Wild boar in Europe, such as this male in Bavaria, Germany, help spread and maintain African swine fever virus.

PERSPECTIVES

INFECTIOUS DISEASE

No hasty solutions for African swine fever

African swine fever vaccines could pose risk of causing disease and spreading the virus further

By **Dolores Gavier-Widén[1], Karl Ståhl[1], Linda Dixon[2]**

An epidemic of African swine fever (ASF), a lethal viral hemorrhagic disease of swine, is devastating pig production in Asia and is a global threat. The ASF virus (ASFV) reached the European Union (EU) in 2014, affecting pig production. ASFV continues to spread through wild boar (*Sus scrofa*), which form interconnected populations across Europe and which maintain the infection and can cause infection in pigs. A vaccine is not yet available and is urgently needed, both for pigs and wild boar. Live attenuated virus (LAV) vaccines are the most promising way forward in the short term (*1*), and recent advances have been made in constructing gene-deleted LAV vaccines. Naturally attenuated LAVs have also been shown to confer protection as vaccines in pigs and wild boar. However, previous experience with vaccination failures using naturally attenuated LAVs emphasizes the need for caution because of safety concerns.

ASF was first described in Kenya in 1921 (*2*) and today is endemic in most countries of sub-Saharan Africa. Local dispersion of the virus can occur through contact between animals, whereas long-distance spread results from the movement of contaminated pork products, in which the virus can survive for months or years depending on temperature. Feeding of food waste to pigs can thus establish new foci of infection. Twenty-four

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genotypes of ASFV have been identified. A genotype I ASFV escaped twice from West Africa into Portugal in 1957 and 1960. The later infection affected the Iberian Peninsula, where the virus persisted for more than 30 years, spreading sporadically to other countries in Europe, the Caribbean, and Brazil. ASF was eradicated from most of these countries by the mid-1990s through culling and movement bans of pigs and their products. However, genotype I ASFV still persists in the Italian island of Sardinia.

A new transcontinental spread of ASFV, this time genotype II, occurred from southeast Africa into Georgia in 2007, probably through catering waste brought by a ship (*3*). Subsequently, the virus spread to the Caucasus, the Russian Federation, Ukraine, and Belarus. It entered the EU Baltic states and Poland in 2014, where the virus is maintained in wild boar populations. Continued spread to other EU countries, including Romania and Bulgaria, has also involved the domestic pig population, with outbreaks mainly in small farms. The natural movements of infected wild boar result in local expansion of the virus; the infection front has been estimated to advance at ~1 to 2 km per month (*4*). In 2018, genotype II ASFV entered China, which contains nearly half of the world's pig population, with catastrophic socioeconomic consequences, particularly for small and underprivileged pig farmers (*5*) who comprise ~30% of the 26 million pig farmers in China (*6*). It then dispersed further to Southeast Asia. A year after its incursion into Asia, genotype II ASFV had caused the death or destruction of ~5 million pigs (*6*) and an estimated reduction of 40% of the Chinese pig herd, thus affecting global food markets (*7*).

It was not until genotype II ASFV entered the EU in 2014 that the capacity of wild boar to maintain circulation of the virus independently of outbreaks in domestic pigs was revealed (*8*). Control of ASFV in wild boar is challenging and has not been achieved in most of the affected countries. There are exceptions, however: The Czech Republic was declared officially free from ASF by the EU ~18 months after the first report, and disease spread seems to have halted in Belgium. Early detection, prompt and coordinated implementation of measures to restrict movements of potentially infected wild boar, and public-access restrictions to infected areas to prevent further ASFV spread are key factors for success. Such measures include carcass finding and removal, fencing, and strategic wild boar hunting and culling operations (*4*).

A combination of direct transmission between wild boar and indirect transmission by contact with infected wild boar carcasses or wild boar scavenging on carcasses (intraspecies scavenging) provides long-term persistence of ASFV in the environment (*8*). Thus, infection in pigs can potentially occur not only from their contact with wild boar—for example, in outdoor holdings—but also from transmission of ASFV from the environment through, for example, vehicles, shoes, and feed. High-biosecurity pig production is better protected from ASF, but it is put at risk if the environment around farms is contaminated, and even such establishments have been infected in Europe (*9*).

Populations of wild boar have been expanding throughout Europe during the past 40 years (*10*). Sustainable reduction in free-ranging wild boar populations is very difficult because wild boar have a high reproductive rate, such that culling results in compensatory growth of the population and influx from adjacent areas. In addition, intensive hunting leads to dispersion of wild boar and can result in expansion of the infected area. ASFV has also been reported in wild boar in China, Far East Russia, and the northern region of South Korea (*11*). However, information about populations of wild boar and the epidemiology of ASF in Asia is scarce.

Developing an ASFV vaccine presents many challenges. ASFV is a large, double-stranded DNA virus of the Asfarviridae family (*12*). The virus is complex; its genome is about 170 to 190 kilobases in length and encodes ~170 proteins, of which ~70 are packaged into the multilayered virus particle (*12*). Identification of antigens that might elicit vaccine-mediated protection among this very large number of proteins is difficult. Immune correlates of protection in swine to enable evaluation of vaccine candidates are insufficiently identified. Moreover, current experimental testing of vaccine candidates can only be conducted in pigs and wild boar and in high-containment facilities.

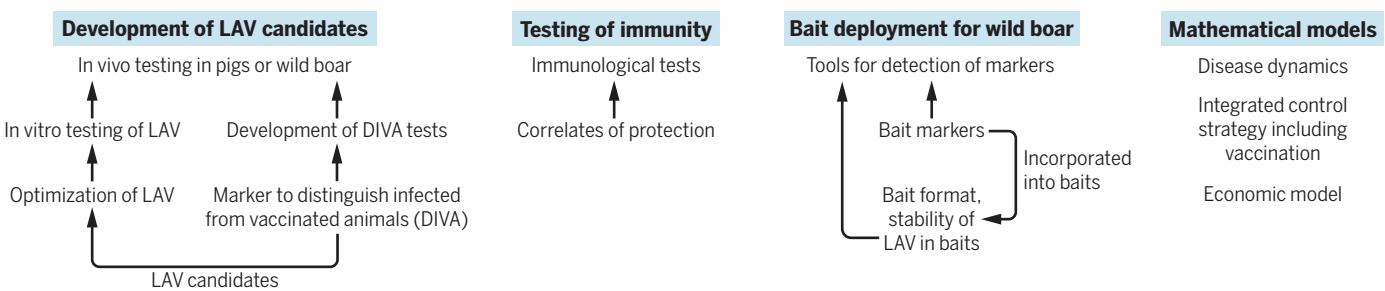
An ASFV vaccine for wild boar must also overcome the challenges of vaccinating wildlife. The approach is likely to involve oral vaccination using baits, which must be deployed in the field and thus be stable and effective in a broad range of environmental settings, including hot Iberian summers and cold Nordic winters, and similarly, but at a larger geographical and climatic scale, in Asia. Baits that are palatable, stable, safe, and inexpensive are needed.

The planning of any ASFV vaccination strategy must also consider the complex epidemiology of ASF, which will vary depending on where the vaccine is applied. For this purpose, mathematical models are essential to assess the efficacy, efficiency, and feasibility of vaccination as a single measure or as a component of an integrated disease management strategy, including, for example, zoning, movement restrictions, and culling of affected premises. However, information on domestic pig farms and their management structure as well as on wild boar populations and habitats is needed for accurate modeling.

ASFV vaccines based on inactivated virus have proven ineffective, even when used with immunogenic adjuvants, because they fail to induce cellular immunity. Subunit vaccines contain only antigenic fragments

Development of an African swine fever vaccine

Safety and efficacy of live attenuated virus (LAV) vaccine candidates and their elicited immune responses have to be tested in vivo in pigs or wild boar. Although domestic pigs can be vaccinated by injection, wild boar are more feasibly vaccinated by oral baits. Mathematical models should be used to plan the vaccination strategy and to assess the efficacy, efficiency, and feasibility of vaccination in the control of African swine fever (ASF).



GRAPHIC: VERONICA FALCONIERI/*SCIENCE*

of the virus and are therefore safe, but their development has been hindered by the limited identification of antigens. Attempts to use either recombinant proteins or DNA vaccination have induced only partial protection or no protection.

In the 1960s, it was observed that recovery from infection with less virulent ASFV isolates protected pigs against subsequent challenge with related virulent ASFV. This is because almost all virus proteins are expressed in infected cells, thus inducing a cellular immune response against a range of virus epitopes in addition to antibody responses to the native virus particle. This demonstrated the potential for LAVs as vaccines. The introduction of ASFV to Portugal and Spain in 1960 provided impetus to produce LAVs for vaccination. LAVs are produced by selecting attenuated ASFV resulting from passage in cells, which results in genome modifications. Vaccines derived by this procedure were used for an extensive vaccination campaign (*13*). However, these vaccines were insufficiently tested and caused a debilitating chronic disease in many vaccinated pigs, resulting in vaccine withdrawal. Other naturally attenuated ASFV strains have conferred different levels of protection but also caused unacceptable postvaccination reactions (*1*).

The current status of ASFV vaccine development shows some encouraging results. The most advanced vaccine candidates are LAVs in which virulence genes are deleted, resulting in a weakened virus that still replicates (so it can trigger immunity) and can be amplified in cell culture for vaccine production. However, a licensed cell line in which a LAV can be stably grown and produced on a large scale is still required. Deletion of ASFV genes that inhibit host antiviral type I interferon responses has been an effective strategy to attenuate the virus and induce protection. These interferon inhibitory proteins include members of multigene family (MGF) 360 and MGF 505. Genetic modification allows for fine-tuning of safety and efficacy and the introduction of markers to distinguish infected from vaccinated animals (DIVA). This is needed to monitor vaccine efficacy and to confirm disease eradication. Several gene-deleted genotype I and genotype II LAV vaccine candidates have shown promising results in preliminary testing (*1*). However, these require further testing and scale-up of production before completing larger-scale safety and efficacy testing in vivo (see the figure).

Although LAVs have the potential to be effective vaccines and have been used for the eradication of smallpox and rinderpest, there are safety concerns. These include induction of ASF-like symptoms and dispersal of the vaccine virus. The vaccine may not protect enough animals to stop the epidemic. Moreover, vaccinated animals may spread the virulent virus to uninfected animals. These safety issues were also observed using a naturally attenuated ASFV strain from Latvia (Lv17/WB/Rie1) (*14*). This virus caused clinical signs of ASF in pigs, including joint swelling, which is associated with a chronic form of ASF (*15*). In addition, the vaccine replicated to high concentrations in blood and spread to pigs on contact. Replication of the virulent virus was not sufficiently controlled, and the pigs shed the virulent virus sporadically and could therefore spread ASF to other animals (*14*), potentially failing to stop the epidemic. Such safety issues should be considered during animal testing of vaccine candidates.

The race to develop an ASFV vaccine may overshadow comprehensive efficacy and safety testing, thus potentially investing in the wrong vaccine development strategy and in unnecessary use of animals for experiments. Additional caution should be taken when developing LAV vaccines to be spread in nature in oral baits. The challenge of ASFV vaccine development, including vaccination of wild boar, should not be underestimated and requires the cooperation of many disciplines in the early stages of vaccine development. ■

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MEMBRANES

Porous crystals as membranes

Microporous crystalline membranes are designed for gas separation and potential scale-up

By **Moises A. Carreon**

Chemical separations account for about half of the United States' industrial energy use and as much as 15% of total U.S. energy consumption (*1*). Most of these industrially employed separations, including distillation, evaporation, and drying, are thermally driven. Energy-efficient separation technologies require reducing heat consumption. Non–thermally driven membrane technology could play a key role in gas separations that are less energy-intensive, making them potentially economically feasible. On page 667 of this issue, Li *et al.* (*2*) illustrate a powerful example using a microporous crystalline membrane to separate water from light gases, with subsequent carbon dioxide conversion to liquid fuels by hydrogenation.

Porous crystals grown as membranes with equally sized micropores or with limiting pore apertures are highly appealing materials to effectively separate gas molecules by size exclusion. Li *et al.* designed a sodium aluminosilicate microporous crystalline molecular sieve NaA zeolite membrane displaying precise water conduction nanochannels that allow water to effectively permeate through a continuous crystalline membrane and restrict the diffusion of gas molecules. This strategy may be useful for many industrially important processes where water is present.

The precise gate effect of the membrane can be exploited for the separation of other industrially relevant gas mixtures, including ammonia separation from light gases. For instance, this zeolite composition has a pore entrance size that should be ideal to effectively sieve ammonia from hydrogen and nitrogen. Furthermore, the pore entrance of NaA zeolite promotes favorable charge-dipole interaction with polar molecules. The higher polarizability of ammo-

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