

ANIMAL VACCINE TRIALS

U.S. TEST LANGUISHES, EUROPEANS PROCEEDING

WASHINGTON, D.C.—A year after receiving provisional approval from officials at the U.S. Department of Agriculture (USDA) for a field test of a genetically engineered rabies vaccine, researchers at the Wistar Institute (Philadelphia, PA) still are waiting to begin (*Bio/Technology*, 7:541, June '89). Currently, officials from Wistar and the Nature Conservancy (Arlington, VA) are negotiating over the proposal. The Nature Conservancy owns Parramore Island, Virginia, the site for the proposed test, and its officials say they are continuing to evaluate the Wistar proposal.

Meanwhile, the potential impact of the rabies vaccine developed by Wistar, as well as several other experimental vaccines, was the subject of a workshop, "Rabies: A World-wide Problem for Humans and Wildlife, the Potential for Commercially Viable Vaccines," sponsored by the National Audubon Society [with support from The Joyce Foundation] and held in Washington, D.C., late in March. Several types of orally administered rabies vaccines are in different stages of development. Of the two most advanced, one is based on the rabies virus itself and the other is based on vaccinia virus (VRG). Yet another prototype rabies vaccine, based on human adenovirus, is at a much earlier stage of development.

The development and testing of such vaccines are "setting the stage for the future," says Audubon's senior staff scientist Jan Beyea. In particular, he adds, the design and conduct of the field tests will help establish an important precedent for the deliberate release of other genetically engineered products.

Wistar researchers, in collaboration with scientists at Rhone-Merieux (Lyon, France), developed a genetically engineered anti-rabies vaccine that is based on vaccinia virus. The Wistar version carries the gene for a key glycoprotein found on the surface of the rabies virus. When animals of several species are treated in laboratory tests with VRG to stimulate production of the rabies virus glycoprotein, the animals develop immunity to rabies.

VRG also has been tested in chimpanzees as a way of estimating its safety in humans, according to Charles Ruprecht of Wistar. There were "no adverse signs" in the animals tested, he says. Moreover, the caretakers who were indirectly exposed to the vaccine were free of effects, except for the triggering of an

anamnestic response (meaning that exposure stimulated a deep-seated immune response to prior smallpox vaccinations).

A good deal of information about the safety of VRG in animals is accumulating from the extensive field trials and use of VRG in Europe, according to Philippe Desmettre, director of research at Rhone-Merieux. The scale of the European VRG open-field trials began small but has escalated considerably.

For example, an early test in October 1987 was confined to six square kilometers within a Belgian military base, and 250 bait samples were set out. The scale of a 1989 field trial

The question is what kind of use--broad or limited--and what the costs involved are for these rabies vaccines.

**-Maureen Hinkle,
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encompassed an area of 2,200 square kilometers in which 25,000 bait samples were distributed by helicopter and other means. A large-scale trial in France, comparable in scope to the 1988 Belgian open-field trial (over 435 square kilometers), also began late in 1989. In both cases, according to Desmettre, preliminary findings indicate that the VRG-containing baits were taken up extensively, and the captured animals showed "no abnormalities in morbidity and mortality, and no specific pox lesions." In the Belgian studies, the reported incidence of rabies in both domestic and wild animals in test areas shows a steady decline from 1987 to 1989, he adds.

The attenuated live rabies virus vaccine is also mixed with baits to attract wild animals; it is used for vaccinating foxes in Europe, according to Anne Flamand of Centre National de la Recherche Scientifique (Gif-Sur-Yvette, France). The vaccine has proved "very effective" in Switzerland. The reduction in reports of rabid animals correlates with the distribution of vaccine-laced baits. More than one million doses of such bait have been distributed in Switzerland, and more than six million in Germany, she notes. The low incidence of rabies still being reported may be caused by the live virus vaccine itself, which can revert from its attenuated

state to cause the disease.

"We are trying to improve the safety" of the product, Flamand says. One such approach involves analyzing and then modifying the already attenuated rabies virus at the molecular level. Such analysis indicates that changes in a particular amino acid residue in one of the rabies virus outer proteins are critical for determining virulence, she reports. "If arginine-333 is changed, virulence is eliminated." Once this change is made, the "avirulent virus is protective" of animals that are treated with it, she adds. The molecular change probably reduces the ability of the virus to penetrate mucous membranes of infected hosts.

Flamand and her collaborators are trying to find other secondary ways of changing the virus so that it becomes less likely to revert into a disease-causing virus. Meanwhile, the molecularly modified rabies virus has been tested—and proved effective in preventing rabies when administered orally in baits to foxes. It now is being tested in Switzerland in open fields along the French border.

Canadian researchers recently began developing yet another vaccine to prevent rabies that makes use of adenovirus, according to Stephen Smith of the University of Waterloo (Ontario, Canada). In laboratory tests, the experimental vaccine produces "high antibody titers in skunks when administered orally," he says. By contrast, most other vaccines, including VRG, do not perform well in skunks. Thus, the vaccine appears promising, although many tests remain to be conducted.

"I see using [VRG] for controlling rabies in raccoons," says Wistar's Ruprecht. "We are living with an epidemic of rabies in raccoons, and we have local goals. It is up to other disease management groups to decide how else to use the vaccine. I am not advocating wholesale inoculation of U.S. wildlife, just control of rabies in special areas."

Other geographic regions present special problems. In Ontario, Canada, for instance, there are two distinct wild animal populations with rabies—foxes and skunks, points out Smith, who chairs the Ontario Rabies Advisory Committee. The government says that it is "worth trying to eliminate rabies in foxes, even in the absence of a strategy for treating skunks," he says. Ideally, although a single vaccine is preferable to use of several, no vaccine yet meets that requirement.

—Jeffrey L. Fox