

Can a Vaccine Save the World's Pigs from African Swine Fever?

A devastating outbreak of the virus across East Asia has made the long-neglected pathogen a top research priority, but many challenges lie ahead.

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In the fall of 2017, a year before an unfamiliar virus captured the world's attention with an explosive outbreak in East Asia that left tens of millions of pigs dead, immunologist [Waithaka Mwangi](#) and his graduate students were already aware of the culprit and its imminent threat to the swine industry. Behind the glass of a biosafety cabinet at Kansas State University's Biosecurity Research Institute—one of two sites in the US authorized to conduct research on the deadly pathogen—they carefully extracted a few milliliters of fluid from a test tube containing live African swine fever virus (ASFV) collected from the spleens of infected pigs. In another room down the hall, the researchers administered droplets of the fluid into the nostrils of piglets. In total, more than 60 young pigs were exposed to the virus, and the team waited to see how they'd fare.

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ASFV is typically harmless to humans, but it can be devastating to domestic pigs (*Sus scrofa domestica*), and this particular strain of the virus, known as Georgia 2007 after appearing in the country that year, was typically fatal. Within a week, infected animals would succumb to a lethal hemorrhagic fever, the same type of illness as that caused by Ebola and Marburg viruses in humans. But a few days earlier, Mwangi's team had given 32 of the piglets a cocktail of proteins that they hoped would help the animals survive the infection.

This prototype vaccine consisted of an inactivated adenovirus—a mostly harmless pathogen that is often used as a vector for therapeutics—that had been genetically engineered to express one of two combinations of ASFV proteins. Mwangi knew from his group's [previous studies](#) that the modified adenovirus

There's so many pigs in China, it was just a matter of time.

—Dirk Pfeiffer, City University of Hong Kong

could trigger the porcine immune system to attack those proteins and generate antibodies against them. Now, he was about to find out if that was enough to fight off an infection with the deadly Georgia 2007 strain.

The scientists monitored the animals daily, checking their temperature and general health. To the team's dismay, after only a few days, some of the piglets began to huddle together, a sign of feverish chills. The first group of vaccinated pigs developed a fever and deteriorated quickly, even faster than control animals that hadn't received the adenovirus, and had to be put down. Eight of 10 animals that had received a different protein cocktail also fell ill and were euthanized. A different formulation of this cocktail showed a little promise, with five of nine animals surviving. But **overall**, "it was disappointing that we didn't get a positive outcome," Mwangi tells *The Scientist*.

The results foreshadowed worse news to come. Almost exactly a year later, China reported an outbreak of the Georgia 2007 strain in Shenyang, a city in the country's northeast. From there, it swept through the world's largest congregations of pigs and among countless small farms, killing hundreds of thousands of animals across China. By the end of October 2019, nearly **200 million animals** had been culled in a desperate effort to stop the virus, but ASFV continued to **spread**, popping up in Mongolia, Vietnam, **Cambodia, Laos, Myanmar, South Korea**, and the **Philippines**. In October, Mark Shipp, the president of the World Council of Delegates of the World Organization for Animal Health, **told** reporters that around a quarter of the global pig population could die due to the disease.

Unrelated to the East Asian epidemic, new outbreaks have also been **reported** in **Eastern Europe**. There, low levels of the virus have been circulating in wild boar and domestic pig populations for more than a decade since it arrived from its native Africa, where it often leaps from wild pigs to domestic animals. The rapid spread of ASFV across Eastern Europe and Asia alarmed officials in Asia, Western Europe, and North America, concerned that the virus could slip into their countries via contaminated pork products or animal feed imported from infected countries. Seemingly overnight, finding a vaccine for ASFV—a virus that had long stood at the periphery of the scientific community's attention—became a global research priority.

But as Mwangi's results suggest, there are still significant challenges to overcome. The most successful vaccine candidates are not yet appropriate to use in agricultural settings, while safer options, such as Mwangi's cocktail approach, have yet to prove effective. Researchers need to understand more about the virus, its origin, and its interaction with the porcine immune system to complete their mission.

"To get to the stage of making a vaccine that can be used in the field requires a lot more [research]," says **Linda Dixon**, a virologist at the UK's Pirbright Institute, part of the government's Biotechnology and Biological Sciences Research Council. "I don't think there are any [candidates] at that stage yet."

ASFV's lifecycle

ASFV is transmitted by ticks of the genus *Ornithodoros* to common warthogs (*Phacochoerus* spp.) when they feed on the wild animals' blood. Domestic pigs (*Sus scrofa domesticus*) can catch the virus through tick bites in areas of Africa where warthogs exist, as well as through contact with contaminated food or materials. In Eastern Europe, where the disease is also endemic, pigs can contract ASFV by coming into contact with bodily fluids or carcasses of infected wild boar (also *Sus scrofa*).

Since the late 2000s, ASFV is thought to have gained a foothold in Europe, especially the eastern part of the continent where infections often spill over to small-scale pig farms. It's not yet clear whether ASFV has infected wild boar populations in China or other East Asian countries it has spread to. If it has, the virus will be near-impossible to eradicate there.

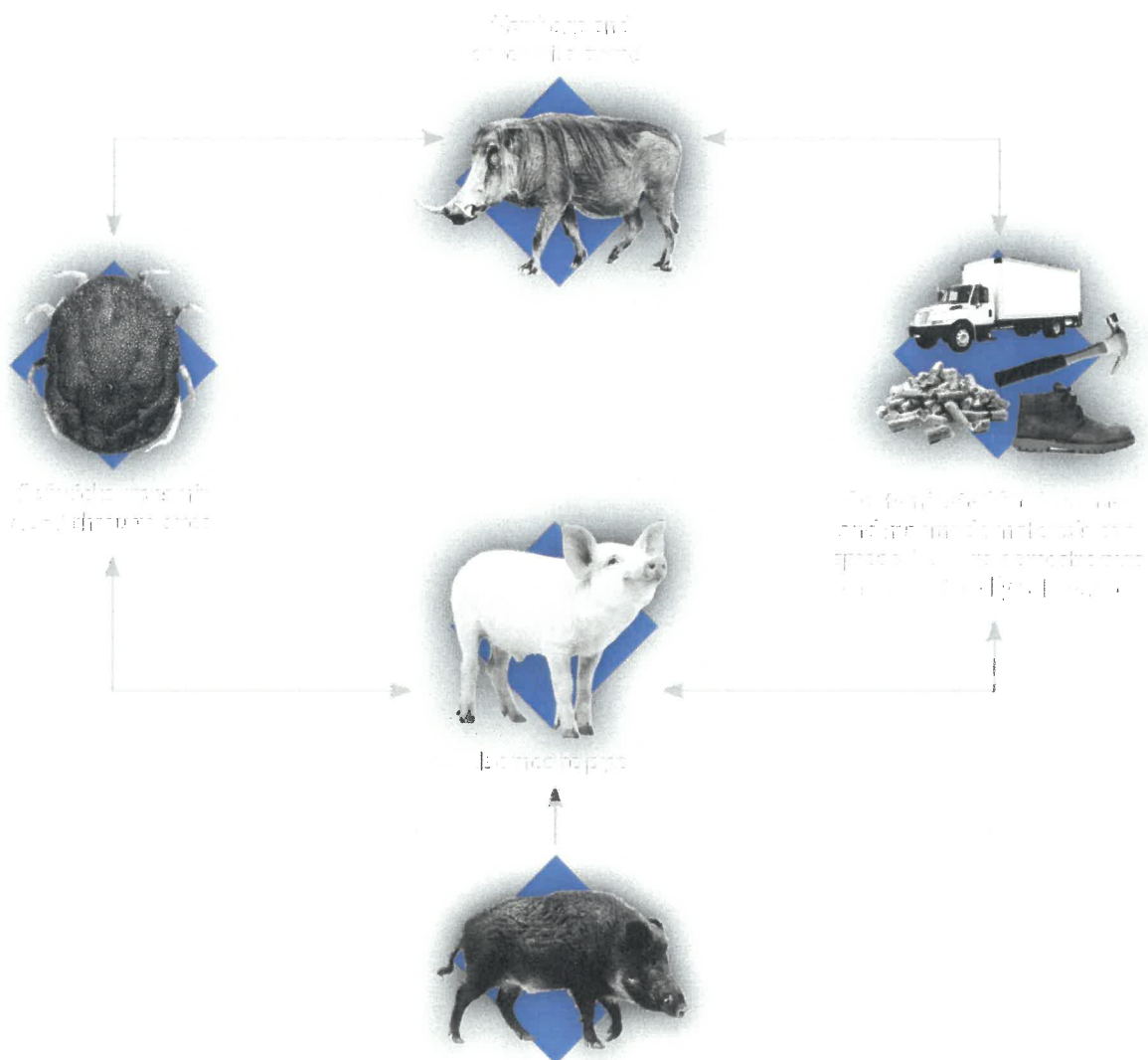


Figure 1. ASFV lifecycle (adapted from [1]).

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Out of Africa

ASFV infection was first documented in the early 20th century in Kenya, then a British colony. People there noted that pigs brought from England quickly succumbed to a “contagious pneumonia,” as veterinarian [Robert Montgomery described](#) it in 1921. When antibodies against classical swine fever, which also causes feverish chills in pigs, failed to offer protection, scientists concluded that a different pathogen, later christened ASFV, must be responsible. (African swine fever is also not to be confused with swine flu, caused by an unrelated virus of the influenza group that can cause respiratory symptoms in pigs and sometimes in people.)

Later [research](#) revealed that ASFV likely arose in eastern and southern Africa and subsequently spread throughout much of Sub-Saharan Africa. It has diversified into at least 24 different genotypes, each of which can encompass many different strains. In eastern and southern Africa, ticks of the *Ornithodoros* genus transmit the virus between common warthogs (*Phacochoerus africanus*) and domestic swine. ASFV infections in African wild species are typically benign, suggesting they’ve coevolved with the virus for a long time, but in domestic pigs, infection unleashes chaos in the animals’ immune systems. Upon infecting macrophages and other white blood cells, many ASFV strains proliferate rapidly and trigger inflammatory reactions while simultaneously releasing proteins that blunt the animals’ immune response. Infection also induces cell death in white blood cells and endothelial cells lining blood vessels. Ultimately, infected pigs develop hemorrhagic shock and die. For farmers in many parts of Africa, “it is devastating,” says [Mary-Louise Penrith](#), a veterinary pathologist at the University of Pretoria in South Africa.

For most of its evolutionary history, ASFV has been limited to its continent of origin. Before the current outbreak in Asia, the virus was known to have journeyed out of Africa only twice: in 1957, when an ASFV genotype 1 strain infected Portuguese pigs that ate food waste from airline flights traveling from Africa; and in 1960, when the same strain revisited the Iberian Peninsula, then crossed the Atlantic to Brazil and several Caribbean island nations. Scientists and public health officials in Spain and Portugal were able to quash the outbreaks through careful surveillance of the disease, culling animals on infected farms, and keeping pigs away from wildlife. By the late 1990s, almost all countries affected by those midcentury outbreaks were virus free, and for the next decade, things were silent.

A Fatal Pig Virus Escapes From Africa



Because of its long-time restriction to Africa, ASFV “probably hasn’t received the amount of attention that it should receive due to the threat it poses,” says [Daniel Rock](#), a virologist at the University of Illinois at Urbana-Champaign. In 2004, the US Department of Homeland Security decided to shutter a research program dedicated to studying the disease, notes Rock, who used to lead the program—ASFV wasn’t considered a priority due to being widely perceived as “an African thing.” That thinking changed in 2007, when the deadly genotype 2 strain of ASFV now making its way across Asia first surfaced in Georgia, possibly arriving via ships from Africa carrying infected pork products that were then fed to domestic pigs. In as little as five years, it swept through the Caucasus and into Russia—a “game-changing moment” for ASFV and the world, says Rock.

That this strain eventually surfaced in China in 2018 was not a surprise to anyone in the field. “There’s so many pigs in China, it was just a matter of time,” veterinary epidemiologist [Dirk Pfeiffer](#) of City University of Hong Kong told [The Scientist](#) last year. By the summer of 2019, the epidemic had escalated into what Pfeiffer calls “the biggest animal disease outbreak ever.” Some feared that it would further escalate into a worldwide pandemic. In response, the field has seen an influx of funding from the [European Union](#), [Bulgaria](#), and [China](#), with governments funding researchers in the hope that they develop a vaccine quickly.

See “[Scientists Race to Build Vaccine for African Swine Fever](#)”

But that’s easier said than done, notes [Luis Rodriguez](#), a virologist at the United States Department of Agriculture’s (USDA) Plum Island Animal Disease Center, which relaunched a long-inactive ASFV program the year after the virus’s spread to Europe in 2007. “We’re doing the best to move

forward as fast as we can in developing these vaccines, but that is a process that takes time and effort, and there are major challenges.”

Live vaccines

As early as 1967, researchers discovered that the traditional approach to making vaccines doesn’t work for ASFV. Pigs injected with killed or inactivated forms of the virus—intended to provoke their B cells into generating virus-targeting antibodies—weren’t protected against virulent forms of the disease. In 2014, a team of German scientists [tried](#) the experiment for themselves, and found that while pigs did develop antibodies against ASFV proteins, it wasn’t enough to fight off the virulent Georgia 2007 strain. “Antibodies alone are not fully effective,” Dixon says.

Over the past few decades, researchers have begun to understand why. [Studies](#) suggest that pigs rely heavily on killer T cells—and potentially other immune cells—to fend off ASFV, and stimulation of T cells can only occur if living viruses infect host cells. Only then are viral peptides processed and presented via cell surface receptors to T cells. This doesn’t happen with the dead viruses traditionally used in the vaccine experiments.

Recognizing this, researchers developed vaccine candidates with live, but weakened, forms of ASFV. They took advantage of the fact that many ASFV strains have mutated over time, becoming less aggressive to domestic pigs and their wild relatives. In 2019, a group of Spanish researchers [injected](#) a number of domestic pigs with a weak strain of ASFV genotype 2 that had been isolated from a wild boar captured in Latvia. The vaccine caused mild, transient symptoms involving fever and joint swelling in some animals, but they all survived after being exposed to pigs that carried the virulent genotype 2 strain Georgia 2007.

To get to the stage of making a vaccine that can be used in the field requires a lot more research.

—Linda Dixon, Pirbright Institute of the UK’s Biotechnology and Biological Sciences Research Council

As researchers have amassed more knowledge about ASFV’s biology, they’ve adopted a more targeted approach in attenuating viruses by removing specific genes that make it so deadly. As Dixon puts it, the goal is to strategically disarm the virus so the porcine immune system has a chance to develop long-lasting antibodies and to prime T cells to attack the virus. In 2016, for example, her group created an attenuated form of ASFV genotype 1 by knocking out eight genes and interrupting two genes the virus uses to dampen pigs’ interferon type 1 response, a pathway that helps curtail viral replication. All five animals immunized with this gene-deleted virus survived a challenge with a virulent genotype 1 strain.

Dixon's team has achieved similar success with [other](#) gene [deletions](#) and, with funding from the Biotechnology and Biological Sciences Research Council in the UK, is working with a New Jersey-based biotechnology company to create a viable vaccine candidate for the Georgia 2007 strain using this approach. Meanwhile, at Plum Island Animal Disease Center, microbiologist [Manuel Borca](#) has developed four gene-deleted vaccine candidates that protect animals against the same strain—each carrying one, [two](#), or three deletions. A [number](#) of other [groups](#), including one in China, are also working on similar gene-deletion approaches.

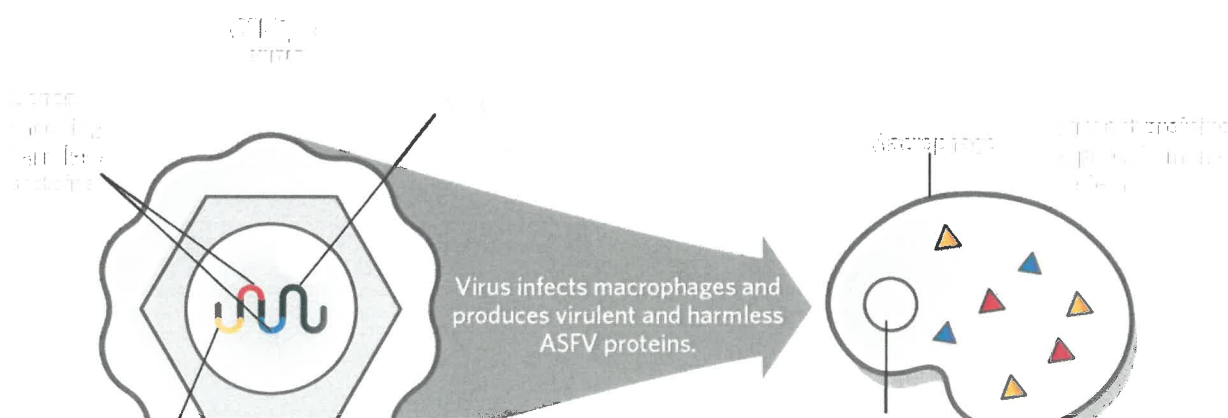
Although they're effective, safety is still a significant concern. Some of Dixon's immunized pigs developed a slight fever, which most veterinary vaccine companies would consider an unacceptable safety risk, though further gene deletions and modifications have greatly reduced or eliminated this fever while maintaining good efficacy, she says. Part of the challenge in identifying the right genes to remove to strike that balance between safety and efficacy is ASFV's unusual genomic complexity. Like some other DNA viruses, ASFV has a very large genome—"this thing is about 190 kilobases," notes Mwangi, making it longer than the RNA genomes of Ebola, HIV, Lassa, Marburg, and rabies viruses put together.

Another major barrier in developing live vaccine candidates is that they're difficult to produce in bulk. ASFV replication requires macrophages, but there aren't any porcine macrophage cell lines that last for more than a few weeks. Instead, researchers have to continuously harvest fresh macrophages from animal blood or other body tissues.

"You will never get a uniform, reproducible [vaccine] product" this way, remarks [Yolanda Revilla](#) of the Spanish National Research Council's Center for Molecular Biology "Severo Ochoa" in Madrid. Finding a cell line that lasts is "one of our most important objectives at the moment."

A Vaccine Hunt

Researchers have tested three main approaches to develop a vaccine candidate for the ASFV strain that is currently killing pigs throughout Asia.

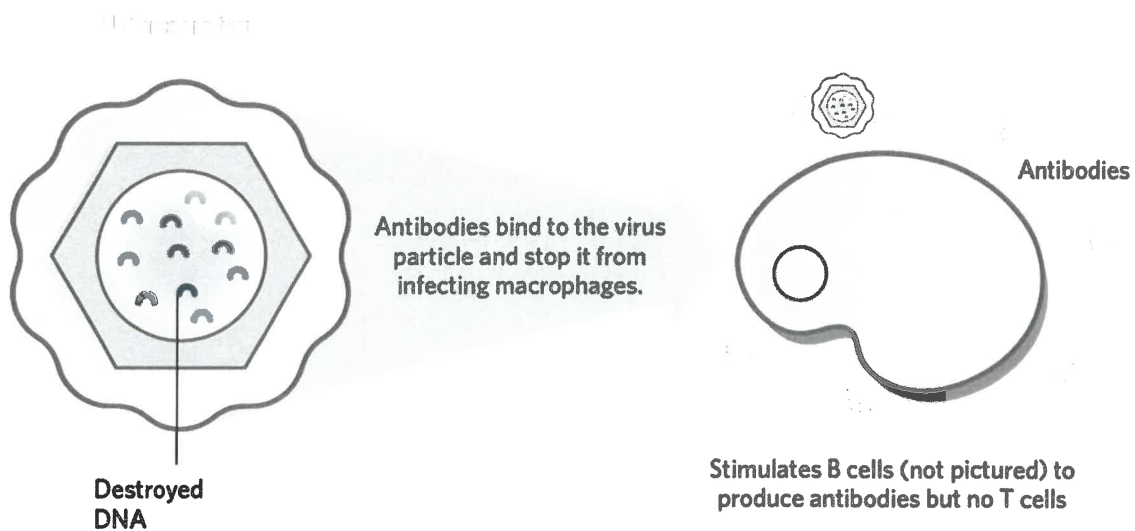




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VACCINE STRATEGY #1: INACTIVATED VIRUSES

The traditional approach involves killing or inactivating viruses—for instance, through UV irradiation—so that they're no longer virulent but retain viral antigens that stimulate the production of protective antibodies.



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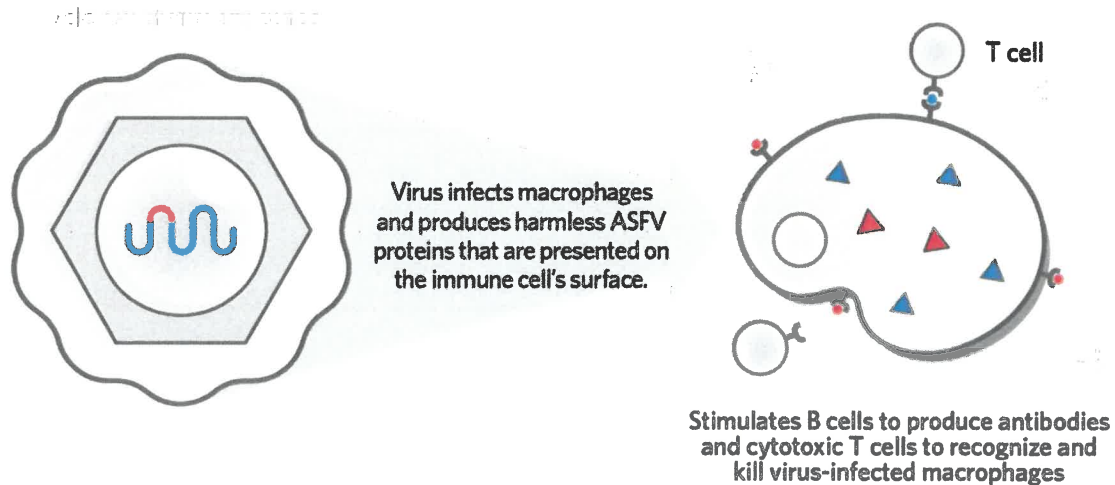
Efficacy: These vaccines stimulate an antibody response in pigs, but they don't protect against intact forms of ASFV. Researchers think this is because inactivated viruses don't activate killer T cells.

Safety: Based on limited studies, no side effects have been shown so far.

Commercial prospects: Researchers have abandoned this approach because of the shortfalls in efficacy.

VACCINE STRATEGY #2: LIVE VIRUSES

Injecting tamer forms of virulent viruses could potentially stimulate antibody production and the all-important T cell responses without killing vaccinated animals.



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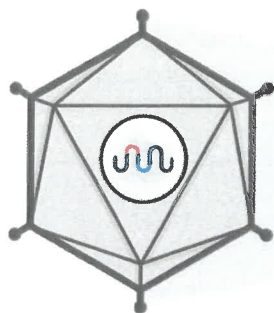
Efficacy: Both gene-deleted and naturally attenuated forms of ASFV stimulate the immune system to generate antibodies and killer T cells and usually offer protection against virulent genotypes of ASFV.

Safety: Vaccinated pigs can develop mild to debilitating symptoms, from fever to joint swelling.

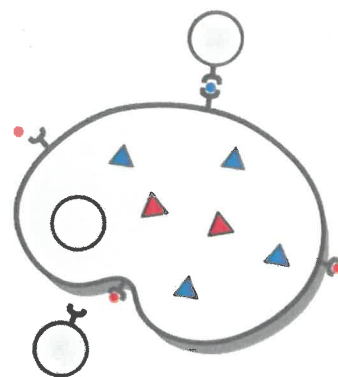
Commercial prospects: Researchers are both testing ASFV strains that have naturally attenuated over time and genetically modifying virulent forms of the virus by removing sequences that code for harmful proteins. Scientists have yet to find a stable cell line capable of generating live vaccine candidates in bulk, but these types of vaccines are expected to be the first to hit the market.

VACCINE STRATEGY #3: SUBUNIT VACCINES

A third approach involves genetically engineering viral vectors such as adenoviruses to express combinations of ASFV antigens. Inside the body, the vector-encoded antigens are produced in the absence of the pathogen.



Viral vector infects antigen-presenting cells such as macrophages and produces harmless ASFV proteins that are presented on the immune cell's surface.



As above, stimulates B cells and T cells

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Efficacy: Inoculations provoke the production of antibodies and killer T cells, but don't seem to protect pigs against virulent forms of ASFV.

Safety: Vaccinated animals typically experience few or no side effects.

Commercial prospects: Researchers are testing different antigen combinations. Many consider this to be the preferred strategy for developed countries, although it's expected to reach the market much later than live virus vaccines. Subunit vaccines can be easily synthesized in bioreactors and rapidly generated in bulk.

See full infographic: [WEB](#) | [PDF](#)

Protein cocktails

To get around these issues, researchers such as Mwangi are trying another strategy: a subunit vaccine. Viral vectors—such as adenoviruses—are engineered to express cocktails of ASFV antigens. Once the vector viruses infect porcine cells, the antigens are presented on the cells' surfaces, triggering B and T cells to target ASFV.

Because the viral vectors are genetically modified so that they're not capable of replicating quickly, there is no risk of uncontrolled spread, Mwangi says. Another advantage of subunit vaccines is that they can be easily grown in bio-reactors using several well-established cell lines, Dixon adds. However, the challenge, as Mwangi's adenovirus study suggests, is finding an antigen combination that induces a protective immune response.

To that end, in 2018 Dixon teamed up with researchers at Arizona State University to design a systematic [screen](#). The scientists selected 47 ASFV proteins with a range of functions and injected pools of as many as 20 of the proteins into pigs. Then, they exposed cytotoxic T cells from the inoculated pigs to each of the proteins in culture. Those proteins to which the lymphocytes

reacted most strongly—releasing interferon gamma, a cytokine that prompts the cells to differentiate into long-lasting memory T cells targeting specific antigens—were selected as good building blocks for a subunit vaccine. From their ranking of the most immunogenic proteins, the team created four different cocktails and engineered vaccinia viruses, a popular vaccine vector, to express one of the cocktails and replicate only a few times.

To Dixon's disappointment, vaccinated pigs that received a lethal dose of ASFV Georgia 2007 died. But the pigs did have reduced concentrations of live ASFV in their blood compared with control animals—a sign that the researchers should be able to refine their approach “to get stronger protective responses,” Dixon says.

[Lucilla Steinaa](#), an immunologist at the International Livestock Research Institute in Nairobi, Kenya, has been conducting a similar screen with her team, focusing on ASFV genotypes 9 and 10, which circulate throughout East Africa. Instead of looking at whole proteins, her team is monitoring T cell responses to specific peptides, to identify the precise amino acid sequences that elicit immune responses. Other organizations, such as the US-based [Phibro](#) Animal Health Corporation and the Madrid-based vaccine company [Algenex](#), are also developing subunit vaccines.

In addition to finding the right proteins, researchers must also consider delivery mechanisms. That vaccinia and adenovirus vectors fail to curtail replication of ASFV virus could be partially due to the fact that the vectors themselves don't induce a strong enough immune response, Dixon explains. For instance, her team has tried [boosting](#) an antigen-encoding adenovirus vaccine with a vaccinia virus containing the same proteins five weeks later, in an attempt to amplify the pigs' immune response. But once again, although this reduced the amount of circulating ASFV, it didn't save the animals from death.

“There's quite a number of approaches that folks are pursuing, but so far, no viable or very promising [delivery mechanism] has been demonstrated,” Mwangi notes.

Preparing for the Next ASFV Outbreak

Many researchers and vaccine companies are concentrating their efforts on finding a vaccine for ASFV genotype 2, the virus responsible for the ongoing outbreak in Asia. But some recognize the need to find defenses against other African genotypes that create immense economic difficulties for African farmers while threatening to unleash havoc on the rest of the world if they escape to another continent. For instance, Linda Dixon, a virologist at the UK's Pirbright Institute, is studying a virulent genotype 1 virus widespread in West and central Africa, while immunologist Lucilla Steinaa of the International Livestock Research Institute is focusing on the dominant genotypes in East Africa, genotypes 9 and 10.

University of Illinois virologist Daniel Rock is taking a different approach by looking for protective antigens—those that trigger specific, long term responses of the immune system—that different ASFV strains have in common, which may help researchers design vaccines that target multiple forms of the virus (*J Gen Virol*, 100:259–65, 2019). Engineering a vaccine that protects against multiple strains and genotypes is “the longer-term goal,” he says.

But even if such a vaccine existed, it wouldn’t be a panacea, warns City University of Hong Kong epidemiologist Dirk Pfeiffer. A major reason why ASFV has spread so explosively in China is that most pigs there live scattered across thousands of small-scale farms with little to no biosecurity—as a report by the United Nations Food and Agriculture Organization (FAO) pointed out just months before Chinese pigs caught the virus. Pfeiffer expects that officials have little chance of eradicating ASFV for good without improving that biosecurity—making sure feed isn’t contaminated with ASFV, for instance. Moreover, if the virus infects Chinese wild boars and becomes endemic in Asia, officials would have to continuously vaccinate pigs for years to safeguard them from spillover from wild animals. A vaccine would “keep a lid on the spread of the virus, but it doesn’t remove the virus,” Pfeiffer says.



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Last year, the Chinese government started providing some biosecurity recommendations to farmers, such as using appropriate cleaning and disinfection on farms and prohibiting feeding of food waste to domestic pigs, although Pfeiffer questions how feasible it is to encourage

compliance among the millions of people involved in the country's giant pork supply chain. Meanwhile, some in the US and Canada, concerned that the virus could slip into North America via contaminated pork products or animal feed imported from countries that have the virus, have taken precautions in their own swine industries. In April 2019, for example, the Canadian Pork Council issued new guidelines for importing swine feed ingredients, recommending that feed be held in a sealed container for up to 100 days, depending on storage temperature, before being accessed, to allow time for the virus to perish.

In the past, many nations outside Africa, including Spain, were able to eradicate the disease through extensive culling of pigs, notes Mary-Louise Penrith, a veterinary pathologist at South Africa's University of Pretoria who has consulted the FAO on how to manage ASFV. And many African farmers, especially in South Africa, succeed in managing the virus's spread through relatively straightforward biosecurity measures such as keeping pigs and their feed away from wildlife.

Preventing ASFV's spread "is not rocket science," Penrith says. But a key to containing the virus is altering human habits—from making sure workers change into clean clothes and footwear once they enter a farm to quarantining sick animals. "All of us are agreeing more and more that what really spreads African swine fever is people. And that's a hard thing to change."

The road ahead

The two main approaches—gene-deleted live vaccines and subunit vaccines—aren't mutually exclusive. To address the rapidly spreading outbreak in China, researchers expect a gene-deleted live vaccine to be the first to enter the market there, optimistically within two years, says Pfeiffer. These approaches already offer good protection against ASFV, and with China's \$130 billion/year pork industry at stake, researchers there may be willing to compromise a little in terms of vaccine efficacy and safety, Rock says—but not by much. Having an unsafe, replication-competent vaccine virus floating around East Asia would spell disaster, he adds.

For other regions that have more time to spare, for example, in the US or Europe, a subunit vaccine would likely be a preferred alternative, [José Escribano](#), founder and chief scientific officer of Algenex, tells *The Scientist* by email. This type of vaccine would be the only approach considered safe enough for regulatory bodies in developed countries to approve, he says.

An effective vaccine for the Georgia 2007 strain would be useful to have on hand for any country, but it won't be the end of the story, Rock warns. There are numerous known ASFV strains circulating in Africa—many of them just as deadly to domestic swine as Georgia 2007—and likely more yet to be discovered, Rock says, and a vaccine against one would be unlikely to protect pigs

against others. With China having an ever-stronger economic presence in Africa, the chances that other strains will find their way to Asia in the future is high, he adds.

“It’s the Georgia [2007] strain today. What’s the strain tomorrow?”

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African swine fever, agriculture, epidemic, infectious disease, outbreak, pigs, vaccine, vaccine design, virus, wild pigs