Innovation brief

RESEARCH PROGRAM ON Livestock

Photo ILRI/Sumy Sadurni

DEVELOPING VACCINES FOR AFRICAN SWINE FEVER

KEY MESSAGES

African swine fever (ASF) is a deadly virus which threatens not only the livelihoods of smallholder farmers in Africa and Asia but pork production worldwide.

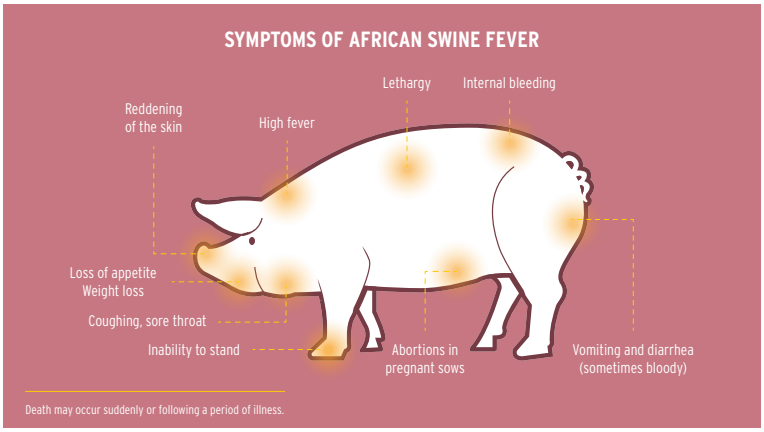
At present there is no treatment for ASF and no vaccine. Developing a vaccine could greatly enhance the control of the disease, especially in areas such as sub-Saharan Africa where pigs are kept in free range scavenging systems.

The International Livestock Research Institute and its partners have made good progress in developing a live attenuated ASF virus vaccine. They are also in the process of developing a subunit vaccine for the disease.

ILRI is developing vaccines that focus on ASF genotypes in Africa. The ASF vaccines currently being developed in either Europe or North America might not work effectively for pigs in Africa.

SUMMARY

Scientists at the International Livestock Research Institute (ILRI) are currently developing vaccines for African swine fever (ASF) which could benefit millions of farmers in sub-Sahara Africa. ASF poses a serious threat to food security. The more virulent strains kill almost all infected pigs, with disastrous consequences for the livelihoods of smallholder farmers. In recent years, the disease has spread to Europe and Asia. There is currently no vaccine to provide protection against ASF.



African swine fever is highly contagious and mortality rates can be as high as 100 percent. Biosecurity measures are therefore important to implement, especially in the absence of a cure and vaccine.

ILRI
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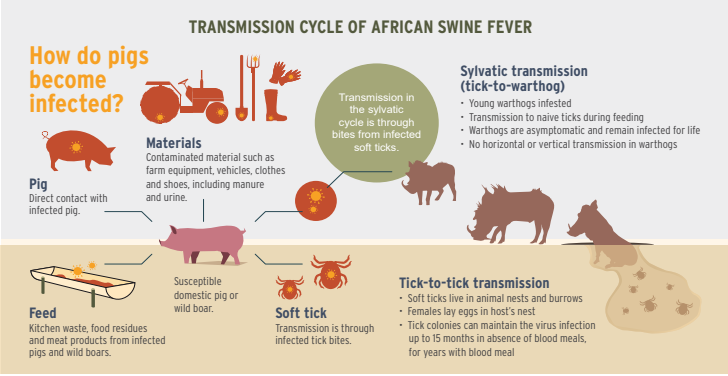
Banner photo: A sow with her piglets in Masaka, Uganda.

INTRODUCTION

ASF is a large DNA virus belonging to the *Asfaviridae* family. Its more virulent strains – there are 24 genotypes in Africa, two of which have spread to Europe and Asia – kill pigs within 6 to 13 days of infection. Outbreaks of the disease can have a devastating impact. Outbreaks of ASF in Côte in 2017 d'Ivoire and Madagascar killed between a third and a half of the national pig herd. But ASF is no longer just an African problem.

The virus was introduced to Portugal in 1957 and it swiftly spread eastwards. By 1995, the disease had been eradicated in Europe except in Sardinia, but its arrival in the Caucasus in 2007 presaged its rapid spread across Europe and Asia. Between 2016 and 2020, eight million pigs were killed by the disease or culled in eradication programmes. The economic costs of the disease are considerable. For example, an outbreak in Russia caused losses of approximately USD 1 billion.

Total eradication of ASF is nearly impossible, not least because the virus is also found in warthogs and other wild pigs in Africa, in wild boar in Europe and Asia, and in soft ticks belonging to the genus *Ornithodoros*. There are various modes of transmission and these are discussed more fully in another innovation brief https://hdl.handle.net/10568/118053. Pigs can develop ASF when they are bitten by infected ticks. The disease can be swiftly passed around herds in secretions and excretions, including urine and faeces. The feeding of infected swill has long been known as a common source of ASF and the virus can also be spread by fomites, such as vehicles, farm equipment, clothes and footwear.



There is no treatment for the disease and the only way to prevent its spread is by using strict biosecurity measures, such as setting up quarantine pens for new animals, washing boots before entering pig farms and ensuring that domestic and wild pigs do not mix. Establishing good biosecurity measures is particularly difficult in African countries where most pigs are kept in free-range scavenging systems. That is why there is an urgent need to develop a vaccine. There are thought to be some 34 million pigs in sub-Saharan Africa – the number is rapidly increasing as pigs are among the most profitable livestock for poor farming families – and an ASF vaccine could benefit 6-17 million smallholder farmers.

Research on vaccines for ASF at ILRI is taking two main approaches. One involves developing a live attenuated vaccine. The other focuses on developing a recombinant subunit vaccine, consisting of fragments of virus which will stimulate an immune response to the virus. The latter is a very safe approach, and attractive if a sufficient level of protection can be obtained, as there are residual safety concerns with live attenuated vaccines.

IN SEARCH OF A LIVE VACCINE

ILRI scientists have been able to take advantage of the latest cutting-edge technologies to fast-track the development of a live attenuated ASF vaccine. By using the CRISPR/Cas9 gene editing system, scientists were able to develop candidates for a live vaccine in less than two months as opposed to six months, which is what it would have taken using conventional techniques. The CRISPR/Cas9 platform was first used under a project which began in 2016. Scientists produced several live vaccine candidates – each a potentially attenuated version of the virus lacking certain genes – which could be the subject of controlled animal experiments. The recombinant viruses were created from a virulent genotype IX virus isolated from an outbreak of the disease on the Kenya–Uganda border in 2013. ASFV-Kenya-IX-1033, as it is known, is the main strain found in East Africa. Several isolates in the area have been sequenced and found to be almost identical.

The first controlled experiment, involving two vaccine candidates, took advantage of work already carried out on genotype II by scientists seeking to establish a vaccine for the strain prevalent in Europe and Asia. Two gene deletions which showed promising results for genotype II were replicated for genotype IX. These were ASFV-1033_ΔCD2v and ASFV-1033_ΔCD2vΔA238L. The attenuated viruses were given by intramuscular injection to two groups of eight animals with a control group receiving salt solution only. Four weeks later, all pigs were inoculated with wild-type virus. The group that was immunized with the salt solution was euthanised after seven days when it became clear that they were not going to survive. The experiment found that ΔCD2v provided a high rate of protection against the disease, namely 87.5%. The virus that had two deletions, ΔCD2vΔ238L, protected 50% of the pigs. However, neither virus was sufficiently attenuated, with both groups of pigs showing clinical signs of ASF.

The aim is to develop a vaccine which stimulates an effective immune response without having a debilitating effect on the vaccinated animal. Scientists are now testing the other candidate vaccines developed by the CRISPR/Cas9 gene editing system and they are also using synthetic biology as a way of building modified ASF viruses from small parts of the genome. This is being done in collaboration with the J. Craig Venter Institute in the US and the Friedrich Loeffler Institute in Germany.

CREATING A SUBUNIT VACCINE

Establishing which antigens in the virus elicit an immune response is a prerequisite for designing a subunit vaccine. In the case of developing a vaccine priming a cellular response to ASF, scientists need to understand the nature of the major histocompatibility complex (MHC) molecules in pigs. These are also known as swine leucocyte antigen (SLA) genes. The MHC antigens bind the T cell epitopes – epitopes being part of the antigen which T cell receptors from killer cells then recognise – and their identification can be essential when formulating a vaccine. The MHC molecules are extremely diverse and vary among individual pigs. This means that the subunit vaccines to tackle ASF will need to contain a spectrum of antigens to trigger the immune response across the herd. During recent years, ILRI scientists sequenced the MHC molecules of indigenous pigs in Africa for the first time and found many new sequences.

To identify the genes needed in a subunit vaccine priming a cellular response, ILRI used a peptide library covering the full proteome/genome of the Kenyan 1033 ASFV isolate, and scientists can now see which antigen/peptide the T cells are reacting to. By studying the reaction pattern of the T cells in 22 different animals – some local and some exotic – they were able to list the top 10-15 antigens recognised by most pigs. At the time of going to press, they were currently in the process of inserting these into Adenovirus and MVA viral vectors, which are now ready to be tested in pigs.

Another approach to developing a subunit vaccine seeks to establish the cause of natural immunity to the disease in warthogs and to find out why some pigs survive the disease while others do not. Instead of focusing on the cellular response once the infection is established, which is the aim of a live vaccine, scientists are hoping to develop a vaccine which will block infection by stimulating the production of antibodies.

In vitro experiments involved the analysis of blood sera taken from three groups of animals: warthogs which showed natural immunity to ASF, pigs which had contracted ASF and survived, and pigs which had been killed by ASF. The working hypothesis is that certain antigens trigger a successful antibody-based immune response and these could be incorporated in a vaccine. Scientists are currently in the process of identifying these. They are also investigating the quality of these antibodies and what activities they undertake to protect the animals. For example, are they blocking the infection or killing infected cells?

There has already been a considerable body of research to identify antigens with stimulate an immune reaction to the ASF virus, but the results have not been convincing and in some cases the antigens used in vaccines have made the disease worse. What makes this approach different is that it seeks to establish which antigens and antibodies are important in conferring immunity in wild pigs like warthogs. It is not that warthogs cannot catch the disease – many young piglets will contract ASF in their burrows after being bitten by the soft ticks that carry the virus – but that a mild dose confers lifelong immunity. It is hoped that a vaccine will do the same. Once the scientists have identified suitable antigen candidates for a vaccine, controlled experiments will be carried out on pigs.

WHAT NEXT?

It is hoped that a live vaccine will be available for commercial development by 2022. This will be targeted at areas in East Africa where genotype IX and the closely related genotype X are prevalent. Preliminary experiments have shown that a gene-deleted live vaccine can confer 100% protection with a reasonably good safety profile, though further tests will continue to be performed. Another area to be explored is the cross-reactivity between different genotypes, to see if the live vaccine can be used in areas where other genotypes are present. However, there may be a reluctance to use a vaccine based on a genotype from elsewhere, as there would be a significant risk of introducing a new strain. This is why ILRI's focus on developing a vaccine for the disease in Africa is so important. Vaccines developed to tackle the genotypes present in other continents might be relatively ineffective in the African context.

Although highly efficacious and often cheap to produce, live vaccines have some disadvantages related to safety, spread in the environment and recombination with existing wild-type strains. Therefore, ILRI researchers are also working to produce a subunit vaccine. The ASF virus is closely related to pox viruses, for which vaccines already exist. These can be freeze dried and are stable for long periods of time. A similar strategy could be undertaken for ASF virus.

Although the subunit vaccines currently under development at ILRI are designed, like the live vaccine, to provide immunity for pigs where genotype IX is prevalent, the vaccine could be relatively easily modified by including the appropriate antigens to be effective for other genotypes. This would certainly make it attractive to countries in Europe and Asia, although recent announcement suggests that good progress has been made in the UK to developing subunit vaccines for genotype II. ILRI recognise that it will be important to produce a vaccine for ASF, whether a live or subunit vaccine, which can be made readily available to smallholder farmers.

Photo ILRI/Kabir Dhanji

Manure management is one important biosecurity measure to keep diseases such as African swine fever at bay from pigs.

Photo ILRI/Sumy Sudarni

A woman feeds her pig gathered forages in Masaka, Uganda.

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