African Swine Fever Poses Risk to U.S. Hogs

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An electron microscope enables microbiologist Thomas Burrage to study the effect of genetically engineered strains of African swine fever virus on the viruses' major target cell, the macrophage. Microbiologists Dan Rock (middle) and Steven Kleiboeker (right) observe. (K7335-4)

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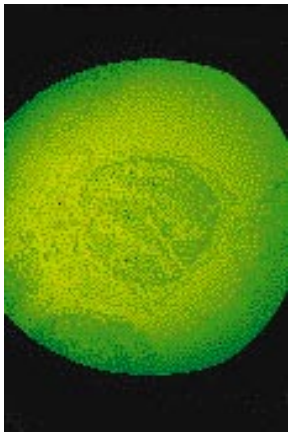

Macrophage cell in early stages of infection with African swine fever virus, magnified about 1,000x. (K7335-17)

It is the stuff of nightmares. Literature—and legend—say the African swine fever (ASF) virus so successfully circumvents an animal's natural disease defenses that a vaccine is an impossibility; that infection with the virus is automatically a death sentence for every exposed pig; that ASF virus is genetically unique.

Dan Rock agrees with the last part. He is a virologist and leader of ARS' African Swine Fever Virus Research Unit at the Plum Island Animal Disease Center, which is set on an 840-acre island in New York's Long Island Sound.

Researchers there tackle the toughest foreign animal disease problems, and Rock has spent the past 5 years sorting African swine fever myth from fact. He says this virus is definitely unique.

"Arboviruses are viruses that can infect both animals and arthropods, such as mosquitoes or ticks," he explains. "ASF is the only known DNA arbovirus—the only one where the genetic material of the virus is composed of double strands of DNA, not RNA. Also, unlike many other viruses that have only a few genes,

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Microbiologist Zhiqiang Lu uses a DNA sequencer to examine genetically engineered African swine fever viruses. (K7334-1)

ASF has about 150. So it's rather complicated, genetically."

Complicated? Yes.

Unconquerable? No.

If virologists sat around late-night campfires telling scary stories, one of the most oft-told might be that once ASF virus invades a pig's body, the animal's immune system never even gets a chance to put up a fight in the form of virus-neutralizing antibodies. The United States has never had an outbreak of ASF—and a good thing, too, the story goes, because this is a struggle American livestock producers could never win.

"The old dogma is that ASF is the only animal virus that doesn't stimulate production of neutralizing antibodies, but that's just not true," says Rock. "We have shown that the wild species of ASF are readily neutralized by antibodies in blood from surviving animals.

"After a pig's been infected, you do see large amounts of antibodies in that pig's blood fairly quickly. The antibodies will provide a protective immune response. But neutralizing antibodies are only one mechanism for protection, and there's no direct evidence they're the critical one. They may be one part of a complicated interaction."

Even with all the myths stripped away, ASF is frightening enough. It's known, for example, that the virus can live at room temperature in blood for 140 days and at a chilly 39°F for 18 months. In tests, virus in blood heated to 122°F for 3 hours still caused infection, and an infected animal's feces kept at room temperature for 11 days still contained virus capable of causing infection.

The most virulent strains of ASF, originating in Africa, are deadly to every infected pig, but weaker strains have emerged in Europe that do leave survivors. The virus frequently hitches a ride from victim to victim in certain ticks of the *Ornithodoros* genus. Some of these, incredibly, can live up to 25 years and have been reported to be infected with the virus for as long as 8 years. But the virus also spreads directly from pig to pig, with no need for help from the tick "middleman."

Once the virus infects a pig, it wreaks havoc ranging from fever, depression, and diarrhea to massive hemorrhaging of internal organs—particularly the lymph nodes, kidney, spleen, and gastrointestinal tissue. Symptoms are similar to those of hog cholera, another devastating animal disease that has fortunately been eradicated in the United States. Currently, the only way to eradicate ASF is by killing all the animals in infected herds.

ASF was first recognized in 1910 by R.E. Montgomery, a British veterinary officer in colonial East Africa. The turning point was the introduction into Africa of European domestic pigs—which, after close contact with the wild African swine, or warthogs, promptly died.

The first known instance of ASF outside Africa occurred in 1957 in Portugal. The flare-up was extinguished, but at a cost of 17,000 pigs dead—either from the infection itself, or from being slaughtered because they had come into contact with sick animals.

Despite that heavy price, the disease reappeared in Portugal in 1960 and spread to Spain. In the ensuing years, it has erupted in France, Italy, Malta, Brazil, Cuba, Haiti, and the Dominican Republic, resulting in swine depopulation programs that in numerous instances have destroyed the only livestock possessed by impoverished rural families.

Rock's research team is pursuing several methods of stopping ASF virus. For starters, they've sorted out the genetic sequence of a virulent

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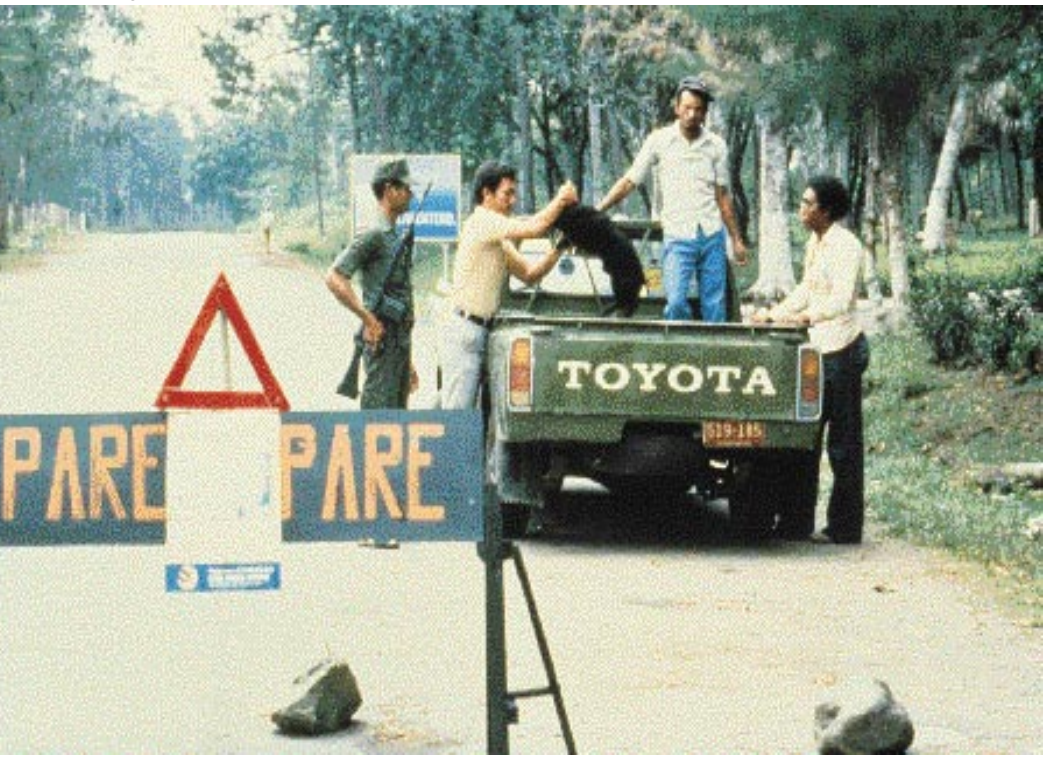

To stop an outbreak of African swine fever, authorities destroyed all pigs throughout the Dominican Republic in 1980.

strain of ASF, with some surprising discoveries.

"There is one gene called *23-NL* that we identified in early 1995," Rock says. "If you take that gene out, the virus will no longer kill pigs. You also have a delay in the appearance of the virus, so that gene may play a role in the ability of the virus to reproduce itself in critical target cells in the pig, such as cells in the animal's lymph nodes and spleen."

Another gene in the virus strongly resembles a protein scientists call CD2. This protein is typically seen on T-lymphocyte cells, which are white blood cells important in fighting disease.

"When we take this gene out of the ASF virus, it doesn't alter the virulence of the virus, but it does slow down its spread in the pig and reduces its ability to reproduce itself," Rock explains. "If the ASF virus is a 'wild' strain that still contains this gene, you find the virus mostly attached to the infected animal's red blood cells. If the gene has been removed, the virus is mostly in the animal's plasma.

"This CD2 protein may be involved in immunosuppression and may play an important role in the tick-warthog cycle. When the virus and host evolve over time, you have an 'arms race.' The virus doesn't kill the pig, for it needs some strategy to ensure its own survival. It must be able to reproduce itself and persist in the pig so there's enough virus present in the blood to infect other ticks that might feed on the pig later. What's interesting is that this is the first time a CD2-like protein has been found in a virus."

Another intriguing ASF discovery is linked to a family of genes known as *BCL-2*. These genes are normally found in all cells and alternately promote or prevent a particular type of cell death called "apoptosis," which is more like a form of suicide than natural cell death.

This is the immune system's version of a firebreak: Just as firefighters may start a back fire in the path of a raging forest fire to stop its advance, virus-infected cells destroy themselves so they don't become a mini-factory in which the virus can replicate itself and spread to uninfected cells.

"A smart virus will carry around genes to interfere with apoptosis and give itself more time to replicate," says Rock. "We've found two interesting genes in ASF virus: one that has a very strong similarity to *BCL-2* and another that's similar to a family of proteins that inhibit apoptosis, separate from *BCL-2*.

"We have evidence that the BCL-2-like protein promotes cell survival and allows a critical amount of time for the virus to replicate. This is only the second time an animal virus has been described to have a gene like this; the first is in Epstein-Barr virus, a human herpes virus.

"It's also important to note," says Rock, "that we have not been able to remove this gene from the virus. You can't make a mutant ASF virus that's minus this gene, so it's likely to be a very critical gene."

Other genes in the virus can be deleted with no apparent impact on its ability to reproduce and spread within the pig. Rock and his fellow researchers surmise that these genes' critical role lies in the virus' ability to survive and thrive in the carrier ticks.

"ASF virus is really a tale of two hosts—the tick and the hog," he says. "The virus has to be successful in both. Maybe by understanding the critical biology of this virus in ticks, a novel strategy for effective control will emerge."

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disease. (K7550-6)

As for vaccine hopes in the near future, "through our genome sequencing, we have identified several proteins that could be involved in stimulating immunity," says Rock. "Studies are under way to immunize pigs with these proteins.

"However, the proteins may be highly variable from one isolate or strain of the virus to another. Let's say a protein turns out to be important in stimulating an immune response, is highly variable from isolate to isolate, and comes in five types. You'd have to immunize against each of those five types."

Although the United States has escaped ASF thus far, an outbreak is not completely implausible. ASF is one of the reasons international travelers are quizzed as to whether they're bringing any meat products into the United States; experiments have shown viable ASF virus can survive in salted, dried meat products for as long as 10 months.

The virus has found its way into many countries via the feeding of ASF virus-infected meat products in garbage to pigs. For example, the Portuguese outbreak of 1957 is believed to have occurred because pigs were fed infected leftovers from an airplane meal that was served on a flight from Angola.

Just as the United States has ready hosts for ASF in its domestic swine population, as well as in wild pigs in Florida and the California Sierras, it also has ticks capable of spreading the virus, says Richard G. Endris.

He is a former ARS veterinary entomologist now working for Mallinckrodt Veterinary, Inc., in Mundelein, Illinois.

"We have *Ornithodoros turicata* found throughout Florida, in southern Georgia, along the Gulf Coast, and from Kansas to East Texas," says Endris. "These ticks will feed on anything, and experimentally they are capable of carrying ASF, but the virus dies out of them in less than a year," he says.

"Throughout the Caribbean basin there's a tick species called *O. puertoricensis* that's capable of transmitting the virus for a couple of years. And there's *O. coriaceus*, found from northern California east at least as far as Reno, Nevada, and south into Mexico.

"*O. coriaceus* also transmits bovine epizootic abortion to cattle and relapsing fever to humans. It is very capable of transmitting ASF to pigs by biting them. Although *O. coriaceus* ticks are capable of living for years, they transmit the virus for only about a year and then disappear from the tick population.

"Fortunately," Endris says, "none of the soft tick species found in and around the United States can transmit ASF virus to their progeny through eggs, as *O. moubata* does in Africa."

"ASF is a very significant threat to swine worldwide," concludes ARS' Rock. "It has a natural, extensive reservoir in ticks and warthogs, the African viruses are highly virulent, and we don't yet have a vaccine.

"The likelihood of an outbreak in the United States is unknown, but it's an international world—you can be in Johannesburg, South Africa, in the morning and, 16 hours later, you're in New York City. We have nasty swine diseases already in this country, but ASF is quite spectacular."—By **Sandy Miller Hays**, ARS.

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