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# Safety studies on an adenovirus recombinant vaccine for rabies (AdRG1.3-ONRAB®) in target and non-target species

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ABSTRACT

A replication-competent human adenovirus vector in which the rabies virus glycoprotein gene was inserted (AdRG1.3-ONRAB®) was given by direct instillation into the oral cavity to representatives of three wildlife vector species of concern in Ontario (red fox, raccoon and striped skunk) and to a variety of non-target wildlife species, domestic and laboratory species. Despite use of a relatively high dose of vaccine, no untoward clinical signs were observed. Subsequent to vaccine exposure, detection of vaccine virus in lung, spleen, intestine, liver, kidney and brain of each animal was attempted using an ONRAB®-specific assay combining PCR with Southern blotting (PCR–SB). Of the 1280 tissue samples obtained from vaccinates or contact animals, 18 (1.4%) were found to be PCR–SB positive. Virus isolation attempts were performed utilizing cell culture for all PCR–SB positive tissues and a selection of PCR–SB negative tissues. Histological examination performed on all PCR–SB positive tissues failed to identify lesions attributed to the vaccine. A quantitative real-time PCR was used to determine the excretion of the vaccine in feces and in the oral cavity with 0.8% of oral swabs and 6.8% of fecal specimens found to be positive. The low rates of recovery of vaccine virus from tissues, feces and the oral cavity suggest that the likelihood of ONRAB® causing a negative impact on wildlife species is unlikely.



## 1. Introduction

Rabies exists in most regions of the world, maintained as self-sustaining enzootics in a limited number of reservoir species [1]. In North America, rabies enzootics occur in certain wildlife species, the most prominent being red fox (*Vulpes vulpes*), striped skunk (*Mephitis mephitis*), raccoon (*Procyon lotor*), and several species of bats (*Chiroptera*) [2]. Traditionally, rabies cases in Canada were most prevalent in Ontario where control of wildlife rabies has included trap-vaccinate-release, depopulation strategies and oral vaccination campaigns [3]. The latter involves distribution of vaccine-laden baits targeting terrestrial rabies reservoir species with the intent to induce a sufficient proportion of immune animals so as to limit rabies virus transmission within the population. Inactivated rabies vaccines fail to generate an acceptable immune response when given orally to foxes [4]. Therefore, attenuated live rabies vaccines including ERA (Evelyn Rokitnicki Abelseth) strain and the closely related SAD (Street Alabama Dufferin) strain were used in the first wildlife vaccination campaigns in Europe [5,6] and subsequently in Ontario [7]. These vaccines are known to have residual pathogenicity in some species and very small numbers of vaccine-induced cases of rabies have been reported both in various European countries [8,9] and in Ontario [10]. Concerns about vaccine safety combined with their lack of efficacy in the raccoon and striped skunk [11], justified the need to develop alternative products.

The use of recombinant viruses overcomes the problem of vaccine-induced rabies. Few of these constructs have been assessed for their efficacy and safety in sufficient detail to permit their use in the field. A vaccinia-rabies glycoprotein recombinant vaccine, V-RG [12], has been used in Europe to control fox rabies [13] and in the US and parts of Canada in an attempt to control raccoon rabies [3,14]. However, this product is not consistently effective for skunks via the oral route [15]. There is a need for an effective vaccine-bait combination for skunks and raccoons. Several recombinant viruses based upon the human adenovirus 5 (HAd5) vector backbone have been described [16,17] and one of these, AdRG1.3, referred to in this paper as ONRAB®, is the subject of this study. This

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recombinant virus contains a rabies virus G gene expression cassette inserted within the E3 region of the adenovirus genome [17]; animals exposed to this virus produce neutralizing anti-rabies virus glycoprotein antibodies critical for protection against this disease [18]. This construct has been shown to be immunogenic when given in baits both to skunks and raccoons [19].

Since this product is intended for widespread environmental distribution in vaccine-laden baits, its safety, both in the intended rabies vector species and in a variety of other non-target species that could potentially come into contact with the virus, must be carefully evaluated. Such studies are the subject of this paper. Animals given ONRAB® by the oral route were examined for (i) the presence of virus in various tissues after administration of massive doses (six fold the normal dose), (ii) horizontal transmission between animals by direct contact, (iii) viral shedding into saliva and feces, and (iv) susceptibility of immunocompromised animals. To facilitate these studies, which required analysis of a large number of biological samples, molecular detection techniques were developed and employed. The biological relevance of such assays was examined on selected samples using more traditional and laborious cell culture isolation procedures (refer to Section 2).

## 2. Materials and methods

### 2.1. Vaccine

ONRAB® was grown in 293 cells, human embryonic kidney cells (Microbix, Toronto, ON) [20], by standard cell culture procedures followed by a concentration step and was stored at −80 °C until used. Virus titres were determined using 293 cells (received from L. Prevec, McMaster University, Hamilton, ON) in 96 well plates (Falcon) with virus detection using a murine monoclonal antibody specific for human adenovirus type 5, 66-7G1-8-10 (CFIA), followed by a FITC-labelled polyclonal anti-mouse antibody (Cappel). Virus titres were calculated using the Spearman–Karber formula [21] and are expressed as the geometric mean of a minimum of five replicated titrations. Virus was used at a titre of $10^{10.3}$ $TCID_{50}$/ml for the safety trials, and at $10^{9.5}$ $TCID_{50}$/ml for the efficacy trial.

### 2.2. Animals

All animals were maintained in accordance with the Canadian Council on Animal Care (CCAC) guidelines with all protocols approved by the Animal Care Committee at Ottawa Laboratory Fallowfield (OLF). Animals were housed in Level 2 biocontainment facilities. See Table 1 for details concerning source, housing and scientific names.

When required, animals were sedated with either a mixture of ketamine hydrochloride (90.9 mg/ml) and acepromazine maleate (2.2 mg/ml) or isoflurane. For euthanasia, Euthanyl Forte (pentobarbital 540 mg/ml), isoflurane or carbon dioxide overdose were used.

The species selected for these studies were divided into five categories representative of the specific risk groups as follows: Category 1, rabies vector wildlife species (striped skunk, red fox, raccoon); Category 2, non-target wildlife (meadow vole, deer mouse, grey squirrel, rabbit, groundhog); Category 3, livestock (horses, sheep, cows, pigs, chickens); Category 4, companion animals (dogs and cats); Category 5, immunocompromised mice (nude and SCID mice) and the cotton rat as a model for HAd5 infection [22] (Table 1).

For the Category 1 animals, feces were collected pre-exposure and at days 0–4, 7, 9, 11, 14, 18 post-exposure. For raccoons, additional samples were collected at days 21 and 28. Oral swabs were collected at days 0, 7, 14 and 21. For the Category 4 animals, swabbing of the oral cavity was conducted at days 0–9, 11, 14 and 18 post-vaccination. Feces were collected from dogs pre-exposure and daily from days 1–11 and at day 14. Cat feces were not collected due to contamination with urine and litter box material. For the cotton rat, feces were collected at days 0–4, and 9 post-exposure.

**Table 1**
Summary of animals used in safety and efficacy studies.

| Cat[a] | Species | Source | Housing | Number of animals | | | Volume (ml) of vaccine |
|---|---|---|---|---|---|---|---|
| | | | | Vaccinate[b] | Contact[b] | Control | |
| 1 | Striped skunk (*Mephitis mephitis)* | Ruby's Fur Farm[c] | Individual | 12 | 0 | 2 | 1.8 |
| 1 | Red fox (*Vulpes vulpes)* | MNR[d] | Individual | 12 | 0 | 2 | 1.8 |
| 1 | Raccoon (*Procyon lotor)* | Ruby's Fur Farm | Individual | 12 | 0 | 2 | 1.8 |
| 2 | Meadow vole (*Microtus pennsylvanicus*) | OLF[e] | Group | 16 | 4 | 2 | 0.18 |
| 2 | Deer mouse (*Peromyscus leucopus*) | OLF | Group | 16 | 4 | 2 | 0.18 |
| 2 | Grey squirrel (*Sciurus carolinensis)* | Wild trapped | Group | 10 | 2 | 2 | 0.90 |
| 2 | Rabbit (*Oryctologus cuniculus*) | Charles River[f] | Group | 10 | 2 | 2 | 1.8 |
| 2 | Groundhog (*Marmota monax*) | North Eastern Wildlife[g] | Group | 10 | 2 | 2 | 1.8 |
| 3 | Cow (*Bos Taurus*) | Stockyard | Group | 4 | 0 | 1 | 9.0 |
| 3 | Horse (*Equus ferus*) | Stockyard | Free stall | 4 | 0 | 1 | 9.0 |
| 3 | Pig (*Sus domesticus*) | OLF | Group | 4 | 0 | 1 | 9.0 |
| 3 | Sheep (*Ovis aries*) | OLF | Group | 4 | 0 | 1 | 9.0 |
| 3 | Chicken (*Gallus domesticus*) | OLF | Group | 10 | 2 | 2 | 1.8 |
| 4 | Dog (*Canis familiaris*) | Liberty Research[h] | Group | 4 | 0 | 1 | 1.8 |
| 4 | Cat (*Felis domesticus*) | Liberty Research | Group | 4 | 0 | 1 | 1.8 |
| 5 | Cotton rat (*Sigmodon hispidus*) | OLF | Group | 16 | 12 | 4 | 0.18 |
| 5 | SCID mouse (*Mus musculus*) | Charles River | Group | 16 | 4 | 2 | 0.18 |
| 5 | Nude mouse (*Mus musculus*) | Charles River | Group | 16 | 4 | 2 | 0.18 |
| | Striped skunk (*Mephitis mephitis*) | Ruby's Fur Farm | Individual | 30 | 0 | 14 | 1.8[i] |

[a] Cat—Category.
[b] Number of animals euthanized was divided equally between day 4, and day 27 or 28.
[c] New Sharon, IA, 50207-8079, USA.
[d] MNR—Ministry of Natural Resources, Codrington, ON, K0K 1R0, Canada.
[e] OLF—Ottawa Laboratory Fallowfield, Ottawa, ON, K2H 8P9, Canada.
[f] St-Constant, QC, J5A 1Y2, Canada.
[g] Harrison, ID, 83833, USA.
[h] Waverly, NY, 14892, USA.
[i] Vaccine presented in an Ultralite bait.