STAR-IDAZ
International Research
Consortium on Animal Health

Roadmaps for African Swine Fever

- **Development of candidate vaccines**
- **Development of diagnostic tests**

SIRCAH Deliverable 3.3

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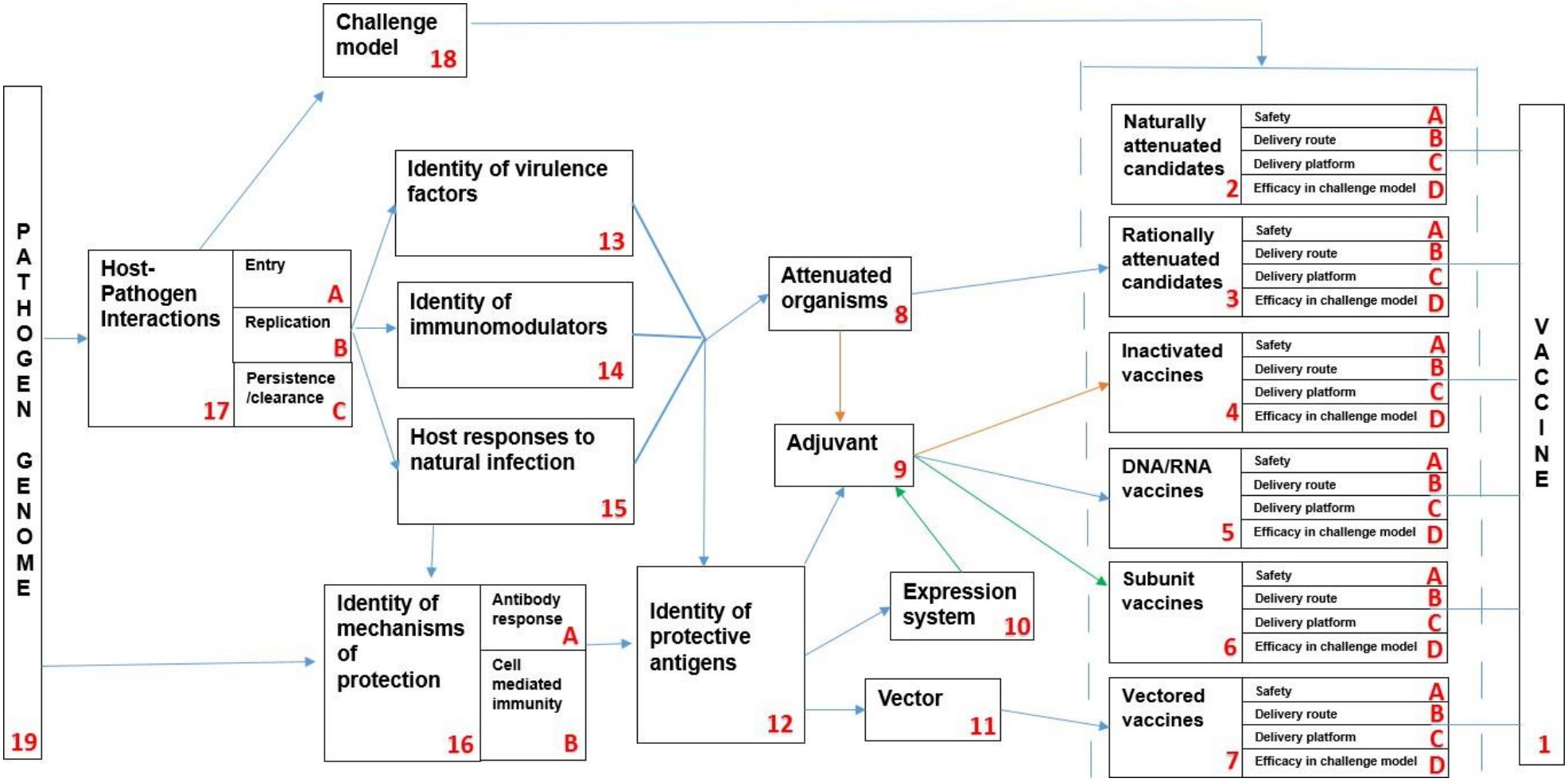
Roadmap for development of a candidate vaccine for ASF



Lead summaries are in draft form until validated by the Global ASF Research Alliance (GARA)

ASF Vaccine - Lead Summary 1

Title:	A cross-protective ASFV vaccine preventing disease, virus transmission and carrier state in vaccinated animals

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Highly efficacious and safe.
Rapid onset of immunity.
Long lasting protection following one shot immunisation (at least 1-year).
Cross-protection against the various ASFV virus strains.
No reversion to virulence.
Virus eradication from a herd or a population
Oral administration for wild suids.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Even homologous protective immunity is very difficult to achieve.
Cross-protection against the various isolates (heterologous protection).
Generation of both a CTC and VN response.
The dominant immunogens may not be protective.

Solution Routes

What approaches could/should be taken to address the research question?

Establish protection levels with various candidate vaccine options, including priming with one vaccine and boosting with a different vaccine.
Establish if pig genetics influences responses.

Dependencies

What else needs to be done before we can solve this need?

Development of an attenuated vaccine that doesn't persist or is excreted.
Development of a subunit vaccine.
Development of a vector vaccine
Development of a DNA vaccine.

State of the Art

Existing knowledge including successes and failures

Several prototypes are presented, but all are far away from a real vaccine candidate.

Projects

What activities are planned or underway?

Lead Summary 2.

Title:	Development of a “naturally” attenuated vaccine that doesn’t persist or is excreted

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The development of a cross protective ASF vaccine by passage in tissue culture or a mutant low pathogenic wild type strain.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Lack of cross protection among different isolates.
Risk of reversion to virulence.
Candidates will need to comply with safety, quality and efficacy requirements described in the ASF chapter of the WOAH Manual of Vaccines and diagnostic Tests 2025
Ensure that the mechanism of attenuation is defined at a molecular level, and its stability is ensured through multiple gene modifications

Solution Routes

What approaches could/should be taken to address the research question?

Perform passage in tissue culture to reduce virulence.
Identify mutant low pathogenic wild strains.

Dependencies

What else needs to be done before we can solve this need?

Identify strains that give greatest cross protection.
Develop suitable cell lines for high titre propagation and licensing.

State of the Art

Existing knowledge including successes and failures

Experimentally, some level of 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.
Challenge models have been evaluated and are available.

Projects

What activities are planned or underway?

Lead Summary 3

Title:	Development of a rationally attenuated vaccine that is safe and effective, prevents transmission, requires only one dose, can be parenterally and oral administration, and provides cross-protection.

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Replicating ASFV are likely to give the most appropriate immune response but wild-type virus manipulates the host response. The aim is to reduce the virulence so that the vaccinated animal can mount a protective immune response. (The vaccine should allow the differentiation between infected and vaccinated animals.)

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Lack of heterologous protection.
Possible link between immunogenicity and pathogenicity of the virus.
Ensure that vaccination prevents excretion of the organism – both the vaccine strain and wild type virus or any combination of the two that may have been generated.
Achieve long duration and fast onset of immunity.
Development of recombinant ASFV is still laborious.
Candidates will need to comply with safety, quality and efficacy requirements described in the ASF chapter of the WOAH Manual of Vaccines and Diagnostic Tests 2025
Attenuation should be designed specifically to ensure minimal risk of regain of function through field strain recombination
Ideally a DIVA element will be incorporated

Solution Routes

What approaches could/should be taken to address the research question?

Identify suitable strains
Create GM organisms lacking virulence and immunomodulatory factors.
Monitor the immune response following immunisation with the various candidates.
Develop improved systems to manipulate ASFV.
Perform challenge experiments with the various vaccine candidates, including challenge with other strains.
Identify more suitable cell lines for ASFV production.

Dependencies

What else needs to be done before we can solve this need?

Generate stable genetically modified attenuated organisms.
Identify virulence factors in ASFV.
Identify immunomodulators in ASFV.
Identify gene function ASFV.
Identify correlates of protection.
(Identify DIVA targets).
Immune response: identify mechanism of immune evasion, and mechanism of protective immunity.
Better understand virus replication.
Develop suitable cell lines for production.

State of the Art

Existing knowledge including successes and failures

Pigs immunised with live attenuated ASF viruses containing engineered deletions of specific ASFV virulence/host range genes could be protected to different levels when challenged with homologous parental virus.

Projects

What activities are planned or underway?

Lead Summary 4

Title:	Development of cross protective/multivalent ASF inactivated/killed vaccine

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The development of an effective killed virus vaccine that provide protection and is safe.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Having it efficacious.
Cross protection.
Stimulating protective response involving both VN Abs and CTC.

Solution Routes

What approaches could/should be taken to address the research question?

Selection of inactivated whole virus preparations
Monitoring the immune response following immunisation with the various candidates.
Challenge experiments with the various vaccine candidates.

Dependencies

What else needs to be done before we can solve this need?

Selection of the right strain, titre and preparation/inactivation to induce a protective immunity.
The availability of suitable adjuvants to stimulate strong CTC and VN-Ab responses.

State of the Art

Existing knowledge including successes and failures

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.

Projects

What activities are planned or underway?

Lead Summary 5, 6 and 7

Title:	Development of a nucleic acid based or subunit vaccine

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The development of a subunit vaccine where ASFV gene/s is cloned into DNA constructs that are used as immunogens, providing broad cross-protective immunity and is safe.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Identify ASFV gene products that can induce a protective immune response to engineer subunit vaccines.
Have it multivalent.
Stimulate protective response involving both VN Abs and CTC.

Solution Routes

What approaches could/should be taken to address the research question?

Multimeric scaffold could be an important research target of priority.

Dependencies

What else needs to be done before we can solve this need?

Identify protective antigens and the antigen combination to give widest protection against the various field isolates.
Select the best adjuvants
Select the best production system
Select the best antigen presentation system, e.g. multimeric scaffold, VLPs etc.
Identify a combination of antigens that would generate protective responses **or** a common single antigen to which immune responses are normally suppressed.

State of the Art

Existing knowledge including successes and failures

Up to now, all subunit candidates failed

Projects

What activities are planned or underway?

Lead Summary 8

Title:	The generation of rationally attenuated genetically modified ASF virus

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To generate organisms that are less virulent in terms of pathological changes that they cause and/or their ability to modulate the host's immune responses – rationally attenuated vaccine.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Ensure the organisms are stable and can be produced in cell culture.
Ensure the organisms can still generate a protective response.
Ensure the organisms give protection against heterologous challenge.
Ensure that the mechanism of attenuation is defined at a molecular level, and its stability is ensured through multiple gene modifications

Solution Routes

What approaches could/should be taken to address the research question?

Identify ASFV genes involved in virulence and in evasion of the host's immune response.
Generate and characterise a range of rationally attenuated organisms
Immune response to the attenuated organisms.

Dependencies

What else needs to be done before we can solve this need?

Identify virulence factors and their genes.
Identify immunomodulators.
Ensure ability to give cross protection against heterologous challenge.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 9

Title:	Identifying suitable adjuvants for subunit and inactivated whole virus vaccine candidates

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Identifying suitable adjuvants that generate an optimal immune response to the various vaccine candidates, possibly resulting in sterile immunity

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Generate both a strong VN Ab and a CTC response

Solution Routes

What approaches could/should be taken to address the research question?

Immune response to antigens delivered on nanoparticles. Immune response following inclusion of various adjuvants with the candidate vaccines.

Dependencies

What else needs to be done before we can solve this need?

Identity of protective antigens

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 11

Title:	Identifying suitable vector for the expression/delivery of protective ASFV antigens

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The identification of a suitable vector for the expression/delivery of protective ASFV antigens.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Ensure the expression of the antigens to induce a protective immune response.
Identify the best vector among those that already went through the regulatory process.
Create stable genetically modified organisms expressing the desired ASFV antigens.

Solution Routes

What approaches could/should be taken to address the research question?

Generate genetically modified organisms (viruses or bacteria) expressing the protective antigens of different ASFV strains.
Use recombinant vaccine strains containing individual ASFV gene/s as vectors.
Use recombinant ASFV subunit vaccines using swinepox virus as vector.
Incorporate molecular adjuvants (expressing CD40 ligand).

Dependencies

What else needs to be done before we can solve this need?

Identify the most appropriate antigens.
Ensure sufficient expression level.
Develop capacity to test the expression.
Identify the most suitable delivery systems (*e.g.* oral for wild suids in Europe).
Identify vectors being suitable for one dose administration.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 12

Title:	Identity of protective antigens of ASF virus

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

The identity of the virus components (epitopes) that the host needs to respond to **prevent** and/or **clear** infection.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

The dominant immunogens may not be the most protective, thus a range of possible antigens will need to be considered.
Investigate gene functions.
Investigate the need of chaperon for proper folding of proteins.
Investigate if different antigens provoke a response in wild boars and pigs.
Induction of a neutralising immune response.

Solution Routes

What approaches could/should be taken to address the research question?

Identify possible protective antigens in the virus genome, their expression and trial in challenge experiments.
Identify the antigens that the host is generating Abs to and their role in protection (preventing and clearing infection).
Identify the antigens that are responsible for protective cellular responses.

Dependencies

What else needs to be done before we can solve this need?

Identify protective mechanisms operating in immune hosts – the role of neutralising Abs and CTCs.
The genome sequence of various virus isolates.
Identify determinant of virulence.

State of the Art

Existing knowledge including successes and failures

Neutralising antibodies directed against virion proteins p30, p54, and p72 have been described but they are not sufficient for antibody-mediated protection.
Partial protection was achieved using a combination of two recombinant proteins, p54 and p30, as well as with recombinant CD2-like protein but failed to give protection against highly virulent isolates.

Projects

What activities are planned or underway?

Lead Summary 13

Title:	To establish the identity of the virulence factors in ASFV that contribute to disease pathology

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Identifying and removal of the factors contributing to pathological changes are essential for generating rationally attenuated vaccines.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Identify molecular differences in the pathogenesis process induced by virus with different degree of virulence.
Identify determinant of virulence.

Solution Routes

What approaches could/should be taken to address the research question?

Swapping of suspected virulence genes between virulent and attenuated viruses.
Generation of a range of knock-out viruses and their use in experimental infections to establish the impact of the changes on virulence.

Dependencies

What else needs to be done before we can solve this need?

Improve understanding of virus macrophage interaction – viral and macrophage gene expression in different in vivo environments (macrophages from naïve and immune hosts).
Perform comparative studies of the response to mild and highly virulent strains.
Identify primary site of viral replication.
Identify viral receptors and co-receptors.
Identify factors of persistence of infection (chronic infection).

State of the Art

Existing knowledge including successes and failures

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.
Deletion of either gene UK (DP96R) and 23-NL (DP71L or l14L), adjacently located in the genome although it does not affect viral growth in macrophages in vitro, does markedly attenuates the virus in swine.

Projects

What activities are planned or underway?

Lead Summary 14

Title:	To establish the identity of the immunomodulatory factors/stealth mechanisms in ASFV

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

ASFV attempts to modulate the host's immune responses so that it can survive and replicate.
Identifying and removal of the factors contributing to the virus stealth mechanisms could contribute to the generation of improved attenuated vaccine candidates

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Identify the mechanisms of immunomodulation and the virus proteins responsible.
Apply the interactomic approach for ASFV infection.

Solution Routes

What approaches could/should be taken to address the research question?

Generation of a range of knock-out viruses where the genes for various immunomodulatory factors or other stealth mechanisms have been removed and their use in experimental infections.
Studies of the modulation of innate immune responses.

Dependencies

What else needs to be done before we can solve this need?

Improve understanding of virus-macrophage interaction – viral and macrophage gene expression in different in vivo environments (macrophages from naïve and immune hosts).

State of the Art

See next page

Projects

What activities are planned or underway?

State of the Art

Existing knowledge including successes and failures

ASFV-infected macrophages mediate changes in cellular immune function and are likely play a role in the severe apoptosis observed in lymphoid tissue. ASFV inhibits phorbol myristic acid-induced expression of pro-inflammatory cytokines such as TNF-α, IFN-α, and IL-8 while inducing production of TGF-β from infected macrophages but TNF-α expression has also been reported after ASFV infection in vitro and in vivo ASFV strains with different virulence phenotypes differ in their ability to induce expression of pro-inflammatory cytokine or IFN-related genes in macrophages early in infection. The ASFV ankyrin repeat-containing protein pA238L (5EL), a homolog of cellular IκB proteins and the cytoplasmic inhibitors of the NFκB/Rel family of cellular transcription factors, is thought to be important in evading host immune responses. NFκB transcriptional pathways play an important role in inducing expression of a wide range of pro-inflammatory 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. Similarly, pA238L inhibits expression of iNOS, and ultimately production of nitric oxide, by a mechanism likely involving p300 transactivation. However, deletion of A238L from pathogenic ASFV does not affect viral growth in macrophages in vitro or viral pathogenesis and virulence in domestic swine. Other proteins involved in modulation of host responses include ASFV 8DR protein (pEP402R), the only known viral homolog of cellular CD2, a T cell protein involved in co-regulation of cell activation. 8DR is necessary and sufficient for mediating haemadsorption by ASFV-infected cells. 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. 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. A potential role for pEP153R in immunomodulation may be subtle, however, since pEP153R does not affect viral pathogenesis or virulence in domestic. 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. 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. 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.

Lead Summary 15

Title:	Characterisation of host responses to natural infection

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

ASF virus cause acute – chronic infection reflecting the virulence of the virus strain or the host (genetics). Understanding the different responses could shed light on the protective mechanisms and virulence factors.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Perform studies in the different hosts.
Apply transcriptomic approach (in wild boars).

Solution Routes

What approaches could/should be taken to address the research question?

Comparative studies of the pathogenic mechanisms of acute and chronic forms induced by high and low virulence ASFV isolates respectively.
Molecular differences in the pathogenesis process induced by virus with different degree of virulence.
Comparative studies of wild type and mutants where specific genes have been deleted.
Apply the interactomic approach for ASFV infection.
The role of specific genomic determinant(s) in disease outcome.

Dependencies

What else needs to be done before we can solve this need?

Improve understanding of the host pathogen interaction including persistence versus clearance.

State of the Art

Existing knowledge including successes and failures

Projects

What activities are planned or underway?

Lead Summary 16

Title:	To identify protective mechanisms in ASFV infected animals

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

Identify the mechanisms operating in immune animals, establishing the role of Abs and CMI in **preventing** and **clearing** infection.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Identify mechanisms responsible for animal survival following infection, including the mechanisms involved in the protective immune responses.
Identify viral genetic patterns that correlate with presence/absence of homologous versus heterologous protection.
Identify mechanism of homologous and heterologous resistance to develop disease upon infection among different species (also disease tolerance and susceptibility).

Solution Routes

What approaches could/should be taken to address the research question?

Identify the role of Ab and CMI in providing protection against infection – passive transfer experiments.
Establish the role of the various cell types and cytokine responses in clearing infection.
Establish the identity of the leukocytes that are effective in eliminating infected macrophages.
Determine patterns of activation of immunologically relevant host genes.

Dependencies

What else needs to be done before we can solve this need?

Improve understanding of host virus interaction at the level of the infected cells.
Genome sequence of various ASFV isolates.

State of the Art

Existing knowledge including successes and failures

Humoral and cellular immunity are significant components of the protective immune response to ASF.

Projects

What activities are planned or underway?

Lead Summary 16A

Title:	The role of antibodies in protection to ASF virus

Research Question
What are we trying to achieve and why? What is the problem we are trying to solve?

To establish the role of antibodies in protection.

Challenge(s)
What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Identify protective / neutralising antibodies.
Stimulate an efficient protective antibody response.
Investigate mucosal antibodies.

Solution Routes
What approaches could/should be taken to address the research question?

Monitor the antibody response to infection with different strains
Passive transfer and challenge experiments

Dependencies
What else needs to be done before we can solve this need?

State of the Art
Existing knowledge including successes and failures

Passive transfer of antibodies from immune pigs conferred partial protection to lethal challenge.

Projects
What activities are planned or underway?

Lead Summary 16B

Title:	The role of cell-mediated immune responses in protection to ASF virus

Research Question
What are we trying to achieve and why? What is the problem we are trying to solve?

To establish the role of cell- mediated immune responses in protection.

Challenge(s)
What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Investigate the role of CTL for different genotypes.
Identify the effectors cells (cell/subtypes population).
Investigate the role of local immunity.

Solution Routes
What approaches could/should be taken to address the research question?

Monitor the cellular response to infection with different strains at different stages of infection and in recovered animals.
Passive transfer and challenge experiments.

Dependencies
What else needs to be done before we can solve this need?

State of the Art
Existing knowledge including successes and failures

CD8 + lymphocytes also appear to have a role in the protective immune response to ASFV infection - Depletion of CD8+ T cells abrogates protection.

Projects
What activities are planned or underway?

Lead Summary 17

Title:	Host Pathogen interaction in ASF infection

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To gain an improved understanding of how ASFV **enters**, **replicates** and **survives** in and is **released** from infected cells.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Establish how the virus interacts with macrophages as to identifying the protective mechanisms and how the virus evades them.
Collect information on viral genomics/host transcriptomics and the determinants responsible for variations in virulence and protective responses.
Identify viral receptors and ligands (identification, how virus interacts).
Identify viral attachment proteins.
Determine patterns of activation of immunologically relevant host genes particularly at early stages after infection.
Identify ASFV genes and genetic determinants (group of genes like multigene families) involved in host range, virulence and pathogenicity.
Development of attenuated ASFV that can be classified as BSL2 agent.

Solution Routes

What approaches could/should be taken to address the research question?

Establish the basis of virulence/pathogenicity - including in high virulence strains – and investigate its relationship with inflammatory response or viral replication.
Identify ASFV genes and genetic determinants (group of genes like multigene families) involved in virulence and pathogenicity – including using knock-out or different strains
Assess viral and macrophage gene expression in different in vivo environments (macrophages from naïve and immune hosts).
Compare responses to highly pathogenic/virulent and mild/attenuated strains of the virus.
Perform comparative genome studies with other large dsDNA viruses, including the *Poxviridae.*
Identify regulatory genes involved in pro-inflammatory cytokines and antibodies production and the assessment of their actual role in the process of virus infection\virulence in swine.

Dependencies

What else needs to be done before we can solve this need?

Genome sequence of various ASFV isolates.

State of the Art

Existing knowledge including successes and failures

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.
Pathological findings in acute ASF include leukopenia B and T cell lymphopenia / thrombocytopenia lymphocyte and mononuclear cell apoptosis.
ASFV exhibit temporal regulation of gene expression.
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.
ASFV 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.

Projects

What activities are planned or underway?

Lead Summary 17A

Title:	Entry of ASF virus into host cells

Research Question
What are we trying to achieve and why? What is the problem we are trying to solve?

Establishing how the virus enters host cells would indicate a possible route that could be blocked by targeted specific immune responses.

Challenge(s)
What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Establish virus components that interact with cell surface receptors.
Identify role of endosomal traffic of the virus.
Establish route of viral replication.

Solution Routes
What approaches could/should be taken to address the research question?

Dependencies
What else needs to be done before we can solve this need?

State of the Art
Existing knowledge including successes and failures

Projects
What activities are planned or underway?

Lead Summary 17B

Title:	Establishing how ASF replicates in the cell and developing suitable cell lines for virus replication.

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To generate knowledge on virus replication, as to allow the identification of the most suitable cell lines for virus replication.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Identify suitable cell lines for virus replication.
Produce an immortalised cell line for ASFV vaccine production
Identify primary site of viral replication.
Identify alveolar macrophage genes that enable ASF viral growth to inform the development of a cell line for vaccine production.

Solution Routes

What approaches could/should be taken to address the research question?

Generation of replication deficient viruses could contribute to the development of rationally attenuated vaccines.

Dependencies

What else needs to be done before we can solve this need?

Identify alveolar macrophage genes that enable ASF viral growth to inform the development of a cell line for vaccine production.

State of the Art

Existing knowledge including successes and failures

ASFV replicates in the cytoplasm of the infected cell, primarily in discrete perinuclear assembly sites.
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.
Implicated in macrophage host range are ASFV proteins involved in nucleotide and nucleic acid metabolism which may provide the deoxynucleotide pools favourable for efficient virus replication
Deletion of the dUTPase (E165R gene) and thymidine kinase (K196R gene) genes from ASFV reduces its ability to replicate in macrophages.

Projects

What activities are planned or underway?

Lead Summary 17C

Title:	Establishing the mechanisms of virus persistence/clearance from infected cells

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

To establish how viruses result in persistent infection.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

Solution Routes

What approaches could/should be taken to address the research question?

Perform studies on natural survivors.

Dependencies

What else needs to be done before we can solve this need?

State of the Art

Existing knowledge including successes and failures

Persistent infection with ASFV is reported to occur in warthogs and in domestic pigs surviving acute viral infection.

Projects

What activities are planned or underway?

Lead Summary 19

Title:	Genome sequence of ASF strains

Research Question

What are we trying to achieve and why? What is the problem we are trying to solve?

There are a range of viruses' strains which differ in terms of virulence. Having virus genome sequences is essential for identifying the host pathogen interactions and how this can be manipulated. Establishing the genomic differences of the various strains will assist in the identification of virulence mechanisms.

Challenge(s)

What are the scientific and technological challenges (knowledge gaps needing to be addressed)?

The annotation and analysis of genomes in the size range of ASFV is difficult and requires specialised tools.

Solution Routes

What approaches could/should be taken to address the research question?

Using current, very robust technologies 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.

Dependencies

What else needs to be done before we can solve this need?

Analyse isolates from different origins and hosts including domestic pigs, wild boar, warthog and ticks.
Analyse field strains from current outbreaks in Africa.

State of the Art

Existing knowledge including successes and failures

ASFV and *Poxviridae* have similar genome structures, including terminal inverted repeats, terminal crosslinks, a central conserved region and variable regions at each end of the genome.
ASFV virion is comprised of more than 50 polypeptides.
External to the inner membrane is the capsid, composed of the structural protein p72, ASFV genome include homologs of cellular ubiquitin conjugating enzyme, trans-prenyltransferase, NifSlike protein, and components of a base-excision repair pathway.
Several of the 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) are among the most variable among multiple field isolates. Currently 22 genotypes have been described, based on the sequencing of a single gene (*p72*). 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.

Projects

What activities are planned or underway?