELISA tests to detect antibodies against *Ornithodoros* tick saliva antigens in bitten animals.
10) Improved knowledge of the role of the survivor pigs as potential shedders by the use of appropriated diagnostic serological and virological tests for identification/detection of these animals.
11) Study the effects and detection of low virulent isolates and persistent infections

EPIDEMIOLOGY

African swine fever was first reported in Africa in 1909 following the introduction of European domestic pigs in Kenya. It was characterized at the time as an acute hemorrhagic disease with mortality rates of 100 percent in domestic pigs (Montgomery *et al.* 1921). It was subsequently recognized that ASF had been present in southern and eastern Africa all along in wild suids (Penrith *et al.*, 2013). Africa has remained endemic and continues to experience a significant number of ASF outbreaks annually (see Table I). Newly infected countries in Africa rapidly become endemically infected; e.g., The Central African Republic, Chad, Ethiopia and a number of countries in West Africa (Achenbach *et al.*, 2016; Brown *et al.*, 2018).

The first spread of ASF outside Africa was in Portugal in 1957 as a result of waste from airline flights being fed to pigs near Lisbon airport (Costard *et al*., 2009; Gallardo *et al.,* 2015). Similar means of ASFV introduction were reported for the outbreak in Brazil in 1978 (Lyra 2006). All ASF introductions outside Africa were successfully eradicated, with the exception of the infection on the Island of Sardinia, Italy. However, in June 2007, an ASF outbreak was notified to the OIE in the Caucasus region by the Republic of Georgia, presumably caused by feeding pigs with ASFV contaminated pork brought in on ships from Africa (Rowlands et al., 2008). Since then, 16 new countries have reported ASF outbreaks, including Russia, Eastern Europe, Baltic countries (Wozniakowski *et al.*, 2016; Nurmoja *et al.*, 2017), Bulgaria, Romania,

Hungary, Czech Republic, Poland, Belgium, and on August 2018, the first report of an ASF outbreak was reported by China (see Table I).

The epidemiology of ASF may vary substantially between affected countries, regions and continents. Two types of transmission cycles have been defined for ASF based mainly on the mode of transmission of the virus among different pig populations: a domestic pig cycle and sylvatic-wild pig cycle (Costard *et al*., 2009). The presence/absence of arthropod vectors (i.e., tick species) in the affected area will impact the spread and maintenance of the virus in the environment (Plowright *et al*., 1994). In sub-Saharan Africa, ASFV is maintained in a sylvatic cycle between warthogs and ticks of the genus *Ornithodoros*. In endemic areas of Africa, infected ticks and warthogs are the source of virus responsible for disease outbreaks in domestic swine. Once established, virus is efficiently contact-transmitted between domestic swine (for review: Tulman *et al*., 2009). Infection through direct contact between domestic pigs and warthogs has not been observed (Costard *et al*., 2009). Thus, ASF may show unique regional patterns of presentation, associated with unique set of risk factors that should be assessed to establish proper surveillance and control strategies.

Twenty-four different *p72 genotypes* have been identified among virus isolates from sub-Saharan African countries. However, the use of *p72* for genotyping only provides an initial characterisation and does not directly provide data on cross immunity between the genotypes or their virulence. Outside the African continent, only isolates belonging to the West African *p72 genotype I,* had been detected. However, the Georgian 2007 outbreak, and all subsequent ASF outbreaks in the Caucasus region were attributed to a new isolate, related to *p72 genotype II,* circulating in South Eastern Africa since 1989. Since then, ASF has spread to neighbouring countries of Armenia, Azerbaijan and the Russian Federation, all the way into Central and Western Europe, and the most recent intrusion declared in China, which has the largest pig population in the world. The 2018 outbreak in Asia confirmed that the threat of ASF spreading to countries outside the African continent is high and is potentially devastating to the global pig industry.

Although the importance of ASF genotypes in the biology of the virus is not well understood, it has contributed to our knowledge of the distribution and evolution of ASFV. It will be important to continue to assess ASF strains in endemic region, as demonstrated in recent reports that have identified new genotypes in Mozambique (Quembo et al., 2017), and Ethiopia (Achenbach et al., 2017).

Based on currently available data, it is possible to delineate the following global distribution of ASFV types:

- West Africa (Genotype I)
- East and Central Africa (all genotypes known)
- Sardinia (Genotype I)
- Caucasus, Russia, Europe (Genotype II)
- China (Genotype II)

Gaps

There is a continuing need for knowledge on the molecular epidemiology of ASFV isolates mainly in relation to wild populations and ticks. The PCR based genotyping might be a tool in endemic areas like sub-Saharan Africa; however, in the event of an outbreaks in new geographical areas, the single most important task is to complete the sequencing of the viral genome. This will provide essential information not only about the potential origin of the virus but possible homologies to other strains.

Research needs

1) Continuing molecular epidemiology studies to monitor both captive and wild suid populations as well as soft tick distribution is essential to effectively address the ASFV problem in endemic areas. These studies are also of great importance for preventive and surveillance programs.
2) The development of ELISA for the detection of tick presence.
3) Need to intensify virus detection, isolation and characterization from sylvatic cycle hosts in Africa for genotyping purposes.
4) Ongoing biological and molecular characterization of currently circulating isolates in Africa and Europe.
5) To identify and apply new phylogenetic markers associated with virus virulence to understand virus evolution in endemic areas.
6) To better understand the socioeconomics of the disease and pig and pork value chains, particularly those related to low biosecurity settings.
7) To better understand the costs (direct and indirect) of ASF, both in epidemic and endemic situations
8) To identify better management tools to control the disease in wild boar.
9) To better understand the role played by the environmental contamination in the disease cycle.
10) The epidemiology of ASF in emergency control programs needs to be assessed and modelled on the level of the individual pig, the herd, and the demographics of the region (low versus high density pig populations).
11) Risk assessments need to be performed with regard to control or spread of ASFV.

SURVEILLANCE

The clinical presentation of ASF in domestic pigs depends on the virulence of circulating virus. Infection of domestic swine results in several forms of the disease, ranging from highly lethal acute manifestations to subclinical depending on contributing viral and host factors (Tulman *et al*., 2009).

Unlike domestic swine, wild African suids infected with ASFV are generally asymptomatic with low viremia titers (Heuschele and Coggins 1969; Montgomery 1921; Plowright 1981; Thomson 1985). These features of ASF presentation and the resemblance of the clinical manifestation to other diseases of swine hamper surveillance based exclusively on clinical signs. Based on the complexity of the epidemiology of ASF and multiple clinical manifestation of the disease it is necessary to develop surveillance activities based on diagnostic testing.

It is worth mentioning that none of the non-invasive methods proposed for pigs or wild boars would be effective for detection of virus because they do not shed virus. If they would chew

ropes it might be possible to detect antibodies. The main thing is that burrow ticks can be used for surveillance in warthogs although a lot have to be tested before a negative result would be considered reliable (Personal communication, Marie Louise Penrith).

Gaps

1) Surveillance is the most important countermeasure to be able to eliminate the disease at the source through early detection and containment of a disease outbreak. However, different surveillance strategies are required to detect the different clinical manifestations resulting from ASFV infections. For acute infection, surveillance activities can be based on clinical signs; however, for mild cases or chronic infections, where recognition of ASF symptoms is less likely, surveillance activities must be based on diagnostic testing to supplement surveillance based on clinical signs
2) Passive surveillance is often the only economically viable solutions for many countries but has many weaknesses due to the difficulty of differentiating ASF from Classical Swine Fever and from other common endemic infectious diseases of swine that may present similar clinical signs.
3) Active surveillance programs are expensive and currently must rely on direct diagnostic tests such as viral isolation and nucleic acid-based assays because of challenges and weaknesses of antibody-based assays.
4) For persistent infections, effective surveillance would be difficult and costly since no signs exist to raise the flag of suspicion. Surveillance activities could be based on herd level stillborn rates or other reproductive parameters. However, such an indicator may lack the specificity to be economically feasible. This category of infection represents a critical vulnerability in the design of a comprehensive ASF surveillance system.

Research needs

1) Evaluate under experimental conditions the performance and overall accuracy of currently available ELISAs and PCR tests for surveillance.
2) Develop and evaluate novel tests such as ELISAs for both antigen and antibody detection for surveillance.
3) Develop tests for detecting ASFV in ticks.
4) To validate outbreak surveillance measures, epidemiological investigations need to be performed on the implementation of emergency control measures and the use of ‘diagnostic tests to detect infected pigs in exposed populations.

FERAL SWINE AND WILD SUIDAE

Feral swine and wild suidae may have an important role in the spread and maintenance of ASF. Research is needed to further our understanding of the potential role of feral swine as a reservoir for ASF.

TICK VECTOR

There is an important need to identify if the ticks in an affected region (where ASF outbreak occurred) could become biological vectors or not. Critical research includes studies to determine whether the new ASFV isolate can productively infect local ticks and whether they become persistently infected. Research is needed to further understand the distribution of soft ticks.

COUNTERMEASURES ASSESSMENT

ASSUMPTIONS

The following captures assumptions made by GARA working groups to assess potential countermeasures to enhance our ability to contain and eradicate an outbreak of ASF.

Situation
Countermeasures assessed for worst case scenario: A coordinated intentional distribution of ASFV-contaminated material in a high density highly populated pig region of an ASF-free country.

Target Population
Countermeasures assessed for target pig production segments in priority order:

1. Backyard pigs
2. Comprehensive commercial swine operations (farrowing, nursery, and finishing)
3. Commercial indoor farrowing operations
4. Large intensive indoor pig farms
5. Valuable commercial genetic swine stock

Scope of Outbreak
Countermeasures assessed for multiple outbreaks occurring simultaneously in backyard pigs, three farrowing commercial operations, a finishing pig commercial operation, a sow replacement operation, and evidence of infection in feral swine.

Vaccine Administration
No vaccine available, therefore the only control strategy would be based in the early detection of infected animals and their elimination, and strict control of the movement of pigs.

DECISION MODEL

The gap analysis working groups used the quantitative Kemper-Trego (KT) decision model to assess available vaccines and diagnostics, including experimental products. Instructions for using the model were provided prior to GARA scientific workshops (see Appendix I). Criteria and weights in the model were modified by the working groups for the purpose of assessing available countermeasures as well as experimental ASF vaccines and diagnostics (See Appendices II, III, IV, and V).

Criteria
The working groups selected critical criteria to enable the comparison of countermeasures using a pertinent and valid analysis, as follows:

Vaccines
- Efficacy
- Safety
- One dose
- Speed to scale-up
- Storage
- Distribution/Supply
- Mass administration
- DIVA compatible
- Withdrawal period
- Cost to implement (cost of goods, cost of replacement, inventory costs, cost to administer)

Diagnostics
- Sensitivity
- Specificity
- Direct (antigen/DNA) detection, DIVA during outbreak
- Indirect (antibody) detection DIVA general and post-outbreak surveillance
- Validation to purpose
- Speed of scale-up
- Throughput
- Pen-side test
- Rapid result
- Need for a confirmatory test
- Easy to perform
- Storage/Distribution/Supply
- Cost to implement

Weight
Each criterion was weighted to allow a quantitative comparison of the impact of the selected interventions.

Product profile
To ensure a consistent and meaningful assessment, the desired product profile (i.e., the benchmark) was identified for each countermeasure:

Desired Vaccine Profile
1. Highly efficacious: prevents transmission; efficacy in all age pigs, including maternal antibody override; one year duration of immunity
2. Safe in all age pigs; no reversion to virulence for live vaccines
3. Only one dose is required
4. Rapid speed of production and scale-up, can deliver finished product quickly, and manufacturing method yields high number of doses
5. Expiration date of 24 months or greater
6. Manufacturer has effective storage and distribution capability
7. Quick onset of protection, 7-days or less
8. DIVA compatible: Can effectively and reliably differentiate infected from vaccinated animals
9. Short withdrawal period for food consumption
10. Cost of goods, cost of administration, cost of storage

Desired Diagnostic Test Profile
1. Detect all ASF genotypes
2. Direct tests for control and eradication
3. Indirect tests for post-control monitoring/detection subclinical cases
4. Rapid test- early detection
5. >95% specificity
6. >95% sensitivity
7. Pen-side test
8. DIVA Compatible
9. Field validated
10. Easy to perform/easily train personnel
11. Scalable
12. Reasonable cost

Values
The values assigned for each of the interventions reflect the collective best judgment of ASF working group members (see Appendices II, III, and IV).

VACCINES

The GARA gap analysis working groups noted that current research into a suitable vaccine for ASFV is limited to only a few research groups worldwide. A summary of experimental ASF vaccines reported in peer-reviewed scientific publications 2012-2018 is provided in Table II. To date, the most promising candidate vaccines are rationally attenuated gene-deleted recombinant live viruses. Previous work has highlighted both virulence and immunomodulation genes, which if removed would provide a strong candidate vaccine strain. The use of live attenuated viruses as vaccines is a well-established system with good protective attributes but evidence of reversion-to-virulence in some of the experimental vaccines tested to date is a concern. The use of recombination technology also allows for the insertion of suitable markers for the development of DIVA vaccines that would be particularly critical in any outbreak situation. There is currently no candidate isolate appropriately attenuated to ensure both safety and efficacy, but in the last few years several laboratories have progressed and are potentially getting closer to developing safe and effective experimental vaccine candidates. The alternative to a live attenuated virus that would remove any risk of reversion to virulence is the use of a subunit vaccine. This would satisfy both safety issues and ensure good DIVA characteristics. Although previous data indicated that such a strategy did not provide efficient protection against ASFV infection, there is currently some promising ongoing research into the feasibility of using such a strategy for producing an ASF vaccine. Collectively, progress in the development of a working vaccine and the preliminary results obtained to date indicate that a first generation vaccine may be feasible in the near future.

Summary

Vaccination against ASF is still not an option, but progress has been made towards the production of a rationally attenuated live virus vaccine.

Assessment of Experimental Vaccines

The GARA gap analysis working groups discussed the characteristics of the different available experimental vaccines. Following is a summary of the group's opinion for each of them.

1) *ASFV recombinant live attenuated gene-deleted vaccine:* Attenuated by deletion of specific genes that have been identified as virulence determinants. As result, attenuated virus strains are produced which has been shown to effectively prevent disease in animals challenged with the parental virulent virus around 28 days post vaccination. The WG recognizes the effectiveness of this experimental vaccine in terms of inducing efficient protection with only one dose, the rapid onset/duration of the induced immunity and the safety of the product along with the molecular basis for the development of DIVA test. Lack of heterologous protection is recognized as its main deficiency, although recent results obtained with some of these vaccines showed the presence of cross protection among genotypically different isolates.

2) *Subunit recombinant ASFV proteins expressed in different vaccine vectors:* Different recombinant vectors containing individual ASFV gene/s have been used; e.g., vaccinia, raccoon pox, Ankara, swine pox, and human adenovirus. Safety, rapid onset of immunity, possibility of developing a DIVA test and the cost of implementation are recognized as the strength of theses vaccine platforms. It is important to remark that so far, with the exception

of a very recent report showing preliminary data, there is no experimental evidence that an individual or a group of AFSV genes vectorized in any way can significantly protect domestic swine against the challenge with the homologous virus. Therefore, development of an ASFV subunit vaccine will depend on further research to identify protective antigens and the virus structures able to induce protection against the infection.

3) *ASFV DNA vaccines:* This is technically also a subunit vaccine where ASFV gene/s is cloned into DNA constructs that are used as immunogens. Its safety and the possibility to develop DIVA compatible diagnostic tests are the only strengths remarked by experts during the GARA Gap Analysis Workshops. As with vaccine candidates analyzed in *(2)* above, no candidate ASFV genes have to date been successfully identified so far to be used in a subunit vaccine.

Based in this assessment the ASFCWG decided that the most promising experimental vaccines are based on the use of rationally attenuated strains of ASFV. Nevertheless, the ASFCWG recognize that this candidate vaccine platform needs a great deal of experimental assessment in several aspects of its basic development, including induction of early immunity, development of a compatible DIVA test, and no reversion to virulence.

DIAGNOSTICS

The GARA gap analysis working groups determined the effectiveness of this countermeasure is high. Early detection of ASF is important to minimize spread of disease and reduce the economic impact. ASF surveillance in the U.S. is accomplished through a combination of passive and active surveillance programs. Diagnostic designed during the recovery phase post-outbreak are also essential.

Summary

- In case of any suspicious of the disease, virus and antibody detection techniques should be performed simultaneously.
- Antibody response to ASFV takes from 7-10 days. Animal surviving
- ASF virus can be detected from 2-3 dpi. The disease antibodies persist for long periods of time.
- Incubation periods, is around 3-15 days. The incubation period is usually 3–15 days. The more virulent strains produce peracute or acute haemorrhagic disease characterized by high fever, loss of appetite, haemorrhages in the skin and internal organs, and death in 3–10 days, sometimes even before the first clinical signs are observed.

Assessment of Laboratory and Commercial Diagnostic Tests (see Appendices III and IV). The GARA working groups identified and assessed six diagnostic tests to be used for surveillance, confirmation, and recovery. These tests are available for use in laboratories worldwide and one test is commercially available. The value of these tests was assessed against the desired diagnostic test profile for ASF control and eradication (See Decision Model, Appendix I).

1) *Virus isolation (VI)*
   Virus isolation in swine macrophages primary cell cultures is a classic technique for the detection of infectious virus. Virus infection is detected by hemadsorption or presence of cytopathogenic assay. The ASFCWG stressed the attributes of VI, including the specificity and sensitivity of the technique as well as the fact that results do not need further confirmation. However, the technique presents disadvantages as it takes several days to run the test, is difficult to scale up, the impossibility to adapt the technique in a throughput system, and the need for technical expertise to perform the test.

2) *Conventional RT-PCR.* This technique is based in the use of specific primers for conserved areas of p72. The technique present good specificity and sensitivity, has been validated, is easily scaled up and results are quickly obtained. Unfortunately, results need to be corroborated by a confirmatory technique and it is necessary to have technical expertise to perform the test.

3) *Real time RT-PCR.* The test present good specificity, results are quickly available, is easy to be adapted in a throughput system and easily scaled up. As with the conventional PCR, results need to be corroborated by a confirmatory technique and it is necessary to have technical expertise to perform the technique.

4) *Fluorescent antibody tests (FAT).* The assay consists of detecting virus in tissues of infected animals using fluorescent anti-ASFV specific antibodies. This test has high specificity, results quickly available, has been validated, is inexpensive, and provides definitive results. The disadvantages of this test are the difficulties for scaling up or set up in a throughput system, and it needs to be performed by a highly trained operator.

5) *Antigen ELISA.* This assay allows the detection of virus using a capture ELISA based on the use of anti-ASFV antibodies on the plate. The specificity is good, although the sensitivity is poor. This technique is easily scaled up as well as adapted to a throughput system. Additionally, it is easy to perform and results are obtained quickly. Beside its poor sensitivity, another disadvantage of the technique is lack of validation, it is expensive and results need to be confirmed by a second technique.

6) *Multiplex PCR assays:* A multiplex conventional RT-PCR is available for simultaneous and differential detection of ASFV and Classical Swine Fever Virus (CSFV) (Agüero *et al.*, 2004). The method is highly sensitive and specific and has been validated using field and experimental porcine clinical material. This test can be useful in case of clinical suspicion of swine hemorrhagic disease, as well as in those countries/areas where both viruses can be co-circulating at any time.

Assessment of Experimental Diagnostic Tests

The GARA gap analysis working groups identified and discussed several new technologies that are being considered for the detection of ASF in the laboratory or as pen-side tests for field use.

1) *Loop mediated isothermal amplification (LAMP)*: LAMP is based on amplification of nucleic acids without the need of PCR equipment. It requires only the combined use of a DNA polymerase with strand-displacement activity and four-six specially designed primers towards six regions of the DNA target (Notomi *et al.,* 2000). LAMP is described as a highly specific and sensitive tool, which allows the detection of amplified products even by the naked eye. The comparative simplicity of the technology makes LAMP adaptable to front-line testing in regional laboratories, simple diagnostic situations and even to pen-side testing as a rapid first-line tool. Several LAMP assays have been developed recently for ASFV detection, and standardisation and validation are currently ongoing (Hertjner and Allan, QUB, Belfast, UK).

2) *Real-time PCR assays using commercial universal probe libraries (UPL)***:** UPL was recently commercialized by *Roche Applied Science*, and is a collection of short hydrolysis DNA probes, originally designed for gene expression analysis and offered as a universal detection system. Currently, UPL probes are being applied also for pathogen detection, main advantages being reasonably low cost, short time of delivery, and ready-to-use format. The combination of a specific primer set and an appropriate UPL probe will allow specific and sensitive detection of ASFV by real-time PCR at a comparably lower cost. Two UPL real-time PCR assays, designed in different viral genome regions, have been developed and standardised recently for ASFV detection (Fernández-Pinero, Gallardo, and Arias, CISA-INIA, Valdeolmos, Spain). Validation for their suitability in diagnosis is in progress.

3) *Linear-After-The-Exponential (LATE)-PCR:* LATE-PCR is an advanced asymmetric PCR producing huge amount of ssDNA molecules, which are detected by the incorporation of a specific low-Tm probe. This tool provides several advantages, such as increased multiplexing capacity and faster thermocycling, compared to currently used PCR chemistries (Sánchez *et al*., 2004). A LATE-PCR method has just been developed for ASFV detection (Hakhverdyan, Stahl, and Belák, SVA, Uppsala, Sweden; in cooperation with Ronish and Wangh, Brandeis University, USA). The LATE technology is exclusively licensed by *Smiths Detection,* and the developed ASF assay will be adapted to their portable PCR platform *BioSeeq* to provide a robust, powerful and simple-to-use diagnostic system for onsite detection of ASFV in a wide range of environmental conditions.

4) *Lateral flow device (LFD)*: A one-step immunochromatographic strip (pen-side test) capable of specifically detecting anti-ASF antibodies in serum specimens is under development. The qualitative assay is based on a direct immunoassay in which the detector reagent is latex micro particles covalently coated with a purified ASFV protein. The capture reagent is a viral protein adsorbed on the nitrocellulose membrane strip to form a test line. A second line created above the test line, by the immobilization of anti-control protein antibodies, is used as a control of the test. A serum specimen is applied to the sample pad. The anti-specific antibodies present in the sample specifically bind to the labelled micro

particles. The antibody-protein binding complex formed migrates until the nitrocellulose membrane by the flow caused by capillary action and reacts with the immobilized viral protein, which generate a visible test line.

DISINFECTANTS/INACTIVATION

The survivability of ASFV in feces and urine of experimentally infected animals was recently investigated by Davies et al., 2017. Based on the calculated half-lives it can be assumed that ASPV remains infectious at 37°C for almost four (urine) or three (feces) days. In a study by Turner and William in 1999, it was shown that at 40°C, the inactivation of ASFV in pig manure is realized after 4 hours, and within 5 minutes at 65°C (See Table 4).

Disinfectants

The use of effective disinfectants for cleaning infected premises, trucks, and fomites is an important measure for preventing new introductions of ASF. However, many of the common disinfectants are ineffective. Care should be taken to use a disinfectant specifically approved for ASFV. A number of inactivation methods and disinfectants have been tested and reported for various materials, including animal waste. See Table IV for a complete list of disinfectants, field application, effective concentrations, exposure time, and references.

Rendering

Rendering of animal byproducts is heavily regulated in Europe and involves sterilization at 3 bar and 133°C for at least 20 min. Any process that exceeds 70°C for 20 min (or 60°C for 30 min) will inactivate ASFV (Plowright, W. and Parker, J., 1967); therefore, the rendering process will inactivate ASFV.

Biogas plant

African swine fever viruses can also be inactivated in a correctly operated biogas plant within hours (thermophilic) or days (mesophilic). The process does not only have temperature effects that inactivate the virus (pH value, metabolites, etc.). Nevertheless, it must be kept in mind that biogas plants are generally not designed in such a way that a strict black/white separation can take place (in contrast to high-security laboratories or modern rendering plants). For this reason, additional measures (preheating, adaptation of the process organization) would be necessary to achieve safe inactivation and protection against recontamination. In the doctoral thesis of Andres Moss (Moss A. 2001), a preheating of materials of animal origin to at least 70°C was proposed. However, this would be difficult to implement under field conditions.

Carcass Burial

Studies to assess ASFV inactivation in buried carcasses and soil are on-going but not yet completed at the time of publishing this report. For soil, the pH found in forest seems to be a limiting factor for virus survival. This report will be updated when the results of these experiments are completed.

ACARICIDES

Acaricides for controlling the soft tick may not be useful as the tick lives off the host and burrows underground as well as crevices in buildings. The best ASF method is to remove the pigs from infected premises.

DRUGS

There are no licensed anti-viral drugs available to treat pigs against ASF.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

ASF is not a human pathogen. PPE should be suitable to prevent farm-to-farm virus spread by animal health officials involved in eradication.

RECOMMENDATIONS

RESEARCH

The GARA recommends the implementation of the following research priorities to advance our ability to rapidly detect, control and respond to an ASF outbreak, including the progressive control and eradication of ASF in endemic settings.

Virology

- African swine fever virus complete genomic sequences from each genotype, viruses with different virulence, and viruses that have replicated exclusively in domestic pigs, wild pigs and ticks
- Generation of reference sequences that have been confirmed by different techniques in different laboratories to account of sequencing errors in repeat regions and other difficult stretches.
- Harmonization of sequencing workflows and validation of different enrichment techniques and host exclusion.
- ASFV bioinformatics resource to establish a comprehensive database that will include full length genome sequence of large number of isolates to replace the current less meaningful genotype based classification.

Viral Pathogenesis

- Basic parameters governing host to host infection, including domestic and wild swine as well as the insect host.
- Study the pathogenesis of ASFV isolates with different virulence in diverse susceptible host.
- Determine patterns of activation of immunologically relevant host genes particularly at early stages after infection.
- Identify ASFV genes and genetic determinants (group of genes like multigene families) involved in host range, virulence and pathogenicity. Correlate transcriptomics and proteomics.
- Continued investigation of determinants of virulence for different genotype and ASFV strains.

Immunology

- Discovery of the immune mechanism mediating effective homologous and heterologous protection against virus infection.
- Identification of viral genetic patterns that correlate with presence/absence of homologous versus heterologous protection.
- Identification of virus protein\s involved in the induction of protective immune response.
- Identify regulatory genes involved in pro-inflammatory cytokines and antibodies production and the assessment of their actual role in the process of virus infection\virulence in swine.
- Explore the development of new assays based on cellular immunity for the early detection of the disease.
- Explore immune-pathogenesis including T-cell responses and MHC presentation.

- Improve our understanding of the role of multigene families in antigenic variability and evasion of immune response.
- Identify and characterize genes related to host protection.

Epidemiology

- A global ASF surveillance system that provides high quality, accurate, and real-time information on ASF risk is needed to cover critical gaps of information of the ASF situation worldwide and to support ASF control and eradication on a global scale.
- Continuing molecular epidemiology studies to monitor both captive and wild suid populations as well as soft tick distribution is essential to effectively address the ASFV problem in endemic areas. These studies are also of great importance for preventive and surveillance programs.
- Conduct field validation of existing ELISA for the detection of tick presence.
- Need to intensify virus detection, isolation and characterization from sylvatic cycle hosts in Africa for genotyping purposes.
- Ongoing biological and molecular characterization of currently circulating isolates in Africa and Europe.
- To identify and apply new phylogenetic markers associated with virus virulence to understand virus evolution in endemic areas.
- To better understand the socioeconomics of the disease and pig and pork value chains, particularly those related to low biosecurity settings.
- To better understand the costs (direct and indirect) of ASF, both in epidemic and endemic situations.
- Identify management tools to control the disease in wild boar
- To better understand the role played by the environmental contamination in the disease cycle.
- Improved knowledge of the role of the survivor pigs as potential shedders by the use of appropriated diagnostic serological and virological tests for identification/detection of these animals.

Surveillance

- Further evaluate performance and overall accuracy of currently available ELISAs and PCR tests under experimental and field conditions. Evaluate under experimental conditions the performance and overall accuracy of currently available ELISAs and PCR tests.
- Automation and standardization of viral genome sequencing for subtyping ASFV strains
- Assess the rate of transmission of strains of ASFV of different virulence in infected-contact animal experiments.
- The epidemiology of ASF in emergency control programs needs to be assessed and modelled on the level of the individual pig, the herd, and the demographics of the region (low versus high density pig populations).
- Risk assessments need to be performed with regard to control or spread of ASFV

Diagnostics

- Support the development of new technologies for pen-side testing
- Evaluate and validate commercially available pen-side tests to "fit for purpose" for surveillance, response, and recovery
- Identify/develop cell lines that replace primary cultures for improved virus isolation techniques.
- Full validation of novel or modified ELISA tests for detection of antibodies in alternative sample types (e.g., blood, exudate´s tissues, oral fluids, meat juice, filter papers, etc.).
- Improved stability of reagents in commercial diagnostic kits (molecular virological and serological assays) regarding shipment and expiration issues. This could be overcome by exploring different strategies such as gelification lyophilization and others.
- Expanded field validation of novel assays, taking into consideration the worldwide scenarios.
- Development and evaluation of non-invasive sampling methodologies in wild suids.
- Validate available penside diagnostic tools to enhance detection and improve surveillance in wild life in Africa.
- Development, evaluation and field validation of commercial confirmatory serological tests.
- Standardization and validation of ELISA tests to detect antibodies against *Ornithodoros* tick saliva antigens in bitten animals.
- Study the effects and detection of low virulent isolates and persistent infections

Vaccines

- ASFV virology and functional genomics studies to inform vaccine discovery research.
- Determine safety characteristics associated with experimental live attenuated vaccines.
- Identify alveolar macrophage genes that enable ASF viral growth to inform the development of a cell line for vaccine production.
- Further explore the engineering of gene-deleted ASFV as potential vaccine candidates.
- There is a need for inter-laboratory testing of vaccine candidates.
- Harmonize challenge tests and read-outs.
- Continue to explore the potential for effective subunit vaccines.
- Research potential antigenic vaccine markers to differentiate infected from vaccinated animals (DIVA).
- Develop baits to enable the effective oral vaccination of wild boars.
- Develop and validate effective parenteral routes for live vaccine administration
- Proof-of-concept testing of needle-free systems for the delivery of new ASF molecular vaccines.

Biotherapeutics

- Testing Ad5-IFN distribution and expression in swine for rapid onset of protection against ASFV infection.

Disinfectants

- Development of low cost commercially available disinfectants for use in the inactivation of ASFV on contaminated surfaces found in farm settings and other susceptible environments.
- Explore the use of disinfectants to reduce the risk of ASFV infections from ASFV-infected carcasses.

Feral Swine and Wild Suidae

- Conduct research projects to further our understanding of the potential role of feral swine and wild suids as a reservoir for ASF.

Tick Vector

- Identify if the ticks in new geographical areas where ASF outbreak occur could become biological vectors or not.
- Determine whether new ASFV isolates can productively infect local ticks and whether they become persistently infected.
- Research is needed to further understand the distribution of soft ticks.

PREPAREDNESS

Many of the countermeasures discussed in this report will require planning, preparation, and integration into a coordinated disease control program. Critical will be funding for veterinary medical countermeasures to be stockpiled for use in an emergency response plan for an outbreak of ASF. The GARA recommends investing in the implementation of research priorities to support preparedness plans and ensure the effective use of countermeasures to prevent, control, and eradicate ASF.

CONCLUSION

African swine fever is a transboundary animal disease that currently threatens swine production worldwide. Even though ASF is an African disease, it is now well entrenched in the Caucasus, Russia, Europe, and currently threatening Asia. The most significant cause of this recent geographical spread is most likely due to the illegal movement of animals, trade, and contaminated products. This places other countries that trade in pig and pig products in danger, including Europe, South America, and North America. Furthermore, the epidemiological implications of ASF outbreaks in new geographical and ecologically unique environments are unknown, complicating control measures. Surveillance programs will be the first line of defense against ASF. Diagnostic tests are available and need to be incorporated in diagnostic laboratories. A key control measure will be vaccines but they are currently unavailable, a major gap in the availability of countermeasures to control ASF outbreaks.

FIGURE 1: ASFV PHYLOGENETIC ANALYSIS

Figure 1: The extent of the ASF virus diversity as revealed by a phylogenetic tree of p72 genotyping revealing ASF Genotypes (Quembo CJ *et al,* 2018)

TABLE I: ASF OUTBREAKS 2007-2018

COUNTRY	DATE OF LAST ASF OUTBREAK	REFERENCE/SOURCE
Belgium	September 2018	https://www.globalmeatnews.com/Article/2018/09/14/African-Swine-Fever-reaches-Belgium
China	August 2018	www.thepigsite.com
Romania	August 2018	https://www.brownsvilleherald.com
Malawi	July 2018	outbreaknewstoday.com/Malawi
Latvia	Aug. 2018	https://eng.lsm.lv/article/.../massive-outbreak-of-swine-fever-in-western-latvia.a28740...
Hungary	April 2018	https://www.pigprogress.net/Health
South Africa	May 2018	https://www.reuters.com
Tanzania	May 2018	www.xinhuanet.com
Moldova	May 2018	https://www.moldpres.md/en/news/2018/05/12/18003882
Poland	Jan. 2018	https://www.pigprogress.net
Uganda	2018	Charles Masembe personal communication
Zambia	2018	https://www.farmersweekly.co.za
Kenya	2018	https://www.nation.co.ke › Business › Seeds of Gold
Ghana	2018	www.thepigsite.com
Guinea-Bissau	2018	http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Burkina Faso	May 2018	http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Nigeria	Feb 2018	http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Estonia	Dec. 2017	www.fao.org/fileadmin/user_upload/reu/europe /documents/.../ASF.../3_estonia.pdf
Burundi	Dec 2017	http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Belarus	Nov. 2017	https://www.pigprogress.net/.../Belarus-independent-media-report-ASF-outbreaks-208...
Côte d'Ivoire	Sept 2017	https://www.oie.int http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Lithuania	July 2017	www.xinhuanet.com/english/2017-07/14/c_136442000.htm
Czech Republic	June 2017	www.izs.it
Siberia	2017	https://wwwnc.cdc.gov/eid
Russia	2017 (Since 2007)	www.thepigsite.com
Ukraine	2017 (Since 2012)	www.thepigsite.com
Central African Rep.	2017	http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Rwanda	2017	http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Mozambique	2016 2017	https://allafrica.com/stories/201608291332.html http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Togo	2016 2017	www.xinhuanet.com/english/2016-06/26/c_135466133.htm http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Congo Rep.	2016	http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail

Mali	Jan. 2016	http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Cape Verde	2015	www.thepigsite.com
Zimbabwe	2015	https://www.wattagnet.com
Benin	Dec 2017	http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Chad	2014	https://www.wattagnet.com/.../20071-chad-has-first-african-swine-fever-outbreak-sinc...
Armenia	2011	https://www.pigprogress.net/.../African-Swine-Fever-outbreaks-in-Armenia-PP004563W...
Angola	2011 2015	www.healthmap.org/site/diseasedaily/article/african-swine-fever-angola-12611 http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Cameroon	2011 2017	https://www.pigprogress.net/.../African-Swine-Fever-hits-Cameroon-100000-animals-co...; http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Madagascar	2010 2017	Charles Masembe personal communication http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Democratic Republic of Congo	2010 2017	Charles Masembe personal communication http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Namibia	2009 2018	https://www.pigprogress.net/ http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Azerbaijan	2008	www.today.az/view.php?id=42715
Georgia	2007	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2634662/
Mauritius	2007	https://www.pigprogress.net/.../African-Swine-Fever-outbreak-in-Mauritius-PP000994W...
Senegal	2007 March 2018	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3204638/ http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail
Gambia	2007 2017	www.africanagriculture.co.zw/2007/08/swine-fever-hits-gambia.html http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail

TABLE II: SUMMARY OF COMPLETE GENOMES AVAILABLE ON NCBI

Name	Assession	Collection date	Country	Host
Odintsovo_02/14	KP843857.1	2014	Russia	Wild Boar
26544/OG10	KM102979.1	2010	Italy: Sardinia	Domestic pig
47/Ss/2008	KX354450.1	2008	Italy: Province of Sassari, Sardinia)	Domestic pig
Georgia 2007/1	FR682468.1	2007	Georgia	Domestic pig
Ken06.Bus	KM111295.1	2006	Kenya	Domestic pig
Ken05/Tk1	KM111294.1	2005	Kenya	Tick
OURT 88/3	AM712240.1	1988	Portugal: Alentejo	Tick
Pretorisuskop/96/4	AY261363	1996	South Africa: Kruger National Park	Tick
Malawi Lil-20/1	AY261361	1983	Malawi:Calaswa	Tick
Mkuzi 1979	AY261362.1	1979	South Africa: Mkuzi Game Resercve	Tick
BA71V	NC_001659.2 U18466.2	1971	Spain	Vero adapted
NHV	KM262846	1968	Portugal	Domestic pig
Tengani 62	AY261364	1962	Malawi: Tengani, Nsanje District	Domestic pig
L60	KM262844	1960	Portugal	Domestic pig
Kenya 1950	AY261360	1950	Kenya	Domestic pig
E75	FN557520.1	1975	Spain	Domestic pig
warthog	AY261366.1	Pre-2003	Namibia	Warthog
Warmbaths	AY261365	Pre-2003	South Africa: warmbaths	Tick
Benin 97/1	AM712239.1	1997	Benin	Domestic pig

TABLE III: ASF EXPERIMENTAL VACCINES

Type of Vaccine	Reference	Protection	Safety
Subunit vaccine: Immunization of pigs by DNA and recombinant vaccinia virus identify ASFV immunogenic proteins.	J Virol. 2018 pii: JVI.02219-17 9	No	Yes
LAV: Protection of pigs with deletion mutant of MGF genes in ASFV Benin by different doses and routes.	Vaccine. 2018 36:707-715	Yes	Not Tested
LAV: Deletion of ASFV Gene DP148R Reduces Virus Virulence in Pigs and Induces Protection against Challenge	J Virol. 2017 91(24). pii: e01428-17	Yes	Not Tested
LAV: BA71ΔCD2: Recombinant Live Attenuated ASFV with Cross-Protective Capabilities.	J Virol. 2017 91(21). pii: e01058-17	Yes	Not Tested
LAV: Adapted ASFV strain Congo is a LAV protecting against parental virulent virus.	Arch Virol. 2017 (10):3081-3088	Yes	Not Tested
Subunit vaccine: Adenovirus-vectored novel ASFV antigens elicit robust immune responses in swine.	PLoS One. 2017 12(5): e0177007	Not Tested	Yes
Subunit vaccine: Safety and immunogenicity of Modified Vaccinia Ankara vectored ASFV subunit antigens in swine.	Vet Immunol Immunopathol. 2017 Mar;185:20-33	Not Tested	Yes
LAV: Naturally attenuated ASFV OURT88/3 protects against virulent homologous field isolate.	Antiviral Res. 2017 138:1-8	Yes	No
LAV: Simultaneous Deletion of the 9GL and UK Genes from the ASFV Georgia 2007 Isolate Increased Safety and Protection against Homologous Challenge.	J Virol. 2016 91(1). pii: e01760-16	Yes	Not Tested
LAV: Deletion of 9GL in Pret4 strain protects against challenge with virulent parental isolate	Viruses. 2016 8(10). pii: E291	Yes	Not Tested
Subunit vaccine: Induction of Immune Responses in Swine by Using a Cocktail of Adenovirus-Vectored ASFV.	Clin Vaccine Immunol. 2016 23(11):888-900	Not Tested	Yes
LAV: Deletion of MGF genes in ASFV Bening isolate reduces virulence in domestic pigs and induces a protective response.	Vaccine. 2016 34(39):4698-4705	Yes	Not Tested
LAV: ASFV Georgia isolate harboring deletions of 9GL and MGF360/505 genes is highly attenuated but does not confer protection against parental virus challenge.	Virus Res. 2016 Aug 2;221:8-14	No	Yes
LAV: ASFV proteins CD2 and Lectine confer serotype-specific protection. A model using adapted attenuated strains.	J Gen Virol. 2015 96(Pt 4):866-73.	Yes	Not Tested
LAV: ASFV Georgia 2007 with a Deletion in 9GL gene Leads to Attenuation and Induces an Effective Protection against Homologous Challenge.	J Virol. 2015 89(16):8556-66	Yes	No
LAV: ASFV Georgia Isolate Harboring Deletions of MGF360 and MGF505 Genes Is Attenuated and Confers Protection against Challenge with Virulent Parental Virus.	J Virol. 2015 89(11):6048-56	Yes	Not Tested
LAV: Naturally attenuated ASFV OURT88/3 Induces Protection Against Challenge with Virulent Strains of Genotype I.	Transbound Emerg Dis. 2016 63(5):e323-7	Yes	No
Subunit vaccine: Inactivated virus and use of Modern adjuvants do not enhance the efficacy of an ASFV vaccine.	Vaccine. 2014 Jun 30;32(31):3879-82	No	Yes
LAV: 9GL and UK genes deletion from attenuated ASFV OUR T88/3 decreases its ability to protect against challenge.	Virology. 2013 Aug 15;443(1):99-105	No	No Tested
Subunit vaccine: DNA immunization partially protects pigs against sublethal challenge ASFV.	Antiviral Res. 2013 98(1):61-5	No	Yes
Subunit vaccine: DNA vaccination partially protects against ASFV lethal challenge.	PLoS One. 2012;7(9):e40942	No	Yes

TABLE IV: DISINFECTANTS FOR INACTIVATING AFRICAN SWINE FEVER VIRUS

Disinfectant	Field of application	Concentration	Exposure time	Reference
Sulfuric acid	surface disinfectant	1%	15 min	Fauser-Leiensetter, 2000
	liquid manure	1%	recommendation: 1 week	Fauser-Leiensetter, 2000
Formic acid	surface disinfectant	1%	15 min	Fauser-Leiensetter, 2000
	liquid manure	4%	recommendation: 1 week	Fauser-Leiensetter, 2000
Peracetic acid	surface disinfectant	2%	15 min	Fauser-Leiensetter, 2000
Formaldehyde	surface disinfectant	0%	15 min	Fauser-Leiensetter, 2000
	liquid manure	1%	recommendation: 1 week	Fauser-Leiensetter, 2000
	liquid manure	0.50%	> 4 days	Shirai et al., 1999, zitiert im EFSA Scientific Review
Sodium dodecyl sulfate	surface disinfectant	3%	15 min	Fauser-Leiensetter, 2000
	liquid manure	3%	recommendation: 1 week	Fauser-Leiensetter, 2000
Glutaraldehyde solution	surface disinfectant	1%	30 min	Fauser-Leiensetter, 2000
	liquid manure	1%	recommendation: 1 week	Fauser-Leiensetter, 2000
	tissue	0.2 %	11 days	Cunliffe et al., 1979, zitiert im EFSA Scientific Review
Sodium hydroxide solution	surface disinfectant	0.50%	30 min	Fauser-Leiensetter, 2000
	liquid manure	4%	recommendation: 1 week	Fauser-Leiensetter, 2000
	liquid manure	1%	150 s (4°C)	Turner und Williams, 1999
	liquid manure	1%	30 min (4 °C)	Turner und Williams, 1999
Citric acid	surface disinfectant	2%	30 min (22°C)	Krug et al., 2012
Caustic lime	dung pack			Bergerdorf et al., 1989, cited by Haas et al., 1995
Iodine		0,015 bis 0,0075 % (potassium iodide)		Shirai et al., 1999
Ortho-phenylphenol		1%	1 h	EFSA Scientific Review; OIE; Stone und Hess, 1973
Chloride, Hypochlorite	surface disinfectant	0,03 bis 0,0075 % sodium hypochloride		Shirai et al., 1999, zitiert im EFSA Scientific Review
	surface disinfectant	2,3 % Chlor	30 min	OIE

	surface disinfectant	0,15 % / 0,2 % als Natrium hypochloride		Krug et al., 2012
Quarterary ammonium compounds	surface disinfectant	0.003%		Shirai et al., 1999, zitiert im EFSA Scientific Review
Lime $Ca(OH)_2$	liquid manure	1%	150 s (4°C)	Turner und Williams, 1999
	liquid manure	1%	30 min (4 °C)	Turner und Williams, 1999
Heat	pig slurry	65 °C	5 min	Turner und Williams, 1999

APPENDIX I: COUNTERMEASURES WORKING GROUP INSTRUCTIONS

Decision Model

We will use a decision model to assess potential countermeasures to stockpile. These countermeasures must significantly improve our ability to control and eradicate an outbreak of African Swine Fever (ASF) in an ASF-free country such as the United States. The decision model is a simple tool that will allow us to focus on critical criteria for the National Veterinary Stockpile, and rank the available interventions relative to each other. The decision model is available as a Microsoft Excel spread sheet, which has been prepared to quantitatively assess the rankings we assign to a set of selected criteria that will lead to the selection of the highest cumulative option. We can use as many criteria as we want but the objective is to get down to the ones that will make or break success. The criteria for each intervention will be selected by the ASF Countermeasures Working Group, but a preliminary set has been identified to expedite the process. You are encouraged to review the criteria prior to coming to the meeting and be prepared to modify the criteria as needed with the working group. The following provides an example of criteria and assumptions for assessing vaccines.

Criteria

If a vaccine is going to be used as an emergency outbreak control tool for ASF, then we need to know: 1) is it efficacious (does it effectively eliminate shedding or just reduce shed by a known log scale); 2) does it work rapidly with one dose (probably do not have time for a second dose); 3) whether it is available today from the perspective of having a reliable & rapid manufacturing process (need to know it can be up & running rapidly and will yield a predictable amount of vaccine; 4) can we get the product to the outbreak site rapidly & safely; 5) once at the site, can we get it into the target population rapidly; 6) type of administration- mass or injected, people and equipment to do the job become important); and 7) are diagnostics available to monitor success and or DIVA compliant. While cost is important, the cost of the vaccine in an outbreak will be small in comparison to the other costs. In addition, how fast the product can be made is important because that will have a big impact on how big a stockpile will be needed. Accordingly, you will see from the Excel sheets that have been prepared for vaccines that the following critical criteria and assignment of weights for each criterion are proposed.

Weight	Critical Criteria
10	Efficacy
6	Safety
8	One dose
6	Speed of Scale up
2	Shelf life
2	Distribution/storage
10	Quick Onset of Immunity
8	DIVA Compatible
2	Withdrawal
2	Cost to Implement

Cyril Gerard Gay, DVM, Ph.D
Senior National Program Leader
Animal Production and Protection
Agricultural Research Service

APPENDIX II: EXPERIMENTAL VACCINES

Experimental Vaccines for ASF

Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed

Weight	Critical Criteria	Recombinant Gene Deleted	Recombinant Vaccinia-vectored	Recombinant Swinepox-Vectored	DNA Vaccine		
10	Efficacy	10	4	4	2		
8	Safety	8	8	4	10		
8	One dose	8	6	6	2		
8	Cross-Protection	2	2	2	2		
10	Onset of Immunity	6	6	6	2		
4	Distribution/Supply	8	8	8	8		
6	Mass Administration	8	8	8	4		
6	Duration of Immunity	8	6	6	4		
8	DIVA Compatible	8	8	8	8		
6	Shelf-Life	6	6	6	8		
6	Cost to Implement	4	8	6	4		

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	mbinant Gene De	binant Vaccinia-v	inant Swinepox-V	DNA Vaccine	0	0
Efficacy	100	40	40	20	0	0
Safety	64	64	32	80	0	0
One dose	64	48	48	16	0	0
Cross-Protection	16	16	16	16	0	0
Onset of Immunity	60	60	60	20	0	0
Distribution/Supply	32	32	32	32	0	0
Mass Administration	48	48	48	24	0	0
Duration of Immunity	48	36	36	24	0	0
DIVA Compatible	64	64	64	64	0	0
Shelf-Life	36	36	36	48	0	0
Cost to Implement	24	48	36	24	0	0
Value	556	492	448	368	0	0

Major Assumptions:

Vaccine Profile

1. Highly efficacious: prevent transmission; efficacy in all age pigs , cross protection across all ASF viral strains; quick onset of immunity; one year duration of immunity, one shot.
2. Safe in all age pigs; no reversion to virulence for live vaccines
3. DIVA compatible
4. Manufacturing method yields high number of doses
5. Mass vaccination compatible to eliminate individual pig inoculation
6. Rapid speed of production and scale-up
7. Reasonable cost
8. Short withdrawal period for food consumption
9. Long shelf life
10. Distribution and supply (determined on need for diluent and freezer versus refrigerated space)
11. Cost to implement, cost of goods, cost of administration, cost of storage

APPENDIX III: DIAGNOSTICS FOR SURVEILLANCE

SURVEILLANCE: Commercial and reference Diagnostics for African Swine Fever

Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed

Weight	Critical Criteria	ELISA K3	ELISA OIE	IB test	IIF test	rtimePCR-King	PCR AGÜERO	VI	DIF	Antigen ELISA K2
8	Sensitivity	8	8	10	8	6	8	8	6	2
10	Specificity	8	6	6	8	8	8	10	8	8
10	Validation to purpose	8	8	8	6	8	10	8	8	2
6	Speed of Scaleup	8	6	2	4	8	8	2	4	8
6	Throughput	8	6	2	2	8	6	2	2	8
2	Pen-Side Test	0	0	0	0	0	0	0	0	0
6	Rapid Result	8	8	8	8	8	8	2	8	8
8	Definitive results	2	2	8	8	4	4	8	8	2
6	Easy to perform	8	8	8	4	6	6	2	4	8
6	Expertise	10	8	6	2	4	4	2	2	10
4	Cost to Implement	2	10	4	8	4	6	6	8	2

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	ELISA K3	ELISA OIE	IB test	IIF test	rtimePCR-King	PCR AGÜERO	VI	DIF	Antigen ELISA K2
Sensitivity	64	64	80	64	48	64	64	48	16
Specificity	80	60	60	80	80	80	100	80	80
Validation to purpose	80	80	80	60	80	100	80	80	20
Speed of Scaleup	48	36	12	24	48	48	12	24	48
Throughput	48	36	12	12	48	36	12	12	48
Pen-Side Test	0	0	0	0	0	0	0	0	0
Rapid Result	48	48	48	48	48	48	12	48	48
Definitive results	16	16	64	64	32	32	64	64	16
Easy to perform	48	48	48	24	36	36	12	24	48
Expertise	60	48	36	12	24	24	12	12	60
Cost to Implement	8	40	16	32	16	24	24	32	8
Value	500	476	456	420	460	492	392	424	392

Major Assumptions for surveillance:

Diagnostic Test Profile

1. Detect all ASFV isolates.
2. Direct and indirect tests.
3. >95% specificity
4. >95% sensitivity
5. Validated
6. Rapid test.
7. Easy to perform
8. Scalable
9. Reasonable cost
10. Pen-side test
11. Expertise

APPENDIX IV: DIAGNOSTICS FOR RESPONDING TO A DISEASE OUTBREAK

OUTBREAK: Commercial and Reference Diagnostics for African Swine Fever

Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed

Weight	Critical Criteria	ELISA K3	ELISA OIE	IB test	IIF test	timePCR-King	PCR AGÜERO	VI	DIF	ntigen ELISA K2
10	Sensitivity	8	8	10	8	8	8	8	6	2
8	Specificity	8	6	6	8	8	8	10	8	8
8	alidation to purpos	8	8	8	6	8	10	8	8	2
8	Speed of Scaleup	8	6	2	4	8	8	2	4	8
8	Throughput	8	6	2	2	8	6	2	2	8
2	Pen-Side Test	0	0	0	0	0	0	0	0	0
10	Rapid Result	8	8	8	8	10	8	2	8	8
6	Definitive results	2	2	8	8	4	4	8	8	2
6	Easy to perform	8	8	8	4	6	6	2	4	8
8	Expertise	10	8	6	2	4	4	2	2	6
4	Cost to Implement	2	10	4	8	4	6	6	8	2

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	ELISA K3	ELISA OIE	IB test	IIFtest	timePCR-King	PCR AGÜERO	VI	DIF	ntigen ELISA K2
Sensitivity	80	80	100	80	80	80	80	60	20
Specificity	64	48	48	64	64	64	80	64	64
Validation to purpos	64	64	64	48	64	80	64	64	16
Speed of Scaleup	64	48	16	32	64	64	16	32	64
Throughput	64	48	16	16	64	48	16	16	64
Pen-Side Test	0	0	0	0	0	0	0	0	0
Rapid Result	80	80	80	80	100	80	20	80	80
Definitive results	12	12	48	48	24	24	48	48	12
Easy to perform	48	48	48	24	36	36	12	24	48
Expertise	80	64	48	16	32	32	16	16	48
Cost to Implement	8	40	16	32	16	24	24	32	8
Value	564	532	484	440	544	532	376	436	424

Major Assumptions in outbreak:

Diagnostic Test Profile

1. Detect all ASFV isolates.
2. Direct and indirect tests.
3. >95% specificity
4. >95% sensitivity
5. Validated
6. Rapid test.
7. Easy to perform
8. Scalable
9. Reasonable cost
10. Pen-side test
11. Expertise

APPENDIX V: EXPERIMENTAL DIAGNOSTICS FOR SURVEILLANCE

Surveillance: Experimental Diagnostics for African Swine Fever						
Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed						
Weight	**Critical Criteria**	**HT-rELISA**	p30-rIB	HT-rIB	IPT test	Fast rtimePCR-King
8	Sensitivity	10	8	8	8	8
10	Specificity	8	10	10	8	8
10	Validation to purpose	8	8	8	6	8
6	Speed of Scaleup	8	2	2	4	8
6	Throughput	8	2	2	2	10
2	Pen-Side Test	0	0	0	0	0
6	Rapid Result	6	8	8	8	8
8	Definitive results	2	8	8	8	6
6	Easy to perform	6	8	8	4	8
6	Expertise	6	8	8	2	4
4	Cost to Implement	4	2	2	8	4

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	HT-rELISA	p30-rIB	HT-rIB	IPT test	Fast rtimePCR-King
Sensitivity	80	64	64	64	64
Specificity	80	100	100	80	80
Validation to purpose	80	80	80	60	80
Speed of Scaleup	48	12	12	24	48
Throughput	48	12	12	12	60
Pen-Side Test	0	0	0	0	0
Rapid Result	36	48	48	48	48
Definitive results	16	64	64	64	48
Easy to perform	36	48	48	24	48
Expertise	36	48	48	12	24
Cost to Implement	16	8	8	32	16
Value	476	484	484	420	516

Major Assumptions for surveillance:

Diagnostic Test Profile

1. Detect all ASFV isolates.
2. Direct and indirect tests.
3. >95% specificity
4. >95% sensitivity
5. Validated
6. Rapid test.
7. Easy to perform
8. Scalable
9. Reasonable cost
10. Pen-side test
11. Expertise

APPENDIX VI: EXPERIMENTAL DIAGNOSTICS FOR RESPONDING TO A DISEASE OUTBREAK

Outbreak: Experimental Diagnostics for African Swine Fever						
Rank each Intervention (2,4,6,8, or 10) as to its importance to making a decision, only one "10" rankings allowed						
Weight	**Critical Criteria**	**HT-rELISA**	p30-rIB	HT-rIB	IPT test	Fast rtimePCR-King
10	Sensitivity	10	10	10	8	8
8	Specificity	8	8	8	8	8
8	Validation to purpose	8	8	8	6	8
8	Speed of Scaleup	8	2	2	4	8
8	Throughput	8	2	2	2	8
2	Pen-Side Test	0	0	0	0	0
10	Rapid Result	8	8	8	8	10
6	Definitive results	6	8	8	8	6
6	Easy to perform	6	8	8	4	8
8	Expertise	6	8	8	2	4
4	Cost to Implement	4	2	2	8	4

Rank each Criteria 2,4,6,8 or10 on each criterion -- no more than two "10" rankings allowed

Critical Criteria	HT-rELISA	p30-rIB	HT-rIB	IPT test	Fast rtimePCR-King
Sensitivity	100	100	100	80	80
Specificity	64	64	64	64	64
Validation to purpose	64	64	64	48	64
Speed of Scaleup	64	16	16	32	64
Throughput	64	16	16	16	64
Pen-Side Test	0	0	0	0	0
Rapid Result	80	80	80	80	100
Definitive results	36	48	48	48	36
Easy to perform	36	48	48	24	48
Expertise	48	64	64	16	32
Cost to Implement	16	8	8	32	16
Value	572	508	508	440	568

Major Assumptions in outbreak:

Diagnostic Test Profile

1. Detect all ASFV isolates.
2. Direct and indirect tests.
3. >95% specificity
4. >95% sensitivity
5. Validated
6. Rapid test.
7. Easy to perform
8. Scalable
9. Reasonable cost
10. Pen-side test
11. Expertise

APPENDIX VII: REPORT CONTRIBUTORS

Covadonga Alonso (GARA Chief Executive Officer, 2013-2019)
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Otra. de la Coruña Km 7,5, Madrid, Spain
Tel: +34 91 347 6896
E-mail: calonso@inia.es

Marisa Arias, Ph.D
Director
Animal Health Research Centre -Centro de Investigación en Sanidad Animal-
EU Reference Laboratory for African Swine Fever
FAO Reference Centre for African Swine Fever
Instituto Nacional de Investigación y Tecnología
Agraria y Alimentaria (INIA-CISA),
Valdeolmos, 28130, Madrid, SPAIN.
Tel: +34 916202300
Fax:+34 916202247
e-mail: arias@inia.es

Jonathan Arzt, DVM, Ph.D
Veterinary Pathologist
Foreign Animal Disease Unit
Plum Island Animal Disease Center
Agricultural Research Service (ARS)
United States Department of Agriculture (USDA)
P.O. Box 848
Greenport, NY 11944
Tel (631) 631-323-3249
jonathan.arzt@ars.usda.gov

Emmanuel Awosanya, DVM, PhD
Senior lecturer (Veterinary Epidemiology)
Department of Veterinary Public Health and Preventive Medicine,
University of Ibadan, Ibadan, Nigeria.
Phone: +234(0) 8072009400
Email: ae.awosanya@mail1.ui.edu.ng
emmafisayo@yahoo.com

Daniel Beltran-Alcrudo, DVM, Ph.D
Animal Health Officer (veterinary epidemiologist)
FAO, Regional Office for Europe and Central Asia
Mozsár utca 14; 1066 Budapest; Hungary
phone: +36-18141262
E-mail: daniel.beltranalcrudo@fao.org

Amanda Bastos
University of Pretoria
Pretoria, South Africa
Tel: +27 12 4204612
E-mail: ADBastos@zoology.up.ac.za

Richard Bishop, Ph.D
Senior Molecular Biologist
International Livestock Research Institute (ILRI)
PO Box 30709, Old Naivasha Road
Nairobi 00100, Kenya
Telephone: 254 20 422 3000 (switchboard) 422 3359 (direct line)
Fax: 254 20 422 3001
Mobile telephone: 0710 831 851
E-mail : r.bishop@cgiar.org

Sandra Blome, DVM, Ph.D (GARA Scientific Director, 2016-2019)
Friedrich-Loeffler-Institut
Institute of Diagnostic Virology
Suedufer 10
17493 Greifswald – Insel Riems
Germany
Phone: +49-38351-7-1144
E-mail: sandra.blome@fli.de

Prof. Fernando Boinas,
Universidad de Lisboa
E-mail: fboinas@fmv.utl.pt

Manuel V. Borca, D.V.M., Ph.D
Foreign Animal Disease Research Unit
Plum Island Animal Disease Center
Agricultural Research Service (ARS)
United States Department of Agriculture (USDA)
P.O. Box 848 Greenport, NY 11944, USA
Tel. (631) 323 3135
Fax: (631) 323 3006
E-mail: manuel.borca@ars.usda.gov

Anette Bøtner
DTU National Veterinary Institute
Lindholm
DK 4771- Kalvehave
E-mail: aneb@vet.dtu.dk

Bruce Carter, DVM
Global Vaccine Manager
Center for Veterinary Biologics
Licensing and Policy Development
Animal and Plant Inspection Services (APHIS)
United States Department of Agriculture (USDA)
510 S. 17th Street, Suite 104, Ames, IA 50010 USA
Tel (515) 232-5785 Ext. 141
Fax (515) 232-7120
E-mail: bruce.carter@aphis.usda.gov

Consuelo Carrillo, DVM, PhD
Senior Animal Health Advisor
DSS-FADDL-STATS-VS-NVSL
APHIS-USDA
PIADC, PO Box 848, Greenport
11944 NY
E-mail: Consuelo.Carrillo@aphis.usda.gov

Tengiz Chaligava
National Food Agency (NFA) of the Ministry of Agriculture (MoA)
6 Marshal Gelovani Ave
Tbilisi, Georgia
E-mail: Tengiz.Chaligava@nfa.gov.ge

Dave Chapman, Ph.D
ASF Virus group
Institute for Animal Health, Pirbright Laboratory
Ash Road, Pirbright, Woking
Surrey, GU24 0NF, UK
Tel : +44 (0)1483 231084 (Direct Line)
E-mail: Dave.chapman@bbsrc.ac.uk

Solenne Costard
E-mail: scostard@epixanalytics.com

Ana De La Torre
INIA-CISA
Ctra Algete - El Casar s/n
Valdeolmos, Madrid, Spain
Tel: +34 91 6202300
E-mail: torre@inia.es

Linda Dixon (GARA Scientific Director, 2013-2016)
Head African swine fever virus group
Head of OIE ASF Reference Laboratory
The Pirbright Institute
Ash Road, Pirbright, Woking, Surrey, UK
linda.dixon@pirbright.ac.uk

Tea Enukidze
Laboratory of the Ministry of Agriculture
#49, Vaso Godziashvili street
Tbilisi, Georgia
E-mail: tea.enukidze@lma.gov.ge

Eric Etter
University Of Pretoria / CIRAD
Private Bag X04. Onderstepoort, South Africa
Tel: +27 (0)12 529 84 67
E-mail: eric.etter@cirad.fr

Folorunso O. Fasina, D.V.M., Ph.D
Department of Veterinary Tropical Diseases, University of Pretoria & Emergency Center for Transboundary Animal Diseases, Food and Agriculture Organization of the United Nations
House H Sida, Ali Hassan Mwinyi Road, Ada Estate
Dar es Salaam, United Republic of Tanzania
P.O. Box 2 Dar es Salaam, Tanzania
Tel. +255 686 132 852
Fax: +255 22 266 7286
E-mail: folorunso.fasina@fao.org

Dr. Jovita Fernández Pinero
EU- Community Reference Laboratory for African Swine Fever
Centro de Investigación en Sanidad Animal – Animal Health Research Center- (CISA)
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Valdeolmos, 28130
Madrid, Spain
Tel. +3491602300
fpinero@inia.es

Dr. Carmina Gallardo Frontaura
Laboratory Coordinator
EU- Community Reference Laboratory for African Swine Fever
Centro de Investigación en Sanidad Animal – Animal Health Research Center- (CISA)
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Valdeolmos, 28130, Madrid, Spain
Tel. +3491602300
gallardo@inia.es

Cyril Gerard Gay, DVM, Ph.D (GARA Executive Secretary, 2013-2019)
Senior National Program Leader
Animal Production and Protection
Agricultural Research Service
United States Department of Agriculture
cyril.gay@ars.usda.gov

Dolores Gavier-Widén, DVM, MsC, PhD, Ass. Prof.
Department of Pathology and Wildlife Diseases
Head of Department
National Veterinary Institute (SVA), Sweden
Tel: +46 (0)18-674215 (office),
+46 703787892 (mobile)
E-mail: dolores@sva.se

Douglas Gladue, Ph.D
Foreign Animal Disease Research Unit
Plum Island Animal Disease Center
Agricultural Research Service (ARS)
United States Department of Agriculture (USDA)
P.O. Box 848 Greenport, NY 11944, USA
Tel. (631) 323 3135
Fax: (631) 323 3006
E-mail: Douglas.Gladue@usda.gov

Ana Gulbani
Laboratory of the Ministry of Agriculture
#49, Vaso Godziashvili street
Tbilisi, Georgia
E-mail: tea.enukidze@lma.gov.ge

Livio Heath (GARA President, 2013-2016)
Onderstepoort Veterinary Institute
South Africa
E-mail: heathl@arc.agric.za

Denis Kolbasov, Ph.D, DVM
Director, Russian National Institute of Veterinary Virology and Microbiology
Pokrov Vladimir region, Russia 601120
Tel/Fax +7-49243-62125
denvk@land.ru

Marie Frederique LePotier (GARA President, 2016-2019)
Anses – Laboratoire de Ploufragan/Plouzané Laboratory
Swine virology and immunology unit
ZOOPOLE LES CROIX
22440 Ploufragan, France
Tel : +33 (0)2 96 01 62 90
Fax : +33 (0)2 96 01 62 94
Email: marie-frederique.lepotier@anses.fr

Willie Loeffen
Central Veterinary Institute
Houtribweg 39, Lelystad, The Netherlands
Tel: +31 320 238696
E-mail: willie.loeffen@wur.nl

Alexander Malogolovkin
E-mail: Malogolovkin@inbox.ru

Carlos Martins, DVM, Ph.D
Coordinator ASFRISK
Office C4-102
Faculdade de Medicina Veterinária
Av. da Universidade Técnica
1300-477 Lisboa, Portugal
cmartins@fmv.utl.pt

Beatriz Martinez
University of California, Davis
E-mail: beamartinezlopez@ucdavis.edu

Charles Masembe (GARA Finance Director, 2016-2019)
Makerere University
Kampala, Uganda
Tel : +256712455987
E-mail: cmasembe@cns.mak.ac.ug

Dr. Marta Martinez Aviles, UCM-CISA
Universidad Complutense, Facultad de Veterinaria
Avda. Puerta de Hierro
28040 Madrid, Spain
and , Centro de Investigación en Sanidad Animal – Animal Health Research Center- (CISA)
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Valdeolmos, 28130
Madrid, Spain
Tel. +3491602300
E-mail: marta@sanidadanimal.info

Ioseb Menteshashvili
National Food Agency (NFA) of the Ministry of Agriculture (MoA)
6 Marshal Gelovani Ave
Tbilisi, Georgia
E-mail: stkhelia@ch2m.com

Gerald Misinzo
Sokoine University of Agriculture
PO Box 3019, Morogoro, Tanzania
Tel: +255767058805
E-mail: gmisinzo@gmail.com

Imbi Nurmoja
Estonian Veterinary and Food Laboratory
Kreutzwaldi 30, Tartu, Estonia
E-mail: imbi.nurmoja@vetlab.ee

Mary-Louise Penrith, PhD, DSc, BVSc (Hons)
Extraordinary Professor
Department of Veterinary Tropical Diseases
Faculty of Veterinary Science
University of Pretoria
South Africa
E-mail: marylouise@vodamail.co.za

Tamas Petrovic, D.V.M. M.Sc. Ph.D.
Scientific Veterinary Institute "Novi Sad"
Director Deputy for research
Rumenacki put 20
21000 Novi Sad
Serbia
Phone: + 381 21 4895 321;
Mob: +381 64 818 5410
E-mail: tomy@niv.ns.ac.rs

Fernando Rodriguez
E-mail: fernando.rodriguez@irta.cat

Luis Rodriguez, DVM, Ph.D
Research Leader
Foreign Animal Disease Research Unit
Agricultural Research Service
United States Department of Agriculture
Orient Point, New York
luis.rodriguez@ars.usda.gov

Guillermo Risatti, DVM, Ph.D
University of Connecticut
61 N. Eagleville Road, Unit 3089, Storrs, CT, USA
Tel: +1 860 486 6150
E-mail: guillermo.risatti@uconn.edu

Bob Rowland, Ph.D (GARA Finance Director, 2013-2016)
Kansas State University
1800 Denison Avenue, Manhattan, Kansas, USA
E-mail: browland@vet.k-state.edu

Karl Stahl
National Veterinary Institute (SVA)
Uppsala, Sweden
E-mail: karl.stahl@sva.se

Carolina Stenfeldt, DVM, Ph.D
Veterinary Virologist / Visiting Scientist
Foreign Animal Disease Research Unit
Plum Island Animal Disease Center
Agricultural Research Service (ARS)
United States Department of Agriculture (USDA)
P.O. Box 848
Greenport, NY 11944
Tel (631) 631-323-3260
carolina.stenfeldt@ars.usda.gov

Hermann Unger
IAEA
Vienna, Austria
E-mail: h.unger@iaea.org

Chris Upton, Ph.D
Biochemistry and Microbiology
University of Victoria, Canada

Victoria, BC
Research page: www.virology.ca
Home page: www.virology.ca/node/333
E-mail : cupton@uvic.ca

Dr. Pedro José Sánchez-Cordón,
Fac. Veterinaria, University of Cordoba, Spain
E-mail: an2sacop@uco.es

Isabel Mínguez Tudela, DVM, Ph.D (In memory – deceased)
Scientific Officer
Directorate General Research
European Commission
Office SDME 8/96
1049 Brussels
BELGIUM

Dr. Yolanda Revilla-Novella, Ph.D
Department of Immunology and Virology
Centro de Biologia Molecular Severo Ochoa
C/Nicolás Cabrera 1 Campus de la Universidad Autónoma de Madrid
28049-Madrid, Spain
Tel. +34-911964401
yrevilla@cbm.uam.es

Juergen A. Richt, DVM, PhD
Regents Distinguished Professor
Kansas State University
Diagnostic Medicine/Pathobiology Dept.
College of Veterinary Medicine
K224B Mosier Hall
Manhattan, KS 66506-5601
Tel: 785-532-4408
E-mail: mailto:dclouser@vet.k-state.edu

Dr. Paloma Rueda, Ph.D
Research Manager
INGENASA (Inmunología y Genética Aplicada S.A.)
Av/Institución Libre de Enseñanza, 41
28037-Madrid, Spain
Tel. +34-913680501
prueda@ingenasa.com

Dr. Patricia Sastre, Ph.D
Research Department
INGENASA (Inmunología y Genética Aplicada S.A.)
Av/Institución Libre de Enseñanza, 41
28037-Madrid, Spain
Tel. +34-913680501
E-mail: psastre@ingenasa.com

Juanita Van Heerden (PhD, Pri.Sci.Nat.)
Onderstepoort Veterinary Institute
South Africa
E-mail: vanheerdenj@arc.agric.za

Nino Vepkhvadze
Laboratory of the Ministry of Agriculture
49, Godziashvili Street
Tbilisi, Georgia
E-mail: stkhelia@ch2m.com

Arvo Viltrop
Estonian University of Life Sciences
Kreutzwaldi 1, Tartu, Estonia
E-mail: arvo.viltrop@emu.ee

Prof. Dr. José-Manuel Sánchez-Vizcaíno, DVM, Ph.D
Catedrático de Sanidad Animal
Director del Laboratorio de Referencia de la OIE
Universidad Complutense Facultad de Veterinaria
Avda. Puerta de Hierro
28040 Madrid, Spain
jmvizcaino@visavet.ucm.es

David Williams, Ph.D
Group Leader – Emergency Disease Laboratory Diagnosis
CSIRO Australian Animal Health Laboratory
5 Portarlington Road
Geelong, Victoria
Australia 3220
Tel. +61-3-5227-5364
d.williams@csiro.au

Hovakim Zakaryan
Institute of Molecular Biology
7 Hasratyan, St. Yerevan, Armenia
E-mail: h_zakaryan@mb.sci.am

Laszlo Zsak, DVM, Ph.D (Retired)
Plum Island Animal Disease Center
Agricultural Research Service (ARS)
United Department of Agriculture (USDA)
P.O. Box 848 Greenport, NY 11944, USA

REFERENCES

Abworo EO, Onzere C, Oluoch Amimo J, Riitho V, Mwangi W, Davies J, Blome S, Peter Bishop R. (2017) Detection of African swine fever virus in the tissues of asymptomatic pigs in smallholder farming systems along the Kenya-Uganda border: implications for transmission in endemic areas and ASF surveillance in East Africa. J Gen Virol. 98 (7):1806-1814.

Abrams CC., Goatley L, Fishbourne E, Chapman D, Cooke L, Oura CA, Netherton CL, Takamatsu HH, Dixon LK. (2013). Deletion of virulence associated genes from attenuated African swine fever virus isolate OUR T88/3 decreases its ability to protect against challenge with virulent virus. Virology. 2013 443(1):99-105. doi: 10.1016/j.virol.2013.04.028.

Achenbach JE, Gallardo C, Nieto-Pelegrín E, Rivera-Arroyo B, Degefa-Negi T, Arias M, Jenberie S, Mulisa DD, Gizaw D, Gelaye E, Chibssa TR, Belaye A, Loitsch A, Forsa M, Yami M, Diallo A, Soler A, Lamien CE, Sánchez-Vizcaíno JM. (2017). Identification of a New Genotype of African Swine Fever Virus in Domestic Pigs from Ethiopia. Transbound Emerg Dis. 64(5):1393-1404.

Afonso CL, Alcaraz C, Brun A, Sussman MD, Onisk DV, Escribano JM, Rock DL (1992) Characterization of p30, a highly antigenic membrane and secreted protein of African swine fever virus. Virology 189:368–373

Afonso CL, Piccone ME, Zaffuto KM, Neilan JG, Kutish GF, Lu Z, Balinsky CA, Gibb TR, Bean TJ, Zsak L, Rock DL (2004) African swine fever virus multigene family 360 and 530 genes affect host interferon response. J Virol 78:1858–1864

Agüero, M, Fernández, J, Romero, L, Zamora, MJ, Sánchez, C. Belák, S, Arias, M, Sánchez-Vizcaíno JM (2004) 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. Vet Res 35:551–563

Alcami A, Angulo A, Lopez-Otin C, Munoz M, Freije JM, Carrascosa AL, Viñuela E (1992) Amino acid sequence and structural properties of protein p12, an African swine fever virus attachment protein. J Virol 66:3860–3868

Anderson EC, Williams SM, Fischer-Hoch SF, Wilkinson PJ (1987) Arachidonic acid metabolites in the pathophysiology of thrombocytopenia and haemorrhage in acute African swine fever. Res Vet Sci 42:387–394

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

Andres G, Simon-Mateo C, Viñuela E (1997) Assembly of African swine fever virus: role of polyprotein pp220. J Virol 71:2331–2341

Andres G, Garcia-Escudero R, Simon-Mateo C, Viñuela E (1998) African swine fever virus is enveloped by a two-membraned collapsed cisterna derived from the endoplasmic reticulum. J Virol 72:8988–9001

Andres G, Garcia-Escudero R, Viñuela E, Salas ML, Rodriguez JM (2001) African swine fever virus structural protein pE120R is essential for virus transport from assembly sites to plasma membrane but not for infectivity. J Virol 75:6758–6768

Andres G, Alejo A, Salas J, Salas ML (2002) African swine fever virus polyproteins pp220 and pp62 assemble into the core shell. J Virol 76:12473–12482.

Argilaguet J.M., Pérez-Martín E., Nofrarías M., Gallardo C., Accensi F., Lacasta A., Mora M., Ballester M., Galindo-Cardiel I., López-Soria S., Escribano J.M., Reche P.A., Rodríguez F. (2012). DNA vaccination partially protects against African swine fever virus lethal challenge in the absence of antibodies. PLoS One. 2012; 7(9):e40942. doi: 10.1371/journal.pone.0040942

Argilaguet J.M., Pérez-Martín E., López S., Goethe M., Escribano J.M., Giesow K., Keil G.M., Rodríguez F. (2013). BacMam immunization partially protects pigs against sublethal challenge with African swine fever virus. Antiviral Res. 2013 98(1):61-5. doi: 10.1016/j.antiviral.2013.02.005

Arias, M and Sanchez-Vizcaino, JM (2002) African swine fever. In Trends in emerging viral infections of swine (eds A Morilla, KJ Yoon and JJ Zimmerman), pp. 119–124. Ames, IA: Iowa State Press

Atuhaire DK, Afayoa M, Ochwo S, Mwesigwa S, Mwiine FN, Okuni JB, Olaho-Mukani W, Ojok L. (2013) Prevalence of African swine fever virus in apparently healthy domestic pigs in Uganda. BMC Vet Res. 9:263.

Atuhaire DK, Afayoa M, Ochwo S, Mwesigwa S, Okuni JB, Olaho-Mukani W, Ojok L. (2013) Molecular characterization and phylogenetic study of African swine fever virus isolates from recent outbreaks in Uganda (2010-2013).Virol J. 10:247.

Awosanya, E. J., Olugasa, B., Ogundipe, G. & Grohn, Y. T. (2015) Sero-prevalence and risk factors associated with African swine fever on pig farms in southwest Nigeria. *BMC Vet Res*, 11, 133.

Ballester, M., Rodriguez, F., 2015. In situ hybridization with labeled probes: assessment of african Swine Fever virus in formalin-fixed paraffin-embedded tissues. Methods Mol Biol 1247, 209-218.

Banjara S., Caria S., Dixon L.K., Hinds M.G., Kvansakul M. (2017). Structural insight into African Swine Fever virus A179L mediated inhibition of apoptosis. J Virol. 2017 doi:10.1128/JVI.02228-16

Barongo, M. B, Stahl, K., Bett, B., Bishop, R. P., Fevre, E. M., Aliro, T., *et al.*(2015) Estimating the Basic Reproductive Number (R0) for African Swine Fever Virus (ASFV) Transmission between Pig Herds in Uganda. *PLoS One*,10, e0125842.

Barrado Gil L., Galindo I., Martinez Alonso D., Viedma S., Alonso C. (2017). The ubiquitine proteasome system is required for African swine fever replication. Plos One. 2017 12(12):e0189741. doi: 10.1371/journal.pone.0189741.

Bastos AD, Penrith ML, Cruciere C, Edrich JL, Hutchings G, Roger F, Couacy-Hymann ER, Thomson G (2003) Genotyping field strains of African swine fever virus by partial p72 gene characterisation. Arch Virol 148:693–706

Baylis SA, Dixon LK, Vydelingum S, Smith GL (1992) African swine fever virus encodes a gene with extensive homology to type II DNA topoisomerases. J Mol Biol 228:1003–1010

Baylis SA, Twigg SR, Vydelingum S, Dixon LK, Smith GL (1993b) Three African swine fever virus genes encoding proteins with homology to putative helicases of vaccinia virus. J Gen Virol 74:1969–1974

Bishop, R. P., Fleischauer, C., de Villiers, E. P., Okoth, E. A., Arias, M., Gallardo, C., *et al.*(2015) Comparative analysis of the complete genome sequences of Kenyan African swine fever virus isolates within p72 genotypes IX and X. *Virus Genes*,50, 303-309.

Blasco R, Lopez-Otin C, Munoz M, Bockamp EO, Simon-Mateo C, Vinuela E (1990) Sequence and evolutionary relationships of African swine fever virus thymidine kinase. Virology 178:301–304

Blome S, Gabriel C, Dietze K, Breithaupt A, Beer M. (2012) High virulence of African swine fever virus caucasus isolate in European wild boars of all ages. Emerg Infect Dis. 18(4):708.

Blome S, Gabriel C, Beer M. (2013) Pathogenesis of African swine fever in domestic pigs and European wild boar. Virus Res. 173(1):122-30

Blomstrom, A. L., Stahl, K., Masembe, C., Okoth, E., Okurut, A. R., Atmnedi, P., *et al.* (2012) Viral metagenomic analysis of bushpigs (Potamochoerus larvatus) in Uganda identifies novel variants of Porcine parvovirus 4 and Torque teno sus virus 1 and 2. *Virol J,* 9, 192.

Boinas, FS, Hutchings, GH, Dixon, LK and Wilkinson, PJ (2004) Characterization of pathogenic and non-pathogenic African swine fever virus isolates from *Ornithodoros erraticus* inhabiting pig premises in Portugal. J Gen Virol 85:2177–2187

Boklund, A, Toft, N, Alban, L, and Uttenthal, A (2009). Comparing the epidemiological and economic effects of control strategies against classical swine fever in Denmark. Prev Vet Med 90:180-93

Borca MV, Irusta P, Carrillo C, Afonso CL, Burrage TG, Rock DL (1994a) African swine fever virus structural protein p72 contains a conformational neutralizing epitope. Virology 201:413–418

Borca MV, Kutish GK, Afonso CL, Irusta P, Carrillo C, Brun A, Sussman M, Rock DL (1994b) An African swine fever virus gene with similarity to the T-lymphocyte surface antigen CD2 mediates hemadsorption. Virology 199:463–468

Borca MV, Carrillo C, Zsak L, Laegreid WW, Kutish GF, Neilan JG, Burrage TG, Rock DL (1998). Deletion of a CD2-like gene, 8-DR, from African swine fever virus affects viral infection in domestic swine. J Virol 72:2881–2889.

Borca, MV, O'Donnell V, Holinka LG, Rai DK, Sanford B, Alfano M, Carlson J, Azzinaro PA, Alonso C, Gladue DP. (2016). The Ep152R ORF of African swine fever virus strain Georgia encodes for an essential gene that interacts with host protein BAG6. Virus Research (2016) 223:181-189

Bosch, J., Iglesias, I., Munoz, M. J. & de la Torre, A. (2016) A Cartographic Tool for Managing African Swine Fever in Eurasia: Mapping Wild Boar Distribution Based on the Quality of Available Habitats. *Transbound Emerg Dis*

Bosch, J., Rodriguez, A., Iglesias, I., Munoz, M. J., Jurado, C., Sanchez-Vizcaino, J. M., *et al.* (2016) Update on the Risk of Introduction of African Swine Fever by Wild Boar into Disease Free European Union Countries. *Transbound Emerg Dis*

Boshoff, C. I., Bastos, A. D., Dube, B. M. M. & Heath, L. (2014) First molecular assessment of the African swine fever virus status of Ornithodoros ticks from Swaziland. *Onderstepoort J Vet Res,* 81, E1-5.

Boursnell M, Shaw K, Yanez RJ, Viñuela E, Dixon L (1991) The sequences of the ribonucleotide reductase genes from African swine fever virus show considerable homology with those of the orthopoxvirus, vaccinia virus. Virology 184:411–416

Braae UC, Johansen MV, Ngowi HA, Rasmussen TB, Nielsen J, Uttenthal Å. (2015) Detection of African swine fever virus DNA in blood samples stored on FTA cards from asymptomatic pigs in Mbeya region, Tanzania. Transbound Emerg Dis. 62(1):87-90.

Breese SSJ, DeBoer JC (1966) Electron microscope observations of African swine fever virus in tissue culture cells. Virology 28:420–428

Brookes SM, Sun H, Dixon LK, Parkhouse RM (1998b) Characterization of African swine fever virion proteins j5R and j13L: immuno-localization in virus particles and assembly sites. J Gen Virol 79:1179–1188

Brown, A.A., Penrith, M.L., Fasina, F.O., Beltrán-Alcrudo, D. (2018). The African swine fever epidemic in West Africa, 1996-2002. Transbound Emerg Dis. 65(1):64-76.

Camacho A, Viñuela E (1991) Protein p22 of African swine fever virus: an early structural protein that is incorporated into the membrane of infected cells. Virology 181:251–257

Carpintero R, Alonso C, Pineiro M, Iturralde M, Andres M, Le Potier MF, Madec F, Alava MA, Pineiro A, Lampreave F (2007) Pig major acute-phase protein and apolipoprotein A-I responses correlate with the clinical course of experimentally induced African swine fever and Aujeszky's disease. Vet Res 38:741–753

Carrasco L, de Lara FC, Martin de las Mulas J, Gomez-Villamandos JC, Perez J, Wilkinson PJ, Sierra MA (1996) Apoptosis in lymph nodes in acute African swine fever. J Comp Pathol 115:415–428

Carrascosa JL, Carazo JM, Carrascosa AL, García N, Santisteban A, Viñuela E (1984) General morphology and capsid fine structure of African swine fever virus particles. Virology 132:160–172

Carrascosa JL, Del Val M, Santaren JF, Viñuela E (1985) Purification and properties of African swine fever virus. J Virol 54:337–344

Carrascosa JL, Gonzalez P, Carrascosa AL, Garcia-Barreno B, Enjuanes L, Viñuela E (1986) Localization of structural proteins in African swine fever virus particles by immunoelectron microscopy. J Virol 58:377–384

Carrascosa, A.L., Bustos, M.J., de Leon, P., 2011. Methods for growing and titrating African swine fever virus: field and laboratory samples. Current protocols in cell biology / editorial board, Juan S. Bonifacino ... [et al.] Chapter 26, Unit 26 14.

Carrillo C, Borca MV, Afonso CL, Onisk DV, Rock DL (1994) Long-term persistent infection of swine monocytes/macrophages with African swine fever virus. J Virol 68:580–583

Chenais, E., Boqvist, S., Sternberg-Lewerin, S., Emanuelson, U., Ouma, E., Dione, M., *et al.* (2017) Knowledge, Attitudes and Practices Related to African Swine Fever Within Smallholder Pig Production in Northern Uganda. *Transbound Emerg Dis*, *64*, 101-115.

Chenais, E., Sternberg-Lewerin, S., Boqvist, S., Emanuelson, U., Aliro, T., Tejler, E., *et al.*(2015) African Swine Fever in Uganda: Qualitative Evaluation of Three Surveillance Methods with Implications for Other Resource-Poor Settings. *Front Vet Sci*, *2*, 51.

Chenais, E., Sternberg-Lewerin, S., Boqvist, S., Liu, L., LeBlanc, N., Aliro, T., *et al.* (2017) African swine fever outbreak on a medium-sized farm in Uganda: biosecurity breaches and within-farm virus contamination. *Trop Anim Health Prod*, *49*, 337-346.

Childerstone A, Takamatsu H, Yang H, Denyer M, Parkhouse RM (1998) Modulation of T cell and monocyte function in the spleen following infection of pigs with African swine fever virus. Vet Immunol Immunopathol 62:281–296

Coggins L (1974) African swine fever virus. Pathogenesis. Prog Med Virol 18:48–63

Colgrove GS, Haelterman EO, Coggins L (1969) Pathogenesis of African swine fever in young pigs. Am J Vet Res 30:1343–1359

Conceicao JM (1949) Estudo das zoonoses porcinas de ngola; primeiro relatorio. A zoonose porcina africana de virus filtravel. Pecuaria 1:217–245

Costard S, Wieland B, de Glanville W, Jori F, Rowlands R, Vosloo W, Roger F, Pfeiffer DU, Dixon LK (2009) African swine fever: how can global spread be prevented? Philos Trans R Soc Lond B Biol Sci. 364:2683-96

Costard S, Mur L, Lubroth J, Sanchez-Vizcaino JM, Pfeiffer DU. (2013) Epidemiology of African swine fever virus. Virus Res. 173(1):191-7.

Creig A, Plowright W (1970) The excretion of two virulent strains of African swine fever virus by domestic pigs. J Hyg, Cambridge 68:673–682

Cubillos C, Gómez-Sebastian S, Moreno N, Nuñez MC, Mulumba-Mfumu LK, Quembo CJ, Heath L, Etter EM, 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 Res.* 173(1):159-67. 2013

Cuesta-Geijo M.A., M. Chiappi, I. Galindo, L. Barrado-Gil, R. Muñoz-Moreno, José L. Carrascosa and C. Alonso. (2016). Cholesterol flux is required for endosomal progression of African swine fever virus. Journal of Virology 90 (3), 1534-1543.

Cuesta-Geijo MA., Barrado Gil L, Galindo I, Munoz Moreno R, Alonso C. (2017). Redistribution of endosomal membranes to the African swine fever virus replication site. Viruses 2017 9(6). pii: E133. doi: 10.3390/v9060133

Davies K., Goatley LC., Guinat C., Netherton CL., Gubbins S., Dixon LK., Reis AL. (2017). Survival of African Swine Fever Virus in Excretions from Pigs Experimentally Infected with the Georgia 2007/1 Isolate. Transbound Emerg Dis.;64(2):425-431. doi: 10.1111/tbed.12381.

de Carvalho Ferreira HC, Weesendorp E, Elbers AR, Bouma A, Quak S, Stegeman JA, Loeffen WL. (2012) African swine fever virus excretion patterns in persistently infected animals: a quantitative approach. Vet Microbiol. 160 (3-4):327-40

de Carvalho Ferreira HC, Weesendorp E, Quak S, Stegeman JA, Loeffen WL. (2013) Quantification of airborne African swine fever virus after experimental infection.Vet Microbiol. 165(3-4):243-51.

de Carvalho Ferreira HC, Weesendorp E, Quak S, Stegeman JA, Loeffen WL. (2014) Suitability of faeces and tissue samples as a basis for non-invasive sampling for African swine fever in wild boar. Vet Microbiol. 172(3-4):449-54.

Dee SA, Bauermann FV, Niederwerder MC, Singrey A, Clement T, de Lima M, Long C, Patterson G, Sheahan MA, Stoian AMM, Petrovan V, Jones CK, De Jong J, Ji J, Spronk GD, Minion L, Christopher-Hennings J, Zimmerman JJ, Rowland RRR, Nelson E, Sundberg P, Diel DG. (2018) Survival of viral pathogens in animal feed ingredients under transboundary shipping models. PLoS One. 13(3):e0194509.

DeKock G, Robinson EM, Keppel JJG (1940) Swine fever in South Africa. Onderstepoort J Vet Sci Animal Industry 14:31–93

de León, P., Bustos, M.J., Carrascosa, A.L., 2013. Laboratory methods to study African swine fever virus. Virus Res 173, 168-179.

del Val M, Viñuela E. (1987). Glycosylated components induced in African swine fever (ASF) virus-infected Vero cells. Virus Res 7:297–308

Denyer, MS, Wileman, TE, Stirling, CMA, Zuber, B and Takamatsu, HH (2006) Perforin expression can define CD8 positive lymphocyte subsets in pigs allowing phenotypic and functional analysis of natural killer, cytotoxic T, natural killer Tand MHC un-restricted cytotoxic T-cells. Vet Immunol Immunopathol 110:279–292

Detray DE (1957) Persistence of viremia and immunity in African swine fever. Am J Vet Res 18:811–816

Detray DE (1963) African swine fever. Adv Vet Sci Comp Med 8:299–333

Detray DE, Scott GR (1957) Blood changes in swine with African swine fever. Am J Vet Res 18:484–490

Dixon LK, Wilkinson PJ (1988) Genetic diversity of African swine fever virus isolates from soft ticks (*Ornithodoros moubata*) inhabiting warthog burrows in Zambia. J Gen Virol 69:2981–2993

Dixon LK, Costa JV, Escribano JM, Rock DL, Viñuela E, Wilkinson PJ (2000) Family Asfarviridae. Virus taxonomy: seventh report of the International Committee on Taxonomy of Viruses. In: van Regenmortel MHV, Fanquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeohh DL, Pringle CR, Wickner RB (eds), Academic, San Diego, pp 159–165

Dixon, LK, Escribano, JM, Martins, C, Rock, DL, Salas, ML, and Wilkinson, PJ (2005) Asfarviridae. In Virus taxonomy. VIIIth Report of the ICTV (eds CM Fauquet, MA Mayo, J Maniloff, U Desselberger and LA Ball), pp. 135–143. London, UK: Elsevier/Academic Press.

Dixon, LK, Abrams, CC, Chapman, DG, and Zhang, F. (2008) African swine fever virus. In Animal viruses molecular biology (eds TC Mettenleiter & F Sobrino), pp. 457–521. Norwich, UK: Caister Academic Press.

Edwards JF (1983) The pathogenesis of thrombocytopenia and haemorrhage in African swine fever. PhD Thesis, Cornell University. Ithaca, New York

Edwards JF, Dodds WJ, Slauson DO (1985) Mechanism of thrombocytopenia in African swine fever. Am J Vet Res 46:2058–2063

Erickson A, Fisher M, Furukawa-Stoffer T, Ambagala A, Hodko D, Pasick J, King DP, Nfon C, Ortega Polo R, Lung O. (2018) A multiplex reverse transcription PCR and automated electronic microarray assay for detection and differentiation of seven viruses affecting swine. Transbound Emerg Dis. 65(2):e272-e283.

Estevez A, Marquez MI, Costa JV (1986) Two-dimensional analysis of African swine fever virus proteins and proteins induced in infected cells. Virology 152:192–206

Esteves A, Ribeiro G, Costa JV (1987) DNA-binding proteins specified by African swine fever virus. Virology 161:403–409

Fasina FO, Lazarus DD, Spencer BT, Makinde AA, Bastos AD (2012). Cost implications of African swine fever in smallholder farrow-to-finish units: economic benefits of disease prevention through biosecurity. Transbound Emerg Dis. 59(3):244-55.

Fernandez-Pinero, J., Gallardo, C., Elizalde, M., Robles, A., Gomez, C., Bishop, R., Heath, L., Couacy-Hymann, E., Fasina, F.O., Pelayo, V., Soler, A., Arias, M., 2013. Molecular diagnosis of African Swine Fever by a new real-time PCR using universal probe library. Transbound Emerg Dis 60, 48-58.

Forman AJ, Wardley RC, Wilkinson PJ (1982) The immunological response of pigs and guinea pigs to antigens of African swine fever virus. Arch Virol 74:91–100

Frączyk M, Woźniakowski G, Kowalczyk A, Niemczuk K, Pejsak Z. (2016) Development of cross-priming amplification for direct detection of the African Swine Fever Virus, in pig and wild boar blood and sera samples. Lett Appl Microbiol. 62(5):386-91.

Freije JM, Lain S, Viñuela E, Lopez-Otin C (1993) Nucleotide sequence of a nucleoside triphosphate phosphohydrolase gene from African swine fever virus. Virus Res 30:63–72

Freitas FB, Frouco G, Martins C, Leitao A, Ferreira F. (2016). *In vitro* inhibition of African swine fever virus-topoisomerase II disrupts viral replication. Antiviral Res. 2016 134:34-41

Freitas FB, Frouco G, Martins C, Ferreira F. (2018). African swine fever virus encodes for an E2-ubiquitin conjugating enzyme that is mono- and diubiquitinated and required for viral replication cycle. *Scientific Reports* 2018 (2018) 8:3471 DOI:10.1038/s41598-018-21872-2

Frouco G, Freitas FB, Coelho J, Leitao A, Martins C, Ferreira F. DNA-Binding Properties of African Swine Fever Virus pA104R, a Histone-Like Protein Involved in Viral Replication and Transcription. J Virol. vol. 91 no. 12e02498-16

Gabriel C, Blome S, Malogolovkin A, Parilov S, Kolbasov D, Teifke JP, Beer M. (2011) Characterization of African swine fever virus Caucasus isolate in European wild boars. Emerg Infect Dis. 17(12):2342-5.

Gallardo, C., Mwaengo, D. M., Macharia, J. M., Arias, M., Taracha, E. A., Soler, A., *et al.* (2009) Enhanced discrimination of African swine fever virus isolates through nucleotide sequencing of the p54, p72, and pB602L (CVR) genes. *Virus Genes*, 38, 85-95.

Gallardo, C., Okoth, E., Pelayo, V., Anchuelo, R., Martin, E., Simon, A., *et al.* (2011) African swine fever viruses with two different genotypes, both of which occur in domestic pigs, are associated with ticks and adult warthogs, respectively, at a single geographical site. *J. Gen Virol,* 92, 432-444.

Gallardo, C., Nieto, R., Soler, A., Pelayo, V., Fernandez-Pinero, J., Markowska-Daniel, I., Pridotkas, G., Nurmoja, I., Granta, R., Simon, A., Perez, C., Martin, E., Fernandez-Pacheco, P., Arias, M., 2015. Assessment of African Swine Fever Diagnostic Techniques as a Response to the Epidemic Outbreaks in Eastern European Union Countries: How To Improve Surveillance and Control Programs. Journal of clinical microbiology 53, 2555-2565.

Gallardo C., Reoyo-de la Torre, A., Fernández-Pinero, J., Iglesias, I., Muñoz, J., Arias, ML. (2015) African swine fever: a global view of the current challenge. Porcine Health Management 1:21 DOI 10.1186/s40813-015-0013-y

Gallardo C, Sánchez EG, Pérez-Núñez D, Nogal M, de León P, Carrascosa ÁL, Nieto R, Soler A, Arias ML Revilla Y. (2018). African swine fever virus (ASFV) protection mediated by NH/P68 and NH/P68 recombinant live-attenuated viruses. Vaccine. 2018 36(19):2694-2704.

Garcia-Escudero R, Andres G, Almazan F, Vinuela E (1998) Inducible gene expression from African swine fever virus recombinants: analysis of the major capsid protein. J Virol 72:3185–3195

Gil S, Spagnuolo-Weaver M, Canals A, Sepúlveda N, Oliveira J, Aleixo A, Allan G, Leitão A, Martins CL (2003) Expression at mRNA level of cytokines and A238L gene in porcine bloodderived macrophages infected in vitro with African swine fever virus (ASFV) isolates of different virulence. Arch Virol 148:2077–2097

Gomez-Puertas P, Rodriguez F, Oviedo JM, Ramiro-Ibañez F, Ruiz-Gonzalvo F, Alonso C, Escribano JM (1996) Neutralizing antibodies to different proteins of African swine fever virus inhibit both virus attachment and internalization. J Virol 70:5689–5694

Gomez-Puertas P, Rodriguez F, Oviedo JM, Brun A, Alonso C, Escribano JM (1998) The African swine fever virus proteins p54 and p30 are involved in two distinct steps of virus

attachment and both contribute to the antibody-mediated protective immune response. Virology 243:461–471

Gomez-Villamandos JC, Hervas J, Mendez A, Carrasco L, de las Mulas JM, Villeda CJ, Wilkinson PJ, Sierra MA (1995) Experimental African swine fever: apoptosis of lymphocytes and virus replication in other cells J Gen Virol 76:2399–2405

Gomez del Moral M, Ortuno E, Fernandez-Zapatero P, Alonso F, Alonso C, Ezquerra A, Dominguez J (1999) African swine fever virus infection induces tumor necrosis factor alpha production: implications in pathogenesis. J Virol 73:2173–2180

Granja AG, Nogal ML, Hurtado C, Vila V, Carrascosa AL, Salas ML, Fresno M, Revilla Y (2004b) The viral protein A238L inhibits cyclooxygenase-2 expression through a nuclear factor of activated T cell-dependent transactivation pathway. J Biol Chem 279:53736–53746

Grau FR, Schroeder ME, Mulhern EL, McIntosh MT, Bounpheng MA. (2015) Detection of African swine fever, classical swine fever, and foot-and-mouth disease viruses in swine oral fluids by multiplex reverse transcription real-time polymerase chain reaction. J Vet Diagn Invest. 27(2):140-9. doi: 10.1177/1040638715574768.

Guinat, C., Gogin, A., Blome, S., Keil, G., Pollin, R., Pfeiffer, D. U. *et al.* (2016) Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions. *Vet Rec*, 178, 262–267.

Hamdy FM, Dardiri AH (1984) Clinic and immunologic responses of pigs to African swine virus isolated from the Western hemisphere. Am J Vet Res 45:711–714

Haresnape JM, Wilkinson PJ, Mellor PS (1988) Isolation of African swine fever virus from ticks of the *Ornithodoros moubata* complex (Ixodoidea: Argasidae) collected within the African swine fever enzootic area of Malawi. Epidemiol Inf 101:173–185

Heuschele WP, Coggins L (1969) Epizootiology of African swine fever in warthogs. Bull Epizoot Dis Afr 17:179–183

Hingamp PM, Arnold JE, Mayer RJ, Dixon LK (1992) A ubiquitin conjugating enzyme encoded by African swine fever virus EMBO J 11:361–366

Howey E.B., O'Donnell Ferreira H., Borca M.V., Arzt J. (2013). 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 (2013) 328– 339

Hu L, Lin XY, Yang ZX, Yao XP, Li GL, Peng SZ, Wang Y. (2015) A multiplex PCR for simultaneous detection of classical swine fever virus, African swine fever virus, highly pathogenic porcine reproductive and respiratory syndrome virus, porcine reproductive and respiratory syndrome virus and pseudorabies in swines. Pol J Vet Sci. 18(4):715-23.

Iglesias, I., Munoz, M. J., Montes, F., Perez, A., Gogin, A., Kolbasov, D. *, et al.* (2016) Reproductive Ratio for the Local Spread of African Swine Fever in Wild Boars in the Russian Federation. *Transbound Emerg Dis*, 63, e237-e245.

Iglesias, I., Rodriguez, A., Feliziani, F., Rolesu, S. & de la Torre, A. (2017) Spatio-temporal Analysis of African Swine Fever in Sardinia (2012-2014): Trends in Domestic Pigs and Wild Boar. *Transbound Emerg Dis*, 64, 656-662.

Irusta PM, Borca MV, Kutish GF, Lu Z, Caler E, Carrillo C, Rock DL (1996) Amino acid tandem repeats within a late viral gene define the central variable region of African swine fever virus. Virology 220:20–27

Jaing C, Allen J, Thissen JB, Rowland RR, Williams D. (2016). Whole transcriptome analysis of pigs infected with African Swine Fever Virus. 3rd Annual GARA scientific workshop. Ploufragan, France. September 6, 2016.

Jancovich JK, Chapman D, Hansen DT, Robida MD, Loskutov A, Craciunescu F, Borovkov A, Kibler K, Goatley L, King K, Netherton CL, Taylor G, Jacobs B, Sykes K, Dixon LK. (2018). Immunization of Pigs by DNA Prime and Recombinant Vaccinia Virus Boost To Identify and Rank African Swine Fever Virus Immunogenic and Protective Proteins. *J Virol.* 2018 92(8). pii: e02219-17. doi: 10.1128/JVI.02219-17.

Jori, F. & Bastos, A. D. (2009) Role of wild suids in the epidemiology of African swine fever. *Ecohealth*, 6, 296-310.

Jori, F., Vial, L., Penrith, M. L., Perez-Sanchez, R., Etter, E., Albina, E., *et al.* (2013) Review of the sylvatic cycle of African swine fever in sub-Saharan Africa and the Indian ocean. *Virus Res,* 173, 212-227.

Kabuuka, T., Kasaija, P. D., Mulindwa, H., Shittu, A., Bastos, A. D. & Fasina, F. O. (2014) Drivers and risk factors for circulating African swine fever virus in Uganda, 2012-2013. *Res Vet Sci*, 97, 218-225.

Kalenzi Atuhaire D, Ochwo S, Afayoa M, Norbert Mwiine F, Kokas I, Arinaitwe E, Ademun-Okurut RA, Boniface Okuni J, Nanteza A, Ayebazibwe C, Okedi L, Olaho-Mukani W, Ojok L. (2013) Epidemiological Overview of African Swine Fever in Uganda (2001-2012). J Vet Med. 2013:949638.

Keil GM; Goller K; Pollin R; Bishop R; Höper D; Blome S; Portugal R. (2016). Adaptation of African Swine Fever Virus field strains to productively replicate in WSL cells is not necessarily associated with larger deletions within the viral genomes. 3rd Annual GARA scientific workshop. Ploufragan, France. September 6, 2016.

Kihm U, Ackerman M, Mueller H, Pool R (1987) Approaches to vaccination. In: African swine fever. Becker Y (ed) Martinus Nijhoff, Boston, pp 127–144

King, D.P., Reid, S.M., Hutchings, G.H., Grierson, S.S., Wilkinson, P.J., Dixon, L.K., Bastos, A.D., Drew, T.W., 2003. Development of a TaqMan PCR assay with internal amplification control for the detection of African swine fever virus. J Virol Methods 107, 53-61.

Konno S, Taylor WD, Dardiri AH (1971a) Acute African swine fever. Proliferative phase in lymphoreticular tissue and the reticuloendothelial system. Cornell Vet 61:71–84

Konno S, Taylor WD, Hess WR, Heuschele WP (1971b) Liver pathology in African swine fever. Cornell Vet 61:125–150

Konno S, Taylor WD, Hess WR, Heuschele WP (1972) Spleen pathology in African swine fever. Cornell Vet 62:486–506

Kukielka, E. A., Jori, F., Martinez-Lopez, B., Chenais, E., Masembe, C., Chavernac, D., *et al.* (2016) Wild and Domestic Pig Interactions at the Wildlife-Livestock Interface of Murchison Falls National Park, Uganda, and the Potential Association with African Swine Fever Outbreaks. *Front Vet Sci,* 3, 31.

Kuznar J, Salas ML, Viñuela E (1980) DNA-dependent RNA polymerase in African swine fever virus. Virology 101:169–175

Kuznar J, Salas ML, Viñuela E (1981) Nucleoside triphosphate phosphohydrolase activities in African swine fever virus. Arch Virol 69:307–310

Kyyro, J., Sahlstrom, L. & Lyytikainen, T. (2017) Assessment of the risk of African swine fever introduction into Finland using NORA-a rapid tool for semiquantitative assessment of the risk. *Transbound Emerg Dis*

Lacasta A, Ballester M, Monteagudo PL, Rodríguez JM, Salas ML, Accensi F, Pina-Pedrero S, Bensaid A, Argilaguet J, López-Soria S, Hutet E, Le Potier MF, Rodríguez F. (2014). Expression library immunization can confer protection against lethal challenge with African swine fever virus. J Virol. 2014 88(22):13322-32. doi: 10.1128/JVI.01893-14.

Leitao, A, Cartaxeiro, C, Coelho, R, Cruz, B, Parkhouse, RME, Portugal, FC, Vigario, JD and Martins, CLV (2001) The non-haemadsorbing African swine fever virus isolate ASFV/NH/P68 provides a model for defining the protective anti-virus immune response. J Gen Virol 82:513–523

Lewis T, Zsak L, Burrage TG, Lu Z, Kutish GF, Neilan JG, Rock DL (2000) An African swine fever virus ERV1-ALR homologue, 9GL, affects virion maturation and viral growth in macrophages and viral virulence in swine. J Virol 74:1275–1285

Lichoti, J. K., Davies, J., Kitala, P. M., Githigia, S. M., Okoth, E., Maru, Y., *et al.* (2016) Social network analysis provides insights into African swine fever epidemiology. *Prev Vet Med,* 126, 1-10.

Lichoti, J. K., Davies, J., Maru, Y., Kitala, P. M., Githigia, S. M., Okoth, E. *et al.* (2017) Pig traders' networks on the Kenya–Uganda border highlight potential for mitigation of African swine fevervirus transmission and improved ASF disease risk management. *Prev Vet Med*, 140, 87–96.

Liu L, Luo Y, Accensi F, Ganges L, Rodríguez F, Shan H, Ståhl K, Qiu HJ, Belák S. (2017). Pre-Clinical Evaluation of a Real-Time PCR Assay on a Portable Instrument as a Possible Field Diagnostic Tool: Experiences from the Testing of Clinical Samples for African and Classical Swine Fever Viruses. Liu L, Luo Y, Accensi F, Ganges L, Rodríguez F, Shan H, Ståhl K, Qiu HJ, Belák S.Transbound Emerg Dis.;64(5)

Lokhandwala S, Waghela SD, Bray J, Sangewar N, Charendoff C, Martin CL, Hassan WS, Koynarski T, Gabbert L, Burrage TG, Brake D, Neilan J, Mwangi W. (2017). Adenovirus vectored novel ASFV antigens elicit robust immune responses in swine. PLoS One. 2017 12(5):e0177007. doi: 10.1371/journal.pone.0177007.

Lopera-Madrid J, Osorio JE, He Y, Xiang Z, Adams LG, Laughlin RC, Mwangi W, Subramanya S, Neilan J, Brake D, Burrage TG, Brown WC, Clavijo A, Bounpheng MA. (2017). Safety and immunogenicity of mammalian cell derived and Modified Vaccinia Ankara vectored African swine fever subunit antigens in swine. Vet Immunol Immunopathol. 2017 185:20-33. doi: 10.1016/j.vetimm.2017.01.004.

Lopez-Otin C, Simon C, Mendez E, Viñuela E (1988) Mapping and sequence of the gene encoding protein p37, a major structural protein of African swine fever virus. Virus Genes 1:291–303

Lopez-Otin C, Freije JM, Parra F, Mendez E, Viñuela E (1990) Mapping and sequence of the gene coding for protein p72, the major capsid protein of African swine fever virus. Virology 175:477–484

Lubisi BA, Bastos ADS, Dwarka RM, Vosloo W (2003) Genotyping African swine fever virus strains from East Africa. Proceedings of the Conference of Southern African Society of Veterinary Epidemiology Preventive Medicine. Roodevallei, pp 10–14

Luka, P. D., Achenbach, J. E., Mwiine, F. N., Lamien, C. E., Shamaki, D., Unger, H. *et al.* (2016) Genetic Characterization of Circulating African Swine Fever Viruses in Nigeria (2007–2015). *Transbound Emerg Dis*

Lung O, Fisher M, Erickson A, Nfon C, Ambagala A. (2018) Fully automated and integrated multiplex detection of high consequence livestock viral genomes on a microfluidic platform. Transbound Emerg Dis. 10.1111/tbed.12994

Luo, Y., Atim, S. A., Shao, L., Ayebazibwe, C., Sun, Y., Liu, Y. *et al.* (2017) Development of an updated PCR assay for detection of African swine fever virus. *Arch Virol*, 162, 191–199.

Lyra, TM P (2006) The eradication of African swine fever in Brazil, 1978–1984. Rev. Sci. Tech. Off. Int. Epizoot. 25:93–103.

Manso Ribeiro J, Rosa Azevedo F (1961) Réapparition de la peste porcine africaine (PPA) au Portugal. Bull Off Int Epizoot 55:88–106

Martin Hernandez AM, Tabares E (1991) Expression and characterization of the thymidine kinase gene of African swine fever virus. J Virol 65:1046–1052

Martins A, Ribeiro G, Marques MI, Costa JV (1994) Genetic identification and nucleotide sequence of the DNA polymerase gene of African swine fever virus. Nucleic Acids Res 22:208–213

Maurer FD, Griesemer RA, Jones FC (1958) The pathology of African swine fever – a comparison with hog cholera. Am J Vet Res 19:517–539

McVicar JW. (1984) Quantitative aspects of the transmission of African swine fever. Am J Vet Res. 45(8):1535-41.

Mebus CA (1988) African swine fever. Adv Virus Res 35:251–269

Mebus CA, Dardiri AH.(1980) Western hemisphere isolates of African swine fever virus: asymptomatic carriers and resistance to challenge inoculation. Am J Vet Res. 41(11):1867-9.

Mendes AM (1961) Considérations sur le diagnostic et la prophylaxie de la peste porcine africaine. Bull Off Int Epizoot 57:591–600

Misinzo G, Kwavi DE, Sikombe CD, Makange M, Peter E, Muhairwa AP, Madege MJ. (2014) Molecular characterization of African swine fever virus from domestic pigs in northern Tanzania during an outbreak in 2013. Trop Anim Health Prod. 46(7):1199-207.

Miskin JE, Abrams CC, Goatley LC, Dixon LK (1998) A viral mechanism for inhibition of the cellular phosphatase calcineurin. Science 281:562–565

Monteagudo PL, Lacasta A, López E, Bosch L, Collado J, Pina-Pedrero S, Correa-Fiz F, Accensi F, Navas MJ, Vidal E, Bustos MJ, Rodríguez JM, Gallei A, Nikolin V, Salas ML, Rodríguez F. (2017). BA71ΔCD2: a New Recombinant Live Attenuated African Swine Fever Virus with Cross-Protective Capabilities. J Virol. 2017 91(21). pii: e01058-17

Montgomery RE (1921) A form of swine fever occurring in British East Africa (Kenya Colony). J Comp Pathol 34:159–191

Moore DM, Zsak L, Neilan JG, Lu Z, Rock DL (1998) The African swine fever virus thymidine kinase gene is required for efficient replication in swine macrophages and for virulence in swine. J Virol 72:10310–10315

Moss A. (2001) Tenacity of viral infectious agents in biogenic waste during co-fermentation with manure. Inaugural dissertation to obtain the doctorate degree at the Department of Veterinary Medicine of Justus Liebig University Giessen; submitted by Andreas Moss, veterinarian from Tübingen, casting 2001, ISBN 3-933953-87-1

Moulton J, Coggins L (1968a) Comparison of lesions in acute and chronic African swine fever. Cornell Vet 58:364–388

Moulton J, Coggins L (1968b) Synthesis and cytopathogenesis of African swine fever virus in porcine cell cultures. Am J Vet Res 29:219–232

Moura Nunes JF, Vigario JD, Terrinha AM (1975) Ultrastructural study of African swine fever virus replication in cultures of swine bone marrow cells. Arch Virol 49:59–66

Muhangi D, Masembe C, Emanuelson U, Boqvist S, Mayega L, Ademun RO, Bishop RP, Ocaido M, Berg M, Ståhl K. (2015) A longitudinal survey of African swine fever in Uganda reveals high apparent disease incidence rates in domestic pigs, but absence of detectable persistent virus infections in blood and serum. BMC Vet Res. 11:106.

Mujibi FD, Okoth E, Cheruiyot EK, Onzere C, Bishop RP, Fèvre EM, Thomas L, Masembe C, Plastow G, Rothschild M. (2018) Genetic diversity, breed composition and admixture of Kenyan domestic pigs. PLoS One.13(1):e0190080.

Muñoz-Moreno R., M.A., Freije JM, Salas ML, Viñuela E, Lopez-Otin C (1993) Structure and expression in *E. coli* of the gene coding for protein p10 of African swine fever virus. Arch Virol 130:93–107

Muñoz-Moreno R., M.A. Cuesta-Geijo, L. Barrado-Gil, C. Martínez-Romero, I. Galindo, A. García-Sastre and C. Alonso. (2016). Antiviral role of Interferon-induced transmembrane (IFITM) proteins in African Swine Fever Virus infection. PLoS ONE 11(4): e0154366.

Mur, L., Iscaro, C., Cocco, M., Jurado, C., Rolesu, S., De Mia, G. M., *et al.* (2017) Serological Surveillance and Direct Field Searching Reaffirm the Absence of Ornithodoros Erraticus Ticks Role in African Swine Fever Cycle in Sardinia. *Transbound Emerg Dis*, 64, 1322-1328.

Mur, L., Sanchez-Vizcaino, J. M., Fernandez-Carrion, E., Jurado, C., Rolesu, S., Feliziani, F., *et al.* (2017) Understanding African Swine Fever infection dynamics in Sardinia using a spatially explicit transmission model in domestic pig farms. *Transbound Emerg Dis*

Muwonge, A., Munang'andu, H. M., Kankya, C., Biffa, D., Oura, C., Skjerve, E., *et al.* (2012) African swine fever among slaughter pigs in Mubende district, Uganda. *Trop Anim Health Prod*, 44, 1593-1598.

Nantima, N., Davies, J., Dione, M., Ocaido, M., Okoth, E., Mugisha, A., *et al.* (2016) Enhancing knowledge and awareness of biosecurity practices for control of African swine fever among

smallholder pig farmers in four districts along the Kenya-Uganda border. *Trop Anim Health Prod,* 48, 727-734.

Nantima, N., Ocaido, M., Ouma, E., Davies, J., Dione, M., Okoth, E., *et al.* (2015) Risk factors associated with occurrence of African swine fever outbreaks in smallholder pig farms in four districts along the Uganda-Kenya border. *Trop Anim Health Prod* 47, 589-595.

Neilan JG, Lu Z, Afonso CL, Kutish GF, Sussman MD, Rock DL (1993a) An African swine fever virus gene with similarity to the proto-oncogene bcl-2 and the Epstein-Barr virus gene BHRF1. J Virol 67:4391–4394

Neilan JG, Lu Z, Kutish GF, Zsak L, Lewis TL, Rock DL (1997b) A conserved African swine fever virus IKB homolog, 5EL, is nonessential for growth in vitro and virulence in domestic pigs. Virology 235:377–385

Neilan JG, Borca MV, Lu Z, Kutish GF, Kleiboeker SB, Carrillo C, Zsak L, Rock DL (1999) An African swine fever virus ORF with similarity to C-type lectins is non-essential for growth in swine macrophages in vitro and for virus virulence in domestic swine. J Gen Virol 80:2693–2697

Neilan JG, Zsak L, Lu Z, Kutish GF, Afonso CL, Rock DL (2002) Novel swine virulence determinant in the left variable region of the African swine fever virus genome. J Virol 76:3095–3104

Neilan JG, Zsak L, Lu Z, Burrage TG, Kutish GF, Rock DL (2004) Neutralizing antibodies to African swine fever virus proteins p30, p54, and p72 are not sufficient for antibody-mediated protection. Virology 319:337–342

Netherton CL. Viral vectored African swine fever vaccines. GARA Scientific Conference, Sardinia, 2018.

Nieto-Pelegrín E, Rivera-Arroyo B, Sánchez-Vizcaíno JM. (2015) First Detection of Antibodies Against African Swine Fever Virus in Faeces Samples. Transbound Emerg Dis. 62(6):594-602.

Notomi, T, Okayama, H, Masubuchi, H, Yonekawa, T, Watanabe, K, Amino, N, Hase, T (2000). Loop-mediated isothermal amplification of DNA. Nucl Acids Res 28: E63.

Nunes Petisca JL (1965) Etudes anatomo-pathologiques et histopathologiques sur la peste porcine africaine (Virose L) au Portugal. Bull Off Int Epizoot 63:103–142

Nurmoja, I., Petrov, A., Breidenstein, C., Zani, L., Forth, J. H., Beer, M., *et al.* (2017) Biological characterization of African swine fever virus genotype II strains from north-eastern Estonia in European wild boar. *Transbound Emerg Dis*

O'Donnell V, Holinka LG, Krug PW, Gladue DP, Carlson J, Sanford B, Alfano M, Kramer E, Lu Z, Arzt J, Reese B, Carrillo C, Risatti GR, Borca MV. (2015a). African Swine Fever Virus

Georgia 2007 with a Deletion of Virulence-Associated Gene 9GL (B119L), when Administered at Low Doses, Leads to Virus Attenuation in Swine and Induces an Effective Protection against Homologous Challenge. J Virol. (16):8556-66. doi: 10.1128/JVI.00969-15.

O'Donnell V, Holinka LG, Gladue DP, Sanford B, Krug PW, Lu X, Arzt J, Reese B, Carrillo C, Risatti GR, Borca MV. (2015b). African Swine Fever Virus Georgia Isolate Harboring Deletions of MGF360 and MGF505 Genes Is Attenuated in Swine and Confers Protection against Challenge with Virulent Parental Virus. J Virol. 89(11):6048-56. doi: 10.1128/JVI.00554-15

O'Donnell V, Holinka LG, Sanford B, Krug PW, Carlson J, Pacheco JM, Reese B, Risatti GR, Gladue DP, Borca MV. (2016a). African swine fever virus Georgia isolate harboring deletions of 9GL and MGF360/505 genes is highly attenuated in swine but does not confer protection against parental virus challenge. Virus Res. Aug 2;221:8-14. doi: 10.1016/j.virusres.2016.05.014.

O'Donnell V, Risatti GR, Holinka LG, Krug PW, Carlson J, Velazquez-Salinas L, Azzinaro PA, Gladue DP, Borca MV. (2016b). Simultaneous Deletion of the 9GL and UK Genes from the African Swine Fever Virus Georgia 2007 Isolate Offers Increased Safety and Protection against Homologous Challenge. J Virol. 91(1). pii: e01760-16

Okoth E, Gallardo C, Macharia JM, Omore A, Pelayo V, Bulimo DW, Arias M, Kitala P, Baboon K, Lekolol I, Mijele D, Bishop RP. (2013) Comparison of African swine fever virus prevalence and risk in two contrasting pig-farming systems in South-west and Central Kenya. Prev Vet Med. 110(2):198-205.

Olsevskis, E., Guberti, V., Serzants, M., Westergaard, J., Gallardo, C., Rodze, I., *et al.* (2016) African swine fever virus introduction into the EU in 2014: Experience of Latvia. *Prev Vet Sci*, 105, 28-30.

Onisk DV, Borca MV, Kutish GF, Kramer E, Irusta P, Rock DL (1994) Passively transferred African swine fever virus antibodies protect swine against lethal infection. Virology 198:350–354

Oura CA, Powell PP, Anderson E, Parkhouse RMJ (1998a) The pathogenesis of African swine fever in the resistant bushpig. Gen Virol 79:1439–1443

Oura CA, Powell PP, Parkhouse RME (1998b) Detection of African swine fever virus in infected pig tissues by immunocytochemistry and in situ hybridisation. J Virol Methods 72:205–217

Oura CAL, Powell PP, Parkhouse RME (1998c) African swine fever: a disease characterized by apoptosis. J Gen Virol 79:1427–1438

Oura CAL, Denyer MS, Takamatsu H, Parkhouse RME (2005). In vivo depletion of CD8+ T lymphocytes abrogates protective immunity to African swine fever virus. J Gen Virol 86:2445–2450.

Penrith, M.-L., Thomson, G.R., Bastos, A.D.S, Phiri, O.C., Lubisi, B.A., Botha, B., Du Plessis, E.C., Macome, F., Pinto, F., Botha, B. and Esterhuysen, J. (2004). An investigation into natural resistance to African swine fever in domestic pigs from an endemic area in southern Africa. Rev. sci. tech., Off. int. Épiz. 23: 665–677.

Penrith ML, Vosloo W, Jori F, Bastos AD. (2013). African swine fever virus eradication in Africa. Virus Res.;173: 228–46.

Perez-Filgueira M., F. Gonzalez, C. Gallardo, P. Resino., Esther Blanco and J.M. Escribano. Optimization and validation of recombinant serological tests for African swine fever diagnosis based on the p30 protein produced in *Trichoplusia ni* larvae. *J. Clin. Microbiol.* 44, 3114-3121. 2006

Petrov, A., Schotte, U., Pietschmann, J., Drager, C., Beer, M., Anheyer-Behmenburg, H., Goller, K.V., Blome, S., 2014. Alternative sampling strategies for passive classical and African swine fever surveillance in wild boar. Veterinary microbiology.

Petrov A, Forth JH, Zani L, Beer M, Blome S. (2018). No evidence for long-term carrier status of pigs after African swine fever virus infection. Transbound Emerg Dis.. doi: 10.1111/tbed.12881

Pietschmann, J., Guinat, C., Beer, M., Pronin, V., Tauscher, K., Petrov, A., *et al.* (2015) Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate. *Arch Virol,* 160, 1657-1667.

Pietschmann, J., Mur, L., Blome, S., Beer, M., Perez-Sanchez, R., Oleaga, A., *et al.* (2016) African swine fever virus transmission cycles in Central Europe: Evaluation of wild boar-soft tick contacts through detection of antibodies against Ornithodoros erraticus saliva antigen. *BMC Vet Res,* 12, 1.

Plowright, W. and Parker, J. (1967). The stability of African swine fever virus with particular reference to heat and pH inactivation. Archiv fur die Gesamte Virusforchung 21, 383–402.

Plowright W (1981) African swine fever. In: Infectious Diseases of wild animals, 2nd edn. Davis JW, Karstad LH, Trainer DO (eds) Iowa University Press, Ames, IA

Plowright W, Parker J, Peirce MA (1969a) African swine fever virus in ticks (*Ornithodoros moubata* , Murray) collected from animal burrows in Tanzania. Nature 221:1071–1073

Plowright W, Parker J, Pierce MA (1969b) The epizootiology of African swine fever in Africa. Vet Rec 85:668–674

Plowright W, Thomson GR, Neser JA (1994) African swine fever. Infectious diseases of livestock. In: Coetzer JAW, Thomson GR, Tustin RC (eds) Oxford University Press, Capetown pp. 568–599

Popescu L, Gaudreault NN, Whitworth KM, Murgia MV, Nietfeld JC, Mileham A, Samuel M, Wells KD, Prather RS, Rowland RRR. (2017). Genetically edited pigs lacking CD163 show no

resistance following infection with African swine fever virus isolate Georgia 2007/1. Virology 501:102-106

Post J, Weesendorp E, Montoya M, Loeffen WL. (2017) Influence of Age and Dose of African Swine Fever Virus Infections on Clinical Outcome and Blood Parameters in Pigs. Viral Immunol. 30(1):58-69.

Powell PP, Dixon LK, Parkhouse RME (1996) An IKB homolog encoded by African swine fever virus provides a novel mechanism for downregulation of proinflammatory cytokine responses in host macrophages. J Virol 70:8527–8533

Quembo, C. J., Jori, F., Heath, L., Perez-Sanchez, R. & Vosloo, W. (2016) Investigation into the Epidemiology of African Swine Fever Virus at the Wildlife - Domestic Interface of the Gorongosa National Park, Central Mozambique. *Transbound Emerg Dis*, 63, 443-451.

Quembo, C. J., Jori, F., Vosloo, W., Heath, L. (2018) Genetic characterization of African swine fever virus isolates from soft ticks at the wildlife/domestic interface in Mozambique and identification of a novel genotype. Transbound Emerg Dis, 65:420–431.

Quintas A, Pérez-Núñez D, Sánchez EG, Nogal ML, Hentze MW, Castello A, Revilla Y. Characterization of the African swine fever virus decapping enzyme during infection. J Virol. doi:10.1128/JVI.00990-17

Ramiro-Ibañez F, Ortega A, Brun A, Escribano JM, Alonso C (1996) Apoptosis: a mechanism of cell killing and lymphoid organ impairment during acute African swine fever virus infection. J Gen Virol 77:2209–2219

Ravaomanana, J., Jori, F., Vial, L., Perez-Sanchez, R., Blanco, E., Michaud, V., *et al.* (2011) Assessment of interactions between African swine fever virus, bushpigs (Potamochoerus larvatus), Ornithodoros ticks and domestic pigs in north-western Madagascar. *Transbound Emerg Dis*, 58, 247-254.

Reis AL, Abrams CC, Goatley LC, Netherton C, Chapman DG, Sanchez-Cordon P, Dixon LK. (2016). Deletion of African swine fever virus interferon inhibitors from the genome of a virulent isolate reduces virulence in domestic pigs and induces a protective response. Vaccine. 34(39):4698-4705. doi: 10.1016/j.vaccine.2016.08.011.

Reis AL, Goatley LC, Jabbar T, Sanchez-Cordon PJ, Netherton CL, Chapman DAG, Dixon LK. (2017). Deletion of the African Swine Fever Virus Gene DP148R Does Not Reduce Virus Replication in Culture but Reduces Virus Virulence in Pigs and Induces High Levels of Protection against Challenge. J Virol. 30;91(24). pii: e01428-17. doi: 10.1128/JVI.01428-17.

Ribeiro, R., Otte, J., Madeira, S., Hutchings, G. H. & Boinas, F. (2015) Experimental Infection of Ornithodoros erraticus sensu stricto with Two Portuguese African Swine Fever Virus Strains. Study of Factors Involved in the Dynamics of Infection in Ticks. *PLoS One*, 10, e0137718.

Rendleman, CM and Spinelli, FJ (1994) The costs and benefits of African swine fever prevention. Am J Agric Econ 76:1255–125.

Rodriguez CI, Nogal ML, Carrascosa AL, Salas ML, Fresno M, Revilla Y (2002) African swine fever virus IAP-like protein induces the activation of nuclear factor kappa B. J Virol 76:3936–3942

Rodriguez F, Alcaraz C, Eiras A, Yanez RJ, Rodriguez JM, Alonso C, Rodriguez JF, Escribano JM (1994) Characterization and molecular basis of heterogeneity of the African swine fever virus envelope protein p54. J Virol 68:7244–7252

Rodriguez JM, Yanez RJ, Almazan F, Viñuela E, Rodriguez JF (1993a) African swine fever virus encodes a CD2 homolog responsible for the adhesion of erythrocytes to infected cells. J Virol 67:5312–5320

Rodriguez JM, Yanez RJ, Rodriguez JF, Viñuela E, Salas ML (1993b) The DNA polymeraseencoding gene of African swine fever virus: sequence and transcriptional analysis. Gene 136:103–110

Rodriguez JM, Yanez RJ, Pan R, Rodriguez JF, Salas ML, Viñuela E (1994) Multigene families in African swine fever virus: family 505. J Virol 68:2746–2751

Rouiller I, Brookes SM, Hyatt AD, Windsor M, Wileman T (1998) African swine fever virus is wrapped by the endoplasmic reticulum. J Virol 72:2373–2387

Rowlands, R J (2008) African swine fever virus isolate, Georgia, 2007. Emerg. Infect. Dis. 14:1870–1874.

Ruiz-Gonzalvo F, Carnero ME, Bruyel V (1981) Immunological responses of pigs to partially attenuated ASF and their resistance to virulent homologous and heterologous viruses. In: Wilkinson PJ (ed) FAO/CEC Expert Consultation in ASF Research, Rome, pp 206–216

Ruiz-Gonzalvo, F, Rodriguez, F and Escribano, JM (1996) Functional and immunological properties of the baculovirus: expressed hemagglutinin of African swine fever virus. Virology 218:285–289

Salas ML, Kuznar J, Viñuela E (1981) Polyadenylation, methylation, and capping of the RNA synthesized in vitro by African swine fever virus. Virology 113:484–491

Salas ML, Kuznar J, Viñuela E (1983) Effect of rifamycin derivatives and coumermycin A1 on in vitro RNA synthesis by African swine fever virus. Arch Virol 77:77–80

Salguero FJ, Ruiz-Villamor E, Bautista MJ, Sanchez-Cordon PJ, Carrasco L, Gomez-Villamandos JC (2002) Changes in macrophages in spleen and lymph nodes during acute African swine fever: expression of cytokines. Vet Immunol Immunopathol 90:11–22

Salguero FJ, Sánchez-Cordón PJ, Sierra MA, Jover A, Núñez A, Gómez-Villamandos JC (2004) Apoptosis of thymocytes in experimental African swine fever virus infection. Histol Histopathol 19:77–84

Salguero FJ, Sánchez-Cordón PJ, Núñez A, Fernández de Marco M, Gómez-Villamandos JC (2005) Proinflammatory cytokines induce lymphocyte apoptosis in acute African swine fever infection. J Comp Pathol 132:289–302

Sánchez-Cordón PJ, Ceron JJ, Nunez A, Martinez-Subiela S, Pedrera M, Romero-Trevejo JL, Garrido MR, Gomez-Villamandos JC (2007) Serum concentrations of C-reactive protein, serum amyloid A, and haptoglobin in pigs inoculated with African swine fever or classical swine fever viruses. Am J Vet Res 68:772–777.

Sánchez-Cordón PJ, Jabbar T, Berrezaie M, Chapman D, Reis A, Sastre P, Rueda P, Goatley L, Dixon LK. (2018). Evaluation of protection induced by immunisation of domestic pigs with deletion mutant African swine fever virus BeninΔMGF by different doses and routes. Vaccine. 36(5):707-715. doi: 10.1016/j.vaccine.2017.12.030.

Sánchez, JA, Pierce, KE, Rice, JE, Wangh, LJ (2004) Linear-After-The-Exponential (LATE)-PCR: an advanced method of asymmetric PCR and its uses in quantitative real-time analysis. Proc Natl Acad Sci 101:1933–1938

Sánchez-Vizcaino JM, Slauson DO, Ruiz Gonzalvo F, Valero MS (1981) Lymphocyte function and cell-mediated immunity in pigs with experimentally induced African swine fever. Am J Vet Res 42:1335–1341

Sánchez-Vizcaíno, J.M., Mur, L., Gomez-Villamandos, J.C. & Carrasco, L. (2015a). An update on the epidemiology and pathology of African swine fever. *Vet. Comp. Path.*, 152, 9-21.

Sanchez-Vizcaino, J. M., Mur, L., Bastos, A. D. & Penrith, M. L. (2015b) New insights into the role of ticks in African swine fever epidemiology. *Rev Sci Tech*, 34, 503-511.

Sastre P, Pérez T, Costa S, Yang X, Räber A, Blome S, Goller KV, Gallardo C, Tapia I, García J, Sanz A, Rueda P. (2016a) Development of a duplex lateral flow assay for simultaneous detection of antibodies against African and Classical swine fever viruses. J Vet Diagn Invest. 28(5):543-9.

Sastre P, Gallardo C, Monedero A, Ruiz T, Arias M, Sanz A, Rueda P. (2016b) Development of a novel lateral flow assay for detection of African swine fever in blood. BMC Vet Res. 12:206

Schloer GM (1985) Polypeptides and structure of African swine fever virus. Virus Res 3:295-310

Simon-Mateo C, Freije JM, Andres G, Lopez-Otin C, Viñuela E (1995) Mapping and sequence of the gene encoding protein p17, a major African swine fever virus structural protein. Virology 206:1140–1144

Simeon-Negrin, RE and Frias-Lepoureau, MT (2002) Eradication of African swine fever in Cuba (1971 and 1980). In Trends in emerging viral infections of swine (eds A. Morilla, K. J. Yoon & J. J. Zimmerman), pp. 125–131. Ames, IA: Iowa State Press.

Simulundu, E., Chambaro, H. M., Sinkala, Y., Kajihara, M., Ogawa, H., Mori, A., *et al.*(2017) Co-circulation of multiple genotypes of African swine fever viruses among domestic pigs in Zambia (2013-2015). *Transbound Emerg Dis.*

Simon-Mateo C, Freije JM, Andres G, Lopez-Otin C, Viñuela E (1995) Mapping and sequence of the gene encoding protein p17, a major African swine fever virus structural protein. Virology 206:1140–1144

Steyn DG (1928) Preliminary report on a South African virus disease amongst pigs. 13th and 14th Reports of the Director of Veterinary Education and Research, Union of South Africa, pp 415–428

Steyn DG (1932) East African virus disease in pigs. 18th Report of the Director of Veterinary Services and Animal Industry, Union of South Africa 1:99–109

Sumption KJ, Hutchings GH, Wilkinson PJ, Dixon LK (1990) Variable regions on the genome of Malawi isolates of African swine fever virus. J Gen Virol 71:2331–2340 Sun H, Jacobs SC, Smith GL, Dixon LK, Parkhouse RM (1995) African swine fever virus gene j13L encodes a 25–27 kDa virion protein with variable numbers of amino acid repeats J Gen Virol 76:1117–1127

Sun H, Jacobs SC, Smith GL, Dixon LK, Parkhouse RM (1995) African swine fever virus gene j13L encodes a 25–27 kDa virion protein with variable numbers of amino acid repeats J Gen Virol 76:1117–1127

Sun H, Jenson J, Dixon LK, Parkhouse ME (1996) Characterization of the African swine fever virion protein j18L. J Gen Virol 77:941–946

Sussman MD, Lu Z, Kutish GF, Afonso CL, Roberts P, Rock DL (1992) Identification of an African swine fever virus gene with similarity to a myeloid differentiation primary response gene and a neurovirulence-associated gene of herpes simplex virus. J Virol 66:5586–5589

Tabares E, Sanchez Botija C (1979) Synthesis of DNA in cells infected with African swine fever virus. Arch Virol 61:49–59

Tabares E, Marcotegui MA, Fernandez M, Sanchez-Botija C (1980a) Proteins specified by African swine fever virus. I. Analysis of viral structural proteins and antigenic properties. Arch Virol 66:107–117

Takamatsu H, Denyer MS, Oura C, Childerstone A, Andersen JK, Pullen L, Parkhouse RM (1999) African swine fever virus: a B cell-mitogenic virus in vivo and in vitro. J Gen Virol 80:1453–1461

Thomas LF, Bishop RP, Onzere C, Mcintosh MT, Lemire KA, de Glanville WA, Cook EA, Fèvre EM. (2016) Evidence for the presence of African swine fever virus in an endemic region of Western Kenya in the absence of any reported outbreak. BMC Vet Res. 12(1):192.

Thomson GR (1985). The epidemiology of African swine fever: the role of free-living hosts in Africa. Onderstepoort J Vet Res 52:201–209

Thomson GR, Gainaru MD, Van Dellen AF (1979) African swine fever: Pathogenicity and immunogenicity of two non-haemadsorbing viruses. Onderstepoort J Vet Res 46:149–154

Thomson GR, Gainaru M, Lewis A, Biggs H, Nevill E, van Der Pypekamp M, Gerbes L,Esterhuysen J, Bengis R, Bezuidenhout D, Condy J (1983) The relationship between ASFV, the warthog and *Ornithodoros* species in southern Africa. In: Wilkinson PJ (ed) African swine fever. ASF, EUR 8466 EN, Procceedings of CEC/FAO Research Seminar, Sardinia, Italy, September 1981, Commission of the European Communities, Rome, pp 85–100

Thoromo, J., Simulundu, E., Chambaro, H. M., Mataa, L., Lubaba, C. H., Pandey, G. S., *et al.* (2016) Diagnosis and genotyping of African swine fever viruses from 2015 outbreaks in Zambia. *Onderstepoort J Vet Res* 83, a1095.

Titov I, Burmakina G, Morgunov Y, Morgunov S, Koltsov A, Malogolovkin A, Kolbasov D. (2017) Virulent strain of African swine fever virus eclipses its attenuated derivative after challenge. Arch Virol. 162(10):3081-3088

Tulman E, Delhon GA, Ku BK, and Rock DL (2009) African swine fever virus. Curr Top Microbiol Immunol. 328:43-87

Turner C and Williams S.M. (1999). Laboratory-scale inactivation of African swine fever virus and swine vesicular disease virus in pig slurry. Journal of Applied Microbiology 1999, 87, 148–157

Uttenthal Å, Braae UC, Ngowi HA, Rasmussen TB, Nielsen J, Johansen MV. (2013). ASFV in Tanzania: asymptomatic pigs harbor virus of molecular similarity to Georgia 2007. Vet Microbiol. 165(1-2):173-6

van Heerden, J., Malan, K., Gadaga, B. M. & Spargo, R. M. (2017) Reemergence of African Swine Fever in Zimbabwe, 2015. *Emerg Infect Dis* 23, 860-861.

Vilanova M, Ferreira P, Ribeiro A, Arala-Chaves M (1999) The biological effects induced in mice by p36, a proteinaceous factor of virulence produced by African swine fever virus, are mediated by interleukin-4 and also to a lesser extent by interleukin-10. Immunology 96:389–395

Wang J, Wang J, Geng Y, Yuan W. (2017)A recombinase polymerase amplification-based assay for rapid detection of African swine fever virus. Can J Vet Res. 81(4):308-312.

Wambura PN, Masambu J, Msami H (2006) Molecular diagnosis and epidemiology of African swine fever outbreaks in Tanzania. Vet Res Commun 30:667–672

Wardley RC, Wilkinson PJ (1977) The association of African swine fever virus with blood components of infected pigs. Arch Virol 55:327–334

Wardley RC, Wilkinson PJ (1980) Lymphocyte responses to African swine fever virus infection. Res Vet Sci 28:185–189

Wilkinson PJ, Wardley RC, Williams SM. (1981) African swine fever virus (Malta/78) in pigs. J Comp Pathol. 91(2):277-84.

Wilkinson PJ (1989) African swine fever virus. In: Pensaert MB (ed) Virus infections of porcines. Elsevier, Amsterdam, pp 17–35

Wozniakowski, G., Kozak, E., Kowalczyk, A., Lyjak, M., Pomorska-Mol, M., Niemczuk, K., *et al.* (2016) Current status of African swine fever virus in a population of wild boar in eastern Poland (2014-2015). *Arch Virol,* 161, 189-195.

Wu X, Xiao L, Lin H, Chen S, Yang M, An W, Wang Y, Yang Z, Yao X, Tang Z. (2018) Development and application of a droplet digital polymerase chain reaction (ddPCR) for detection and investigation of African swine fever virus. Can J Vet Res. 82(1):70-74.

Xiao L, Wang Y, Kang R, Wu X, Lin H, Ye Y, Yu J, Ye J, Xie J, Cao Y, Wei Y, Liao D, Pan M, Lin Y, Dai Z, Li X. (2018) Development and application of a novel Bio-Plex suspension array system for high-throughput multiplexed nucleic acid detection of seven respiratory and reproductive pathogens in swine. J Virol Methods. 261:104-111.

Yabe, J., Hamambulu, P., Simulundu, E., Ogawa, H., Kajihara, M., Mori-Kajihara, A., *et al.* (2015) Pathological and molecular diagnosis of the 2013 African swine fever outbreak in Lusaka, Zambia. *Trop Anim Health Prod* 47, 459-463.

Yanez RJ, Viñuela E (1993) African swine fever virus encodes a DNA ligase. Virology 193:531–536

Yanez RJ, Boursnell M, Nogal ML, Yuste L, Viñuela E (1993a) African swine fever viru encodes two genes which share significant homology with the two largest subunits of DNA-dependent RNA polymerases. Nucleic Acids Res 21:2423–2427

Yanez RJ, Rodriguez JM, Boursnell M, Rodriguez JF, Viñuela E (1993b) Two putative African swine fever virus helicases similar to yeast 'DEAH' pre-mRNA processing proteins and vaccinia virus ATPases D11L and D6R. Gene 134:161–174

Yanez RJ, Rodriguez JM, Rodriguez JF, Salas ML, Viñuela E (1993c) African swine fever virus thymidylate kinase gene: sequence and transcriptional mapping. J Gen Virol 74:1633–1638

Yates PR, Dixon LK, Turner PC (1995) Promoter analysis of an African swine fever virus gene encoding a putative elongation factor. Biochem Soc Trans 23:139S

Zani L, Nurmoja I, Breidenstein C, Leidenberger S, Martin B; Blome S. (2016). First evidence of an attenuated phenotype of genotype II African Swine Fever Virus in Estonia. 3rd Annual GARA scientific workshop. Ploufragan, France. September 6, 2016.

Zhang F, Hopwood P, Abrams CC, Downing A, Murray F, Talbot R, Archibald A, Lowden S, Dixon LK (2006) Macrophage transcriptional responses following in vitro infection with a highly virulent African swine fever virus isolate. J Virol 80:10514–10521

Zsak L, Onisk DV, Afonso CL, Rock DL (1993) Virulent African swine fever virus isolates are neutralized by swine immune serum and by monoclonal antibodies recognizing a 72-Kda viral protein. Virology 196:596–602

Zsak L, Lu Z, Kutish GF, Neilan JG, Rock DL (1996) An African swine fever virus virulence-associated gene NL-S with similarity to the herpes simplex virus ICP34.5 gene. J Virol 70:8865–8871

Zsak L, Caler E, Lu Z, Kutish GF, Neilan JG, Rock DL (1998) A nonessential African swine fever virus gene UK is a significant virulence determinant in domestic swine. J Virol 72:1028–1035

Zsak L, Lu Z, Burrage TG, Neilan JG, Kutish GF, Moore DM, Rock DL (2001) African swine fever virus multigene family 360 and 530 genes are novel macrophage host range determinants. J Virol 75:3066–3076

Zsak L., Borca M.V., Risatti G.R., Zsak A., French R.A., Lu Z., Kutish G.F., Neilan J.G., Callahan J.D., Nelson W.M., Rock D.L.. 2005. Preclinical diagnosis of African swine fever in contact-exposed swine by a real-time PCR assay. J Clin Microbiol.;43(1):112-9.