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Efficacy of Ontario Rabies Vaccine Baits (ONRAB) against rabies infection in raccoons

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ABSTRACT

In the US, rabies lyssavirus (RABV) only circulates in wildlife species and the most significant reservoir from a public and animal health perspective is the raccoon (*Procyon lotor*). Management of wildlife rabies relies principally on oral rabies vaccination (ORV) strategies using vaccine-laden bait delivery to free-ranging target hosts, in order to reduce the susceptible population to prevent the spread of and eliminate RABV circulation. Our objective was to evaluate efficacy of the Ontario Rabies Vaccine Bait (ONRAB) against a lethal RABV challenge in captive raccoons. Sham or live vaccine baits were offered to 50 raccoons and efficacy was evaluated in 46, split into two trials of 17 and 29 raccoons. Raccoons were challenged with a lethal dose of RABV 180 days post-vaccination and observed for 90 days post-infection. Raccoon bait interactions were assigned increasing integer scores for approach, oral manipulation, puncture, and consumption behaviors. Higher bait interaction scores were observed in the fall compared to the spring trial, indicating that more raccoons consumed baits in the fall. Although animal age did not explain variation in bait interaction scores, the geometric mean rabies virus antibody titers among juvenile vaccinates were higher than adults at all pre-challenge time points. The prevented fraction associated with ONRAB delivery was 0.73 (8/11, 95% CI 0.39–0.94) in the spring trial and 0.91 (21/23, 95% CI 0.72–0.99) in the fall trial. All sham-vaccinated raccoons (12/12) succumbed to rabies infection, in contrast to 15% (5/34) mortality among vaccinated raccoons. Our results indicate a high efficacy of ONRAB bait vaccination in protecting adult and juvenile raccoons against RABV infection for a minimum of six months. These data complement experimental field trials that have also demonstrated the potential of ONRAB for the control and prevention of RABV circulation in free-ranging raccoon populations in the US.

1. Introduction

In the United States (US), rabies lyssavirus (RABV) only circulates in wildlife species and the most significant reservoir from a public and animal health perspective is the raccoon (*Procyon lotor*). The human exposures and animal case burden associated with raccoon RABV is due in part to the ubiquitous nature and high population densities of this peri-domestic species in suburban and urban habitats [1–3]. The current enzootic focus of raccoon RABV extends from a historical area in the southeastern US north to the border with Canada. Management of wildlife rabies historically involved population reduction strategies (e.g. culling), but now focuses on oral rabies vaccination (ORV) strategies to deliver vaccine-laden baits to free-ranging target hosts, in order to reduce the susceptible population [4]. The National Rabies Management Program (NRMP), administered by the US Department of Agriculture (USDA), Animal and Plant Health Inspection Service, Wildlife Services program, has the mission to control and eliminate specific RABV variants circulating in wild carnivores in the US. Coordinated ORV programs to target raccoons have been operational since the 1990s [5]. While ORV has been successful in preventing westward spread of raccoon RABV in the US, post-ORV population immunity levels have averaged 30% across several years and have led to concern about the ability of current ORV products to eliminate RABV circulation in raccoons [5].

The Raboral V-RG® product (Boehringer Ingelheim Animal Health, Athens, Georgia, USA) is the only oral rabies vaccine currently licensed for use with free-ranging raccoons and coyotes (*Canis latrans*) in the US [6]. However, another product has shown promising results for the control of RABV in raccoons and striped skunks (*Mephitis mephitis*) in southern Ontario, Canada [7,8]. The Ontario Rabies Vaccine Bait (ONRAB; Artemis Technologies, Inc.,

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Guelph, Ontario, Canada) is comprised of a sweet attractant matrix that coats a blister pack containing a live recombinant human adenovirus expressing the RABV glycoprotein [9], and is licensed for use with free-ranging striped skunks in Canada. One study demonstrated immunogenicity and efficacy of ONRAB baits for raccoons, but did not meet prevented fraction standards for animal rabies vaccines in the US [10].

We conducted a randomized and blind evaluation of the efficacy of ONRAB ultralite baits (ULBs) in protecting raccoons against lethal RABV infection, to re-assess its potential for meeting efficacy standards for animal rabies vaccines in the US.

2. Materials and methods

2.1. Animals, housing, and restraint

A total of 65 naïve captive-bred raccoons (19 adult males, 20 adult females, 14 juvenile males, 12 juvenile females) were obtained from Ruby Fur Farm (New Sharon, Iowa, USA). Animal use procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the USDA National Wildlife Research Center (NWRC) under protocol 2278. The import and housing of raccoons at the NWRC facility was authorized under Colorado Parks and Wildlife permits 14TR2056A1, 15TR2143, and 16TR2143. During quarantine, individual animal health was inspected by a veterinarian and passive integrated transponders (PIT tags; Avid Identification System, Inc., Norco, California, USA) were subcutaneously injected into each raccoon under anesthesia for unique identification (ID). A total of 50 raccoons (30 adults, 20 juveniles) were randomly assigned to the vaccine efficacy study, which was conducted as two consecutive trials (trial 1, n = 21, March 2015–December 2016; trial 2, n = 29, November 2015–August 2016). At the time of vaccination, juveniles in the first trial were ten months old and in the second trial were six months old. A total of 15 raccoons (9 adults, 6 juveniles) were used in a challenge virus titration study, conducted as two consecutive trials during November 2014 (n = 10) and January 2016 (n = 5). All raccoons were housed in individual elevated pens (1.2 × 2.4 × 1.8 m) in an open-air outdoor building during quarantine and vaccination. Raccoons were moved to individual pens on concrete (3 × 3 × 2.5 m) in an open-air outdoor building during post-vaccination (pv) monitoring. Raccoons were housed in individual cages (0.7X1X1m) in an Animal Biosafety Level 2 room during RABV challenge and post-infection (pi) monitoring. Each pen or cage had a den box attached to the outer edge, and contained other forms of enrichment. Raccoons were fed a daily ration of 200 g of Mazuri omnivore diet (PMI Nutrition International, St. Louis, Missouri, USA) except during vaccination and were provided water *ad libitum*. Raccoons were anesthetized using inhalation delivery of isoflurane gas or intramuscular (IM) injection a 5:1 ratio (20 mg/kg and 4 mg/kg respectively) of ketamine (Ketaset®; Zoetis, Inc., Florham Park, New Jersey, USA) to xylazine (AnaSed®; Akorn, Inc., Lake Forest, Illinois, USA) for the purpose of blood sample collection from a jugular vein and inoculation. For isoflurane anesthesia, induction was accomplished by delivery of 5% isoflurane in oxygen at 5L/min in the den box containing the animal [11]. Upon induction, a cone was then fitted over the oronasal region for maintenance at 2–3% at 1–2 L/min during sample collection. Upon completion of procedures, animals were returned to den box for arousal under ambient conditions, and monitored until bright, alert and responsive to stimuli.

2.2. Challenge virus titration study

A RABV challenge virus was obtained from the USDA Center for Veterinary Biologics. The 92-5A RABV is a New York City dog variant that was most recently passaged in red foxes (*Vulpes vulpes*). This virus was selected for study because it met regulatory requirements for purity, potency, and purpose. The neat titer was $10^{7.9}$ mouse intracerebral lethal doses per mL ($MICLD_{50}$/ml). Virus was diluted using sterile phosphate buffered saline, supplemented with 2% fetal bovine serum (Invitrogen, Carlsbad, California, USA). As data were not available regarding pathogenicity of this virus for raccoons, an initial titration trial was designed so that five raccoons each were randomly assigned, while blocking for sex and age, to receive a dose of $10^{6.9}$ or $10^{5.9}$ $MICLD_{50}$/mL. In a second trial, five additional raccoons received the $10^{5.9}$ $MICLD_{50}$/mL dose to test the repeatability of the initial trial outcome at this dose. Baseline blood samples were collected from each raccoon prior to IM inoculation with 0.5 mL of diluted RABV into each masseter muscle (1.0 mL total). Animals were monitored daily, or twice-daily during the expected clinical period (days 7–35 pi), for 90 days pi. Upon display of two or more clinical signs of rabies, raccoons were anesthetized with an IM injection of ketamine/xylazine. Under heavy anesthesia, a terminal blood sample was collected prior to intracardiac administration of pentobarbital sodium and phenytoin sodium (VetOne Euthanasia Solution; Med-Pharmex, Inc., Pomona, California, USA). Surviving raccoons were euthanized on or after day 90 pi. Brainstem and cerebellum tissues were collected postmortem from individual raccoons.

2.3. Vaccine immunogenicity and efficacy study

Fifty raccoons were randomly assigned, while blocking for sex and age, to one of two treatment groups, live or sham, for presentation of a single ONRAB ULB (lots AdRG1.3 14-01, AdRG1.3 14-01P). Raccoon treatments were assigned by the cooperator (Artemis), and the NWRC research team was blind to the assignments until the conclusion of each efficacy trial. Food was withheld from raccoons for 24 h prior to bait offering. A total of 12 raccoons received a sham bait, and 38 raccoons received a live bait containing 1.8 mL of vaccine at a titer of $10^{9.6}$ $TCID_{50}$/mL during a 24 h presentation window. Bait offering was monitored using motion-activated trail cameras (Reconyx Silent Image, Holmen, Wisconsin) and video cameras (Supercircuits model PC161IR, Supercircuits, Inc., Austin, Texas, USA; Zodiac model CAMZ836IR, Zodiac Light Waves Inc., Ontario, Canada; Polaris model EZ-380VF, Polaris USA, Norcross, Georgia, USA). Plastic sheets were placed underneath the elevated cages of each animal prior to bait offering to collect bait debris and vaccine spillage, and were inspected every 4 hrs to assess bait remains and estimate spillage to the nearest 0.1 mL using a pipette.

Scores were assigned for each raccoon-bait interaction as follows: (0) animal does not approach bait, (1) animal approaches bait, but without oral contact, (2) oral manipulation of bait by animal, but no puncture of blister pack, (3) oral manipulation and puncture of the blister pack, but incomplete consumption, or (4) animal consumes entire bait. Camera and video footage were analyzed post-trial to verify scores.

Baseline blood samples were collected from raccoons prior to vaccination, and then on days 30, 60, 90, and 176 pv. Raccoons were challenged on day 180 pv by IM inoculation with 0.5 mL of diluted RABV into each masseter muscle (1.0 mL total). Animals in the first trial (n = 17) were inoculated with $10^{6.2}$ $MICLD_{50}$/mL of RABV. Based on results of the first trial, the animals in the second trial (n = 29) were inoculated with a lower dose of $10^{5.9}$ $MICLD_{50}$/mL. Animals were monitored as described previously, and blood samples were collected on days 15, 30, and 60 pi. Surviving raccoons were euthanized on days 90 or 91 pi. The procedures for euthanasia and tissue collection were the same as described for the challenge virus titration study.

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