Environmental Assessment
for Field Testing
Rabies Vaccine, Live Raccoon Poxvirus Vector

I. Proposed Action

APHIS is considering granting authorization to ship an unlicensed Rabies Vaccine, Live Raccoon Poxvirus Vector, for field safety testing. Fort Dodge Animal Health, Division of Wyeth, Fort Dodge, Iowa, has requested authorization to conduct field tests under conditions that are typical of dog and cat husbandry in the United States.

Under the provisions of the Virus-Serum-Toxin Act of 1913, as amended in 1985, the USDA must ensure that veterinary biologics are pure, safe, potent, and efficacious and not worthless, contaminated, dangerous, or harmful. Accordingly, APHIS has conducted a risk analysis and has concluded that the safety risks to animals, public health, and the environment are low. A copy of the risk analysis with confidential business information removed is available upon request.

II. Background

Rabies is an acute viral encephalomyelitis found throughout the world and is fatal (in all but one human case) once clinical signs appear. In the US, there are numerous virus variants, generally each associated with a certain species. Although canine rabies has been largely eliminated from dogs in North America and Europe due to successful vaccination programs, rabies is maintained in wildlife. This ongoing source of exposure necessitates continued vaccination. Although no feline rabies virus variant is known, cats are the most frequently reported rabid domestic animal in the US, and reported cases in US domestic cats have outnumbered cases in dogs since 1988.[1]

The raccoon poxvirus is a member of the Poxviridae family and contains a linear and nearly 200 kb double-stranded DNA genome with a hairpin loop at each end. Like other poxviruses, raccoon poxvirus replicates in the cytoplasm and uses its own transcription systems. Poxviruses have been used many times as live vectors to express foreign genes for vaccine development. A live poxvirus vector expressing an immunogen can provide both humoral and cell-mediated immune response against an invading pathogen. Examples of USDA-licensed poxvirus vaccine vectors include vaccinia virus, fowlpox virus, and canarypox virus. Raccoon poxvirus has been used previously as a live vector to express foreign genes in a recombinant vaccine, although those constructs have not received USDA licensure. For this current vaccine construct, no genetic manipulation was made to the wild type parent strain, which is considered to be nonpathogenic for raccoons. It was first isolated from the upper respiratory tract of a raccoon with no obvious clinical symptoms by Y.F. Herman in Aberdeen, Maryland, in 1961-1962. Here, the raccoon poxvirus serves as a vector to express the rabies glycoprotein (G).

The resulting avirulent live vaccine is for use in healthy cats and dogs as an aid in the prevention of rabies virus infection. The proposed field safety test will be conducted in at least three different geographical locations according to instructions on the product circular. Owners of individual cats and dogs will be recruited to participate in the trial; the animals will be handled under normal conditions of small animal husbandry.

III. Need for the Proposed Action

This experimental vaccine represents an attempt by Fort Dodge Animal Health to produce an efficacious and safe vaccine against rabies that protects cats and dogs with one dose administered subcutaneously.

IV. Areas of Concern

The three areas of concern to APHIS are: 1) animal safety, 2) public health, and 3) environmental safety. APHIS has conducted a risk analysis to assess whether risks are associated with the proposal to field test this experimental vaccine. The safety characteristics of this vaccine have been thoroughly evaluated. The conclusions derived from the risk analysis for each of the areas of concern are summarized below.

A. Animal Safety

The risk to animals is low.

1. Studies at CDC and at Fort Dodge indicated that oral administration of raccoon poxvirus-vectored candidate vaccines did not result in reversion to virulence or dissemination into body fluids or feces. No virus was recovered from oral cavity, feces, cervical lymph nodes, liver, spleen, or tonsil when high titer of the vaccine virus was given orally to 20 seronegative cats at 8 weeks of age. Also, no unfavorable reactions were observed after a concentrated stock of the vaccine virus was administered subcutaneously in cats and dogs. Further, in one- and three-year duration-of-immunity studies initiated by the firm, 112 cats and 57 dogs at 12 weeks of age were given one dose of the vaccine subcutaneously with no local or systemic reactions occurring.
2. Animal safety testing in 10 dogs and 10 cats given one dose of vaccine subcutaneously per 9 CFR 113.40 and 9 CFR 113.39, respectively, had satisfactory results.
3. A 10X dose of vaccine was given to dogs and cats in a safety test of the immunogenicity vaccine with no adverse effects in 7 days of observation.
4. The vaccine virus construct was tested in mice by intraperitoneal injection and no adverse reactions were observed.
5. The raccoon poxvirus vector appears to be avirulent for various domestic and wild animals, as shown by safety studies in raccoons, dogs, bobcats, cotton rats, striped skunks, gray foxes, rabbits, domestic cats, American mongooses, sheep, swine, patas (African) and cynomolgus (Asian) monkeys.[2, 3]
6. One report indicated poor antibody response to a raccoon poxvirus vector expressing the rabies G protein and given orally in sheep, implying that if ingested by sheep it would

not be harmful. Transmission of the virus between vaccinated and in-contact controls was not observed or detected by serology.[4]
7. In studies comparing the pathogenicity of a raccoon poxvirus, the same virus with a rabies G insert, the Copenhagen strain of vaccinia virus (vv), and a vv-G construct, the raccoon poxvirus-G construct was 100-fold less pathogenic than its parent strain, 100- to 1,000-fold less pathogenic than vv-G, and 1,000- to 10,000-fold less pathogenic than vv. The results were determined using rabbit skin histopathology, suckling mouse lethality, and oral infection of monkeys and mice.[5]

B. Public Health

The risk to public health is low.

1. There are no indications that special safety measures should be taken to conduct this study. Human exposure will be limited to the qualified personnel administering the vaccine and handling the animals during vaccination.
2. CDC/NIH Guidelines (Appendix B-II-D) recommend for raccoon poxvirus a containment level appropriate for a Risk Group 2 (RG2) agent. There is one reported case of human exposure, caused by a needle prick to the finger of a scientist administering a recombinant raccoon poxvirus expressing *Yersinia pestis* F1 capsular antigen during an experiment. A very small blister developed within 9 days and was healed within 4 weeks.[6] As in this case, RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.
3. Rabies is also classified as a Risk Group 2 agent (Appendix B-II-D), as it is easily prevented if treated in a timely manner. Here, only the glycoprotein gene of rabies virus is present in the construct and by itself is not known to be virulent nor associated with disease in healthy adult humans. The glycoprotein gene has been previously expressed safely by DNA plasmid in *E. coli* host, and in vaccinia virus, fowlpox virus, canarypox virus, and raccoon poxvirus.
4. Although the safety of this experimental vaccine in humans has not been fully evaluated and is therefore unknown, no safety hazards to the public health are expected following vaccination of the intended target species. Since the vaccine virus construct is avirulent and nonreplicative in cats, as demonstrated in the reversion-to-virulence study, the risk to public health is low.

C. Environmental Safety

The risks to the environment are low.

1. The potential for escape and dispersal of this recombinant vaccine is low. The shed/spread capabilities of the vaccine used in dogs and cats are limited even under direct contact exposure. No virus shedding or spread was observed following high titer oral inoculation of cats in the reversion-to-virulence study.
2. The genetic stability of the Master Seed (MS) after five passages under pre-manufacture scale-up procedure was determined to be satisfactory using PCR testing. No

insertion or deletion was detected within the flanking regions and rabies glycoprotein genes. The phenotypic stability of the MS at passage levels X and X+5 was evaluated by immunofluorescent assay (IFA) and blue plaque assay. No significant difference in rabies G protein expression was observed.
3. The potential for horizontal gene transfer of the rabies G gene is low and the risk of recombination between the vaccine virus construct and wild type (wt) poxviruses is low. Cats and dogs are not likely to be co-infected with wt poxvirus strains, which would be required for recombination to occur. Even if recombination should occur, because both the vaccine and wt parent strain are avirulent, recombination would not be expected to generate a more virulent virus.
4. The survivability of the recombinant in the field is not expected to exceed that of the parent strain. The experimental vaccine was stored at 2-7^{o}C and samples of the vaccine removed at specific intervals, to determine 27-month real time stability for lyophilized and liquid forms. Virus titer loss was not great, even for liquid form, indicating this virus is relatively stable when stored under refrigeration.
5. There are no expected adverse ecological effects associated with the proposal to conduct field testing with this experimental vaccine, which is derived from a strain that already exists in nature, in raccoon populations that are not clinically affected by its presence. The vaccine candidate is more attenuated than the parental strain, suggesting its risk to the environment is minimal.

V. Alternatives

Two alternatives were considered. The only alternative considered, other than the preferred action alternative, is not to approve the proposed field tests, the "no action" alternative. We have considered the applicants' goals in light of the agency's public interest and responsibilities and any potential environmental impact. Based upon the results of our risk analysis and the potential applications for this vaccine in disease control, APHIS adopts the alternative that the proposed field tests be approved.

VI. Conclusion

Based upon the risk analysis documented in this Environmental Assessment, APHIS has determined that implementation of the proposal would not significantly affect the quality of the human environment and that the preparation of an Environmental Impact Statement is not required (Finding of No Significant Impact).

References

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