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The safety of ONRAB® in select non-target wildlife

Q1 Tricia L. Fry[a,*], Kaci K. VanDalen[b,1], Colleen Duncan[c,2], Kurt VerCauteren[a,3]

Q2 [a] USDA APHIS WS National Wildlife Research Center, 4101 Laporte Avenue, Fort Collins, CO 80521, United States
[b] USDA APHIS WS National Wildlife Research Center, 4101 Laporte Avenue, Fort Collins, CO 80521, United States
[c] Colorado State University Veterinary Diagnostic Laboratory, 300 West Drake, Fort Collins, CO 80523, United States

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ABSTRACT

ONRAB® is a recombinant human adenovirus type 5 (HAd5) with the rabies glycoprotein gene incorporated into its genome. ONRAB® has been used in Canada as an oral rabies vaccine in target wildlife species such as: red fox (*Vulpes vulpes*), raccoon (*Procyon lotor*), and striped skunk (*Mepthis mephitis*). We evaluated the safety of ONRAB® in non-target wildlife species likely to contact the vaccine baits during oral rabies vaccine campaigns in the United States. We investigated the effects of oral inoculation of high titer ONRAB®, approximately ten times the dose given to target species, in wood rats (*Neotoma* spp.), eastern cottontail rabbits (*Sylvilagus floridanus*), Virginia opossums (*Didelphis virginiana*), eastern wild turkeys (*Meleagris gallopavo silvestri*), and fox squirrels (*Sciurus niger*). We performed real-time polymerase chain reaction (PCR) on fecal swabs, oral swabs, and tissues, including lung, liver, kidney, small intestine, large intestine, and when appropriate nasal turbinates, to detect ONRAB® DNA from inoculated animals. By seven days post-inoculation, turkeys, opossums, and cottontails had all stopped shedding ONRAB® DNA. One wood rat and one fox squirrel still had detectable levels of ONRAB® DNA in fecal swabs 14 days post-inoculation. Real-time PCR analysis of the tissues revealed some ONRAB® DNA persisting in certain tissues; however, there were no significant gross or histologic lesions associated with ONRAB® in any of the species studied. Our results suggest that many non-target species are not likely to be impacted by the distribution of ONRAB® as part of oral rabies vaccination programs in the United States.

1. Introduction

Rabies viruses in the United States once affected domestic animals and wildlife alike, but since the 1970s have been effectively eliminated in domestic animals a result of municipal mandates requiring pets be vaccinated for rabies. Now control of rabies virus in the United States focuses on controlling the pathogen in wildlife reservoirs. Since 1995, the United States National Rabies Management Program (NRMP), administered by the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (WS), has been working to control terrestrial rabies virus through wildlife vaccination. Oral rabies vaccines are distributed by hand and by fixed and rotary-wing aircraft in most states east of the Appalachian Mountains, as well as portions of Ohio, Texas, New Mexico, and Arizona. It is estimated that at least 60% of raccoons (*Procyon lotor*) in an infected population must be immunized against the rabies antigen to eliminate raccoon rabies [1]. At present, it is estimated that 30% of raccoons are inoculated [2] by the Oral rabies vaccination (ORV) program in the United States.

Raboral V-RG® (Merial Ltd., Athens, GA), the vaccine presently used in the ORV program, is a recombinant vaccine comprised of a vaccinia virus with the rabies glycoprotein gene inserted into its genome. Presently, raccoons, gray foxes (*Urocyon cinereoargenteus*), coyotes (*Canis latrans*), and possibly other terrestrial mammals can be inoculated by ingesting this liquid vaccine, which is contained in a palatable sachet [2,3]. Ideally, when the animal bites into the sachet the liquid vaccine coats the buccal lining, entering and replicating in the oral cavity, tonsils, and other surrounding tissues, and stimulates an immune response. However, the immunogenicity of Raboral V-RG, as determined by measurements of circulating rabies virus neutralizing antibodies (rVNA), is limited. Brown et al. [4] reported that of 19 wild raccoons inoculated by Raboral V-RG®, 14 succumbed to a rabies challenge. Additionally, a vaccine comparison study in Canada and the United States found that only 30% of raccoons inoculated in the United States by Raboral V-RG® demonstrated detectable rVNA ≥0.5 IU/mL, while nearly 75% of raccoons administered, ONRAB® (Artermis Technologies, Guelph, Ontario, Canada) produced rVNA titers ≥0.5 IU/mL [5,6].

* Corresponding author. Tel.: +1 970 266 6162.
E-mail addresses: tricia_fry@hotmail.com, tricia@wildlife.org (T.L. Fry), kaci.vandalen@aphis.usda.gov (K.K. VanDalen), colleen.duncan@colostate.edu (C. Duncan), kurt.c.vercauteren@aphis.usda.gov (K. VerCauteren).
[1] Tel.: +1 970 266 6312.
[2] Tel.: +1 970 297 5422.
[3] Tel.: +1 970 266 6193.

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ONRAB® is a recombinant oral rabies vaccine with human adenovirus type 5 (HAd5) as the backbone and the rabies virus glycoprotein gene inserted into its genome. HAd5 is a relatively safe and well-studied virus, which is used in many vaccine formulations [7–9], but does not replicate well in most non-human species [10]. The addition of the rabies virus glycoprotein allows the virus to infect many different species and triggers the host's immune system to produce antibodies against the rabies virus glycoprotein [10]. Studies on the efficacy and safety of the ONRAB® vaccine in animals have been completed in Canada [6,10]. Results of field and laboratory studies indicated that persistence of the virus in animals is short-lived and that ONRAB® is unlikely to have an overall negative impact on wildlife [6,10–12]. Rabies virus seroconversion rates for raccoons in areas baited with ONRAB® averaged 80% in the first two years [13], well above the seroconversion rates detected when the vaccine Raboral V-RG® was used [3,4].

Knowles et al. conducted vaccine safety and immunogenicity studies on 18 species: striped skunks, red foxes (*V. vulpes*), raccoons, meadow voles (*Microtus pennsylvanicus*), deer mice (*Peromyscus leucopus*), gray squirrels (*Sciurus carolinensis*), laboratory rabbits (*Oryctologu cuniculus*), ground squirrels (*Marmota monax*), cattle (*Bos taurus*), horses (*Equus ferus*), domestic swine (*Sus domesticus*), domestic chickens (*Gallus domesticus*), sheep (*Ovis aries*), dogs (*Canis familiaris*), cats (*Felis domesticus*), cotton rats (*Sigmodon hispidus*), SCID mice and nude mice (*Mus musculus*) [9]. For their non-target safety trials, Knowles et al. [10] administered an ONRAB® dose approximately 10 times greater than that needed to vaccinate target species. Animals were monitored daily and all tolerated exposure to the vaccine. Analyses of tissues resulted in viral DNA detected in some lung and kidney samples. Following histopathological analysis, the most marked findings were in the lungs with pulmonary congestion possibly due to vaccine aspiration. Oral swabs and/or fecal samples were collected from skunks, raccoons, foxes, cotton rats, cats and dogs. Viral shedding, if any, was generally complete by 4 days post-inoculation (dpi) except for one skunk, which continued to shed virus in feces through 14 dpi [10].

Our research expands on the species evaluated by Knowles et al. [10] and investigates the vaccine as it relates to its safety in wildlife species likely to come in contact with ONRAB® as part of WS NRMP ORV operations. We investigated the effects of oral inoculation of high titer ONRAB® vaccine in 21 eastern wild turkeys (*Meleagris gallopavo silvestri*), 17 opossums (*Didelphis virginiana*), 14 cottontail rabbits (*Sylvilagus floridanus*), 21 fox squirrels (*Sciurus niger*), and 15 wood rats (*Neotoma* spp.). We preformed post-mortem examination to determine any gross or histological pathology that may be linked to the vaccine. We assessed immunogenicity of ONRAB® in the non-target animals and evaluated the duration of viral DNA shed into the environment via fecal and oral shedding. We also investigated the persistence of ONRAB DNA in select tissues of vaccinated animals.

2. Materials and methods

2.1. Study animals

All animals were wild caught with the exception of the eastern wild turkeys which were purchased from a commercial breeder (McMurry Hatchery, Webster City, IA, USA) and shipped at 2 days old. All animal trapping, handling, maintenance, and sampling techniques were approved by the WS National Wildlife Research Center (NWRC) Institutional Animal Care and Use Committee (QA1882). Animals were trapped and transported under permits issued by the State of Colorado (Permit #11TR1232A1, Permit # 12TR1232) and individually housed throughout the study.

2.2. Vaccination

Artemis Technologies Inc. (Guelph, Ontario, Canada) supplied us with bulk ONRAB® oral rabies vaccine under the United States Veterinary Biological Product Permit No. VB-118764. The 50% cell culture infectious dose ($CCID_{50}$) of the vaccine was $10^{10.44}$ $CCID_{50}$/mL. We followed the methods described by Knowles [10] et al. and orally inoculated animals with 1.8 mL dose of vaccine, which resulted in an infectious virus titer approximately 10 times higher than found in a standard ONRAB® bait.

2.3. Sample collection

We evaluated the safety of the ONRAB® vaccine via numerous tests and observations following the methods of Knowles et al. [10]. On a daily basis we monitored animals for changes in behavior and eating habits. We looked for shedding of ONRAB® DNA via oral swabs on 0, 4, and 7 days post infection (dpi) in turkeys, cottontail rabbits, opossums and fox squirrels and on 0 and 4 dpi in wood rats. We also collected feces on 0–7, 14, 21, and 28 dpi for all species. We collected paired blood samples on 0 and 28 dpi to evaluate sera for Rabies virus neutralizing antibodies (rVNA).

One-half of the study animals for each species were humanly euthanized on 4 dpi, when viral replication should be at or near its peak [10,12,14]. The remaining individuals were euthanized at the conclusion of the study (28 dpi). Turkeys were euthanized by an intravenous injection of an overdose of barbiturates. Opossums were anesthetized with 10 mg/kg ketamine HCl and 2 mg/kg xylazine via intramuscular injection before receiving an intracardiac injection of an overdose of barbiturates. Cottontail rabbits and fox squirrels were anesthetized with isoflurane then given an overdose of barbiturates via intracardiac injection. Wood rats were euthanized by an inhaled overdose of isoflurane.

2.4. Post-mortem examination

Following euthanasia on either 4 dpi or 28 dpi, a standard post-mortem examination was conducted by a veterinary pathologist. Any abnormal tissue and routine sections of lung, liver, kidney, small intestine, and large intestine were preserved in 10% neutral buffered formalin for histology. Tissues were embedded in paraffin, cut at 5 μm, stained with hematoxylin and eosin stain and examined histologically. Fresh tissues were also collected for ONRAB® DNA detection by real-time PCR.

2.5. Serological methods

Serum samples were heat inactivated at 56 °C for 30 min. Rabies virus neutralizing antibodies were detected in the sera by use of the rapid fluorescent focus inhibition test (RFFIT), as described by Smith et al. [15]. Murine neuroblastoma cells were used for infection by the CVS rabies virus strain. Sera with rVNA activity ≥0.2 IU/ml were considered positive samples.

2.6. Real-time polymerase chain reaction

We used the MagMAX™ Viral RNA isolation kit (Life Technologies, Grand Island, NY, USA), which is also recommended for isolating DNA, to extract ONRAB® DNA from fecal and oral swabs. The DNeasy Blood & Tissue kit (Qiagen, Valencia, California, USA) was used to isolate DNA from 10 to 25 mg of lung, liver, kidney, small intestine, large intestine, and nasal turbinates as directed by the manufacturer. Real-time PCR was performed on the extracted DNA using published primers and a dual-labeled probe for an ONRAB target sequence [11]. Twenty-five milliliter PCR reactions containing 1× TaqMan Universal Master mix (Life Technologies,

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