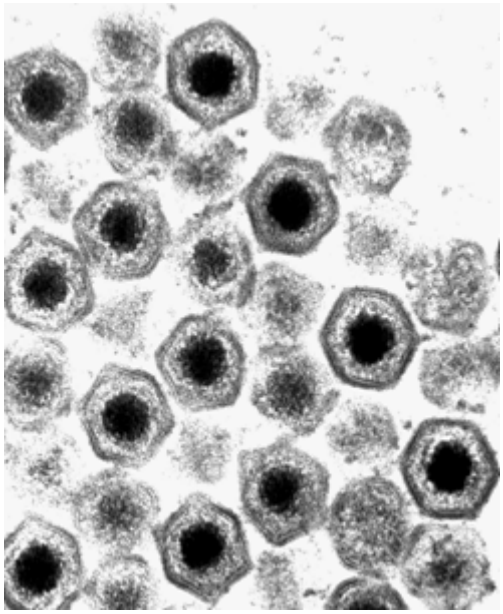
# African Swine Fever (ASF)

# Gap Analysis Workshop

## Madrid, Spain, June 1-2, 2009



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# 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.

PPE:  Personal Protective Equipment

RT-PCR: Reverse transcription-polymerase chain reaction

cRT-PCR:  Conventional RT-PCR

rRT-PCR: Real-time reverse transcription-polymerase chain reaction

VMO:  Veterinary Medical Officer

# EXECUTIVE SUMMARY

A group of international experts on African Swine Fever (ASF) was convened to conduct a gap analysis of our current knowledge of ASF and the available countermeasures to effectively control and mitigate the impact of an outbreak in the United States. The African Swine Fever Countermeasures Working Group (ASFCWG) was organized by Cyril Gerard Gay, Senior National Program Leader, Agricultural Research Service, in collaboration with Professor José-Manuel Sánchez-Vizcaíno, Catedrático de Sanidad Animal, Director del Laboratorio de Referencia de la OIE, Universidad Complutense Facultad de Veterinaria, and the support of Isabel Minguez-Tudela, European Commission, and the European Union (EU) ASFRISK project coordinator, Professor Carlos Martins. The working group met in Madrid, Spain, on 1-2 June, 2009. This report provides the results of the gap analysis and research priorities.

The threat for an introduction of ASF in the United States is significant. ASF is one of the most complex viral disease affecting domestic pigs, wild boars, and wild suids. Soft ticks also have been described as biological reservoir and transmission vectors. ASFV usually induces an unapparent infection in a variety of African wild boar and bush pig population.

Currently, the disease is endemic in more than twenty sub-Saharan African countries. In Europe ASF is still endemic on the island of Sardinia (Italy) and new outbreaks have been declared in the Caucasus region since 2007, affecting Georgia, Armenia, Azerbaijan and Russia. The situation is not under control in these countries yet, increasing the ASF risk of entry towards other countries.

The initial expression of ASF in U.S swine would be 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. 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 makes early detection difficult and a challenge for surveillance programs.

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

## Surveillance

Surveillance 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. The initial expression of ASF in U.S swine would 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. Strains vary from low to highly virulent; and clinical signs range from persistent congenital infections with no apparent signs to outbreaks of acute infection. There must be at least two surveillance programs in place: a ‘syndromic’ surveillance program based on reporting of clinical signs and a laboratory-based surveillance program that includes diagnostic testing of populations at risk.

## 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 has resulted in significant financial implications and the culling of thousands of animals has also become ethically debatable.

## Biosecurity

On-farm biosecurity is a critical countermeasure for controlling 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. 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 including 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.

## 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.

## Diagnostics

ASF 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. Serological differentiation from ASFV-like diseases can be performed by ELISA and Western blot although commercial tests are not currently available. ELISA is fully capable of distinguishing between ASFV-specific antibodies and antibodies to other ASF-like disease of swine.

# INTRODUCTION

African Swine Fever (ASF) is a contagious viral disease of domestic pigs with significant economic consequence. In Africa, ASF virus (ASFV) produces inapparent infections in two species of wild suids: wart hog (*Phacochoerus aethiopicus*) and the bush pig (*Potamochoerus porcus*). The reservoir of ASFV is considered the soft tick *Ornithodoros moubata* (Dixon *et al*., 2005).

ASF virus (ASFV) is a large, enveloped virus containing a double stranded (ds) DNA of approximately 190 kilobase pairs. ASFV 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 iruses) (Costard *et al*. 2009).

ASFV 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. ASF occurs in several forms, ranging from highly lethal to sub-clinical. Acute forms of ASF, associated with highly virulent ASF strains, are characterized by death 3 to 7 days post-infection. 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. Sub-acute and chronic forms of the disease are associated with ASF strains of moderate and low virulence, respectively (van Oirschot 1999)

ASF was considered an endemic disease in the Iberian peninsula that was successful eradicated from the European Union (EU) at the end of the 1990s, with the exception of Sardinia, and the recent 2007 ASF outbreak in the Caucasus. Although most Member States of the EU have successfully managed to eradicate the disease from the domestic pig population, there is the constant threat of the re-introduction into a country or spread to domestic livestock from the import of infected pig products fed as contaminated swill to domestic pigs (Costard *et al*. 2009).

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/eradicate ASF (Costard *et al*. 2009).

# BACKGROUND

## Organization of the African Swine Fever Countermeasures Working Group (ASFCWG)

The Chair of the ASFCWG, Dr. Cyril Gerard Gay, partnered with Professor José-Manuel Sánchez-Vizcaíno, DVM, Ph.D, Catedrático de Sanidad Animal, Director del Laboratorio de Referencia de la OIE, Universidad Complutense Facultad de Veterinaria, to select a team of ASF experts from research institutions, industry, academia, and government to serve on the ASFCWG. A total of 19 experts (see list of working group members on pages 3-9) accepted to serve on the ASFCWG. The ASFCWG met in Madrid, Spain, on 1-2 June, 2009. Instructions (see Appendix I) and several reference materials were provided by the ASFCWG Chair prior to the meeting. The ASFCWG members were tasked by the Chair with assessing the best available countermeasures to rapidly and effectively control and eradicate ASF should an outbreak occur in the United States.

### *Reference Material*

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

1. http://www.asfrisk.eu/
2. http://www.fao.org/docrep/004/y0510e/y0510e00.HTM
3. http://www.oie.int/wahis/public.php?page=disease
4. http://athena.bioc.uvic.ca/organisms/Asfarviridae

# DEFINITION OF THE THREAT

The threat for an introduction of African Swine Fever (ASF) in the United States is significant. ASF is a highly contagious viral disease of domestic pigs and wild boar with 11 countries having notified the OIE of at least one outbreak of ASF on their territory within the last three years. ASF was successfully eradicated from Europe in the 1990s but remains endemic on the island of Sardinia. Although the EU has successfully managed to eradicate the disease from the domestic pig population, there is the constant threat of the re-introduction into a country or spread to domestic pigs from infected wild boar populations.

The initial expression of ASF in U.S swine would be variable and unpredictable due to the myriad of host factors and the broad diversity of virulence among strains of ASF virus. Viral mechanisms involved in induction of disease, tissue tropism, host range, and induction of immune responses are still not well understood. Additionally, the presence of arthropods in the epidemiological chain of the disease would introduce a significant factor in the eradication of the disease in the case of an outbreak. The disease occurs in several forms, ranging from highly lethal to sub-clinical depending of the acting virus strain. There is no vaccine available for ASFV, which constitutes a serious concern in the implementation of a plan for the control/eradication of the disease.

## Economic Impact

The extent of the economic impact of ASFV into the United States is unknown. 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 lost of status for international trade and the implementation of drastic and costly control strategies to eradicate the disease (Costard et al., 2009). There are few examples in the Western Hemisphere, in Cuba, the introduction of the disease in 1980 led to a total cost, including the eradication program, of USD $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, it has been estimated that the net benefit of preventing ASF introduction in the USA amounts to almost US $450 million: nearly 5 per cent of the value of total sales of pork products (Rendleman and Spinelli 1994).

## Virology

African swine fever virus (ASFV) is a large, enveloped virus containing a double stranded (ds) DNA of approximately 190 kilobase pairs. ASFV shares aspects of genome structure and replication strategy with other large dsDNA viruses, including the *Poxviridae, Iridoviridae,* and *Phycodnaviridae* (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) (Costard *et al*. 2009). 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 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 (van Oirschot 1999).

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, NifSlike 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.

## Pathogenesis

Clinical presentation of ASF in domestic pigs depends on the virulence of the circulating virus. ASFV 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 swine 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 such as Erysipelas and Classical Swine Fever hamper surveillance based exclusively on clinical signs.

Infection usually occurs through the oronasal route with a 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 time 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. 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). Typical 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), hemorrhage in lymph nodes, spleen, kidneys, and respiratory and gastrointestinal tracts, congestion of skin and serosae, and severe interlobular lung edema (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). 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 appears to be a critical factor in ASFV virulence. Persistent infection with ASFV is reported to occur in warthogs and in domestic pigs surviving acute viral infection (DeKock *et al*., 1994; Detray 1957). Under experimental conditions, long-term persistent infection is the sequel to infection with ASFV in domestic pigs (Carrillo *et al*., 1994). In these animals, viral DNA was detected in the peripheral blood monocyte fraction more than 500 days p.i. by PCR; however, infectious virus could not be isolated from these samples.

In sub-Saharan Africa, ASFV is maintained in a sylvatic cycle between wild swine (warthogs and bushpigs) and argasid ticks of the genus Ornithodoros (Plowright *et al*., 1969a and 1969b; Thomson *et al*., 1983; Wilkinson 1989). Unlike domestic swine, wild swine 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 demonstrate 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. Large deletion of six MGF360 genes and two MGF530 genes significantly reduce viral replication in macrophages and the virus pathogenesis in swine ((Neilan *et al*., 2002). Implicated in macrophage host range are ASFV proteins involved in nucleotide and nucleic acid metabolism and which, 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, again 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 ), adjacently located in the genome. UK, an early proteins quite variable depending of the virus isolate, lacks similarity to other known proteins and its deletion from pathogenic ASFV, although it does not affect viral growth in macrophages in vitro, does 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) since its deletion from the ASFV E70 strain reduces its virulence in swine without affecting viral replication in macrophages in vitro.

## Immunology

Currently, there is no vaccine available for ASF and the disease is strictly controlled by animal quarantine and slaughter. 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).

# Epidemiology

The epidemiology of ASF may vary substantially between affected countries, regions and continents. Two types of transmission cycles can be 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, 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. The first spread of ASF outside Africa was to Portugal in 1957 as a result of waste from airline flights being fed to pigs near Lisbon airport (Costard *et al*., 2009). Similar means of ASFV introduction were reported for the outbreak in Brazil 1978 (Lyra 2006).

Twenty two different *p72 genotypes* have recently been identified among virus isolates from sub-Saharan African countries. However this 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, in June 2007, an ASF outbreak was notified to the OIE in the Caucasus region, in Georgia, in the Caucasus region presumably by feeding pigs with ASFV contaminated pork brought in on ships (Rowlands et al., 2008). It was attributed to a new isolate, related to *p72 genotype II*, circulating in South Eastern Africa. Since then, ASF has spread to the neighbouring countries of Armenia, Azerbaijan and the Russian Federation, reaching the border with Ukraine. The 2007 outbreak confirmed that the threat of ASF spreading to countries outside the African continent is high and is potentially devastating to the global pig industry. This includes the EU countries, Eastern Europe, the Black Sea basin countries and - in the worst case scenario central Asia and even China, which has the largest pig population in the world.

## Surveillance

Clinical presentation of ASF in domestic pigs depends on the virulence of circulating virus. ASFV 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 swine 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 would be prudent to develop surveillance activities based on diagnostic testing.

The following surveillance programs should be implemented to meet the objective of rapid detection of ASFV in U.S swine:

1. Population-based passive reporting of suspicious ASF cases. Efforts to enhance reporting will be focused on high risk states. High risk areas for ASF include those with garbage feeding operations, backyard swine operations, feral swine hunting clubs, military bases, international air or sea ports, farming operations utilizing an international labor force, corporations engaging in international movement of swine, etc. High risk is also a function of the number of swine in each state and the number of swine imports in each state. Puerto Rico is identified as a very high risk area and 18 States (8 from eastern region and 10 from western region) are recognized as high risk States.

2. Laboratory-based surveillance of serum and tissue submitted from sick pigs with a suspicious hemorrhagic disease. The intended coverage of this surveillance program would be any and all premises where domestic swine exist. This includes all 50 states. Any laboratory or slaughter plant is encouraged to submit tissues from sick pigs to ASFV approved NAHLN laboratories or FADDL for routine ASFV surveillance or submit to FADDL if ASFV is suspected. This plan seeks to enhance the submission of tissues from sick pigs, specifically in high risk states. For the higher risk States, the determination will be made for the most

appropriate source of serum and tissue samples from sick pigs to focus on appropriate target populations.

3. VMO/AHT-based active surveillance of registered waste feeders for ASF. VMO/AHT-based: Waste feeders must be licensed and regularly inspected by State or Federal VMO's and/or AHT's. The intended coverage would be all sites in the continental U.S. feeding waste to swine. This definition of the target population may be too aggressive in some respects and may need to be tightened.

4. Slaughter-based active surveillance of samples collected from swine in TX, FL, and PR. The waste feeders in PR are too numerous to sample directly. Furthermore, not all swine sites in FL or TX are waste feeders but are small herds that may slaughter pigs in state. One exception to this are those feral pigs captured, fed, and sold for slaughter in TX plants. So this population has good overlap with the population being targeted with the added benefit of some monitoring of the feral pig population as well. Ideal samples to collect would be tonsil and spleen. Need input from FSIS on which plants to collect samples from.

5. Population-based active surveillance of high risk herds. Data collected directly from producers in a 1 km area surrounding disposal sites for pork meat scraps of foreign origin, e.g., airports and military bases. Also, data collected directly from producers or practitioners from those production sites importing any type of genetic material from any foreign country within the previous 3 months.

6. Population-based active surveillance of high risk herds. For example, herds importing swine genetic material or near disposal areas of pork meat.

## On-farm Biosecurity

The main goal of an on-farm biosecurity plan is to control disease by decreasing the probability of infection. The purpose is to minimize impact of endemic diseases but to also avoid introduction of new diseases into a swine herd. A set of zoo-sanitary measures should be put in place to accomplish the goals set by the biosecurity plan. A biosecurity plan for a swine operation should include the following elements:

- Location of premises
- Distance between premises
- Design of the facilities
- Perimeter of facilities
- Flow of vehicles, personnel, and visitors in-out of the premises
- Personnel gear
- Load-out-facilities
- Pig handling and flow.
- Introduction of pigs/semen in a herd
- Management of sick and dead animals
- Feed management and distribution within the farm
- Waste management

# 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 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.

# Diagnosis

A wide variety of laboratory techniques are available either for ASF virus and antibody detection. It is important to point out that ASF presents three significant advantages: 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.

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: Lymph nodes, kidney, spleen, lung, blood and serum. Tissues are used for virus isolation (HA test), viral antigen detection (DIF test), and DNA viral detection (PCR test), while blood is 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) test, Direct immunofluorescence (DIF), and since 2000, the molecular detection of ASF virus by PCR. None of these techniques are commercially available with the exception of a PCR kit that has been very recently commercially available, which includes all reagents dried down, as well as a rehydration buffer and a positive control (Zsak et al. 2005).

**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 a small number of field strains showing only cytopathic effect without producing the hemadsorption phenomenon. These strains are identified using the direct immunofluorescence test on the sediments of these cell cultures.

ASF-DNA detection. Since 2000, some PCR tests, based on conventional and Real-time procedures, have been developed and some of them are nowadays already 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.

Direct immunofluorescence (DIF). 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 Ag-technique, the Ag-ELISA. It is well-know that 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.

**Antibody Detection Techniques**

Antibody ELISA. This is the most useful method for large-scale serological studies. It is 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 (Igezim PPA Compact. Prionics), and it has been validated by the Central Reference Laboratory (CRL), Spain. The procedure of an “in house” OIE ELISA as well as a standardized/validated soluble antigen for OIE ELISA test could be also provided by CRL previous a 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 not a 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.

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 less than three hours 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 involved 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 determining the genetic relationships and origin of viruses responsible for disease outbreaks occurred in the last years in Europe (Italy and Caucasus countries) and Africa.

## Vaccines

There is currently no commercial vaccine available for ASFV. In fact, an effective commercial vaccine for ASF has never been available. 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 t ransfer 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 were protected when challenged with homologous parental virus (Lewis *et al*., 2000; Moore *et al*., 1998; Zsak *et al*., 1996 and 1998). Further research is required to develop effective vaccines. Identification of ASFV genes involved in virulence and in evasion of the host's immune response (for review see Dixon *et al*., 2008) makes the

development of rationally attenuated vaccines through sequential deletion of these genes realistic. However, extensive testing of the safety of such vaccines is required.

Alternative approaches based on expression of protective antigens are halted since no viral antigen inducing protection has been identified yet. The 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.

One concern about the use of ASFV vaccines is the genetic diversity of strains circulating in some countries. Although recent experiments have claimed to demonstrate cross-protection between different genotypes (Zsak personal communication), and therefore it may be possible to develop vaccines that can cross-protect against infection with several genotypes, lack of cross-protection among geographical or temporal separated isolates is the rule.

## Summary of Obstacles to Prevention and Control

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

**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 solutions for many countries but has many weaknesses due to the difficulty of differentiating ASF from many common endemic infectious diseases.
- 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.

**Depopulation**

- Depopulation is the primary countermeasure to stop the spread of ASFV in case of an outbreak in a ASF-free country like the United States. However, depopulation would present significant challenges in the event of an outbreak in the United States, starting with the significant financial implications and the culling of thousands of animals.

**Biosecurity**

- Biosecurity is a critical countermeasure both to prevent and protect commercial 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.

**Diagnosis**

- ASF is usually suspected based on clinical signs, but clinical evidence may be nonspecific and difficult to differentiate from infectious diseases endemic to the U.S or other foreign animal diseases. Real time and conventional RT-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.

**Vaccines**

- There are no commercially available vaccines to control ASF outbreaks.

# ASSUMPTIONS

The following captures assumptions made by the ASFCWG to assess potential countermeasures for the NVS to enhance our ability to contain and eradicate an outbreak of ASF in the United States.

***Situation***
Countermeasures assessed for worst case scenario: A coordinated intentional distribution of ASFV-contaminated material in a high density highly populated pig region of the United States.

***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 should be based in the early detection of infected animals and their elimination, and strict control of the movement of pigs.

# DECISION MODEL

The ASFCWG 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 the June 1-2, 2009 meeting (see Appendix I). Criteria and weights in the model were modified by the ASFCWG for the purpose of assessing available countermeasures as well as experimental ASF vaccines and diagnostics (See Appendices II, III, IV, and V).

***Criteria***

The ASFCWG 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 NAHNL's personnel
11. Scalable
12. Reasonable cost

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

# GAP ANALYSIS

The threat for an introduction of African Swine Fever (ASF) in the United States is significant. ASF is a highly contagious viral disease of domestic pigs, wild boar, and wild suids with 11 countries having notified the OIE of at least one outbreak of ASF on their territory in the last three year. ASF was considered an infection that was eradicated in the European Union (EU) at the end of the 1990s with the exception of Sardinia and the 2007 ASF outbreak in the Caucasus in 2007. Although most Member States of the EU have successfully managed to eradicate the disease from their domestic pig population, there is the constant threat of the re-introduction into a country or spread to domestic livestock from the import of infected pig products fed as contaminated swill to domestic pigs.

The initial expression of ASF in U.S swine would be variable and unpredictable due to the myriad of host factors and the broad diversity of virulence among strains of ASF virus. 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 to chronic with all infections being highly lethal. Antibody response elicited by infection with virulent strains of the virus does not begin to appear to detectable levels until at least 15 days, which makes early detection difficult and a challenge for surveillance programs.

## Virology

African swine fever virus (ASFV) is a large, enveloped virus containing a double stranded (ds) DNA of approximately 170-193 kilobase 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). 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. 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).

Gaps

The number of laboratories working on ASFV worldwide over the last 40 years has been minimal. This is primarily due to the fact that 1) the virus does not infect humans, 2) the virus has been, to a large extent, limited to Africa and a small part of Europe, and 3) ASFV has no close viral relatives; i.e., there are no other viruses in the *Asfarviridae.*

Although there is only a single virus species, currently 22 genotypes have been described; 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 22 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.

One important gap is the lack of information on viral genomics and the determinants responsible for variations in virulence and the lack of vaccine cross-protection. Consequences of this lack of genomic data are:

1) Cross-protecting vaccines cannot be made and we are unable to identify or predict conserved epitopes that may provide cross-protection. The possibility of producing effective vaccines is hampered since correlation between patterns of conservancy and divergence among genes is unknown and therefore homologous/heterologous experimental protection cannot be established.
2) Difficulties in assigning significance to particular genes or groups of genes in virus adaptation or tropism for wild/domestic pigs.
3) Since current genotype grouping is based in a restricted group of genes, current genotyping may be biased yielding erroneous information. In addition, genotyping data has yet to be shown to relate to data on vaccine cross-protection.

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 insects.

2) ASFV bioinformatics resource:

The annotation and analysis of genomes in 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 would be highly valuable to establish a comprehensive database, which would include full length genome sequence of large number of isolates to replace the current less meaningful genotype based classification.

# Pathogenesis

In the course of chronic forms, the morbility and mortality rates are lower than in acute and subacute forms of ASF, where severe inflammatory changes responsible for intense tissular 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 a 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 background among different virus strains. Differences in patterns of virus replication, kinetics and severity in presentation of micro and macropathology and patterns of host gene activation should be analyzed in swine infected with each of the paired virus.

Gaps

There is a significant gap in the basic knowledge regarding the identification of:

1) Basic mechanisms governing animal to animal infection and the event in the process of host-virus interaction.
2) Molecular differences in the pathogenesis process induced by virus with different degree o f virulence
3) The role of specific genomic determinant(s) in disease outcome.

Research needs

1) Basic mechanisms governing animal to animal infection and host-pathogen interactions
2) Study the pathogenesis of virulent ASFV isolates in susceptible host.
3) Determine patterns of activation of immunologically relevant host genes particularly at early stages after infection
4) Identify ASFV genes and genetic determinants (group of genes like multigene families) involved in host range, virulence and pathogenicity.
5) Use gene deleted recombinant viruses to determine viral pathogenesis, host responses, and virus-host interactions.

# Immunology

There is no vaccine available for ASF. Attempts to vaccinate animals using many different approaches have 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. Unfortunately, deletion of the genes encoding for those proteins from pathogenic ASFV does not affect viral growth in macrophages *in vitro* or viral pathogenesis and virulence in domestic swine.

Gaps

1) Identification of immune mechanism(s) mediating protection against the infection in swine.
2) Identification of the virus protein(s) responsible for the induction of protective immune mechanism.
3) Understanding the actual role of virus driven host immunomodulation in the process of virus infection in swine.

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.

# Diagnostics

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.

Gaps

1) Current virus isolation techniques take several days, are difficult to scale up, and require technical expertise to perform the tests.
2) Field validation of the real time RT-PCR, currently the single most important tool for detection
3) Validation of serological and virological tests for different epidemiological situations (e.g., low versus virulent ASFV strains)
4) There are no pen side tests available

Research needs

1) Develop new technologies for virus isolation
2) Develop pen side tests for use in disease outbreaks
3) Develop companion diagnostic for next generation vaccines to differentiate infected from vaccinated animals (DIVA).

# Epidemiology

The epidemiology of ASF varies regarding the two types of transmission cycles among swine populations, which can be defined as a domestic pig cycle and sylvatic-wild pig cycle. Additionally, presence or absence of arthropod vectors will impact the maintenance of the virus in the environment. Importantly, once established, virus is efficiently contact-transmitted between domestic swine although infection through direct contact between domestic pigs and warthogs has not been documented. 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.

Molecular epidemiology must be applied in ASFV risk analysis and outbreak tracing, but should be based on multiple genes. Currently, combined sequence analysis of viral genes *p72-p54* and CVR remains the most effective approach to delineate the phylogenetic relationship between isolates. The p30 gene is being investigated as an additional tool for ASF genotyping. The *p72* and *p30* phylogenies yield similar topologies with marginally better resolution using *p30.* Based on currently available data, it is possible to delineate the following global distribution of ASFV types:

- Caucasus and Russia Federation (genotype II)
- Americas (genotype I)
- West Africa (genotype I)
- East and Central Africa (all genotypes known)
- Sardinia (Genotype I)

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.

## Surveillance

Surveillance 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. The initial expression of ASF in U.S swine would be variable and unpredictable due to the myriad of host factors and the broad diversity of virulence among strains of ASF virus. Different surveillance strategies will be required to detect the different clinical manifestations. One of the priorities will be to prepare veterinarians for the clinical recognition of ASF in pigs and feral suids.

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 CSF 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.
2) Evaluate under natural conditions the performance and overall accuracy of currently available ELISAs and PCR tests.
3) Develop and evaluate novel tests such as ELISAs for both antigen and antibody detection.
4) Automation and standardization of viral genome sequencing for subtyping ASFV strains
5) Assess the rate of transmission of strains of ASFV of different virulence in infected-contact animal experiments.
6) Develop tests for detecting ASFV in ticks.

7) 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).
8) Epidemiological investigations should be performed on the implementation of emergency control measures and the use of ‘diagnostic tests to detect infected pigs in exposed populations.
9) Risk assessments need to be performed with regard to control or spread of ASFV

## 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

## *Vaccines*

The ASFCWG determined that no ASF vaccines are commercially available. The current research into a suitable vaccine for ASFV is limited to only a few groups worldwide. The most promising, potential, candidates are rationally attenuated 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. 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. Unfortunately, there is currently no candidate isolate appropriately attenuated to ensure both safety and efficacy; although work is ongoing at the Plum Island Animal Disease Center to produce an appropriate virus for initial testing. 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; however, previous data indicated that such a strategy did not provide efficient protection against ASFV infection. Currently there is some ongoing research into the feasibility of using such a strategy for producing an ASF vaccine. The current research is sadly a long way from producing a working vaccine, although it is hoped that the information generated by the research will be of great use in furthering our understanding of this disease and hence help generate effective vaccines in the future.

<u>Summary</u>

Vaccination against ASF is currently not an option. A focused drive on producing a rationally attenuated live virus vaccine would help to bring a viable vaccine online in the shortest timescale.

<u>Assessment of Experimental Vaccines</u>

The ASFCWG 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 vaccine:* attenuated by deletion of specific gen: these vaccines are created by the deletion of specific genes which have been identified as virulence associated genetic determinants. As result, attenuated virus stains 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.

2) *Subunit recombinant ASFV protein/s vectorized in vaccinia virus:* Recombinant vaccinia strains containing individual ASFV gene/s will be used as vector. Safety, rapid onset of immunity, possibility of developing a DIVA test and the cost of implementation are

recognized as the strength of this vaccine. The efficacy and lack of protection against heterologous viruses would be its main disadvantages. It is important to remark that so far there is no experimental evidence that an individual or a group of AFSV genes vectorized in any way can protect domestic swine against the challenge with the homologous virus. Therefore, development of an ASFV subunit vaccine depends on previous research identifying the virus structures able to induce protection against the infection.

3) *Recombinant ASFV subunit vaccines using swinepox virus as vector:* as the previous one, this is a subunit vaccine where an ASFV gene/s is vectorized by swinepox virus. The assessment of this vaccine is very similar to that of the subunit vaccine using vectorized through vaccinia. The WG found that its safety may be an additional defect for this particular vaccine. Again, no candidate ASFV gene has been identified so far to be used in a subunit vaccine.

4) *ASFV DNA vaccines: this* is 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 accompanying test are the only strengths remarked by the WG. As in vaccine candidates analyzed in *(2)* and *(3)*, no candidate ASFV gene has been 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 needs a great deal of experimental assessment in several aspects of its basic development: as induction of early immunity, development of the accompanying DIVA test, assessment of reversion to virulence.

## *Diagnostics*

The ASFCWG determined that 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 ASFCWG 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. Detection of virus infection is detected by hemadsorption or presence of cytopatogenic 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 present 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 easy to be 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 easy to be 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 easy to be scaled up as well as adapted to a throughput system. Additionally, it is easy to be performed 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 ASFCWG 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 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.

## Other Countermeasures

Disinfectants
Many of the common disinfectants are ineffective. Care should be taken to use a disinfectant specifically approved for ASFV. Sodium hypochlorite (5.25% is household bleach) and some iodine and quaternary ammonium compounds have been shown to be effective.

Acaricides
Acaricides for controlling the soft tick may not be useful as the tick lives off the host and burrows underground and 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.

# CONCLUSION

The United States is vulnerable to an accidental or intentional virulent ASF outbreak. Even though ASF is an African disease, it is now well entrenched on the Caucasus and Russia and the virus is spreading west and east threatening Europe and Asia, respectively. 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 the United States. Furthermore, the epidemiological implications of ASF outbreaks in new geographical ecological 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 our 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

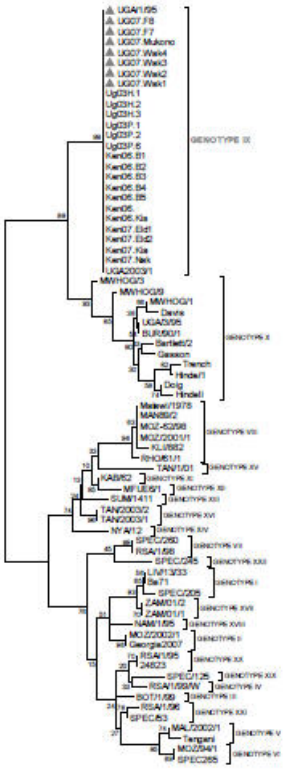

Figure 1: The extent of the ASF virus diversity as revealed by a phylogenetic tree of p72 genotyping revealing 22 ASF Genotypes (Provided by Carmina Gallardo)

# 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 on October 1, 2009, 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 on October 1. 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 |

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Animal Production and Protection
Agricultural Research Service

# APPENDIX II

**Experimental Vaccines For ASF - September 30, 2009**

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 | nbinant Gene D | oinant 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)

# APPENDIX III

**SURVEILLANCE (USA) 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

**OUTBREAK (USA) 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 | 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 | rtimePCR-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

| Surveillance (USA) 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

| Outbreak (USA) 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

# LICENSED DIAGNOSTIC TEST MANUFACTURERS

**PRIONICS AG**
Wagistrasse 27a
CH-8952 Schlieren-Zurich
Switzerland

**Ingezim PPA COMPAC**
Antibody detection ELISA

**Ingezim PPA DAS**
Double antibody sandwich ELISA for the detection of ASFV antigen

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