A vaccinia-vectored rabies vaccine field trial: ante- and post-mortem biomarkers

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Summary: During the field safety evaluation of a vaccinia-rabies glycoprotein recombinant virus vaccine for wildlife, two biomarkers were used to identify potential contact with vaccine-laden baits. Tetracycline, a commonly used and reliable calciphilic tissue marker, was included in a fish-meal polymer bait matrix and was evaluated from post-mortem bone samples. Additionally, an ante-mortem marker was needed to identify, for prospective study, raccoons which had contacted baits and thus, potentially, vaccine. Sulfadimethoxine (SDM) was included in an attractant slurry surrounding the bait, as a novel short-term seromarker. Preliminary laboratory studies in raccoons demonstrated SDM residues for up to one week following ingestion of a single 250 mg dose. During the first six days after bait distribution, 49 individual raccoons were live-trapped in the vaccination area. SDM was detectable in 38 of 49 (77.5%) serum samples. Similarly, 47 of 56 (83.9%) bone samples from raccoons collected in the vaccination area throughout the twelve-month study were tetracycline-positive. Conversely, none of the serum samples (n = 12) from the first six days of the trial nor any of the bone samples (n = 34) from raccoons in the surveillance area were biomarker-positive.

KEYWORDS: Biomarker - Field release - Rabies - Raccoon - Recombinant vaccine - Vaccinia-rabies glycoprotein recombinant virus.

INTRODUCTION

Although the domestic dog continues to be the primary source of human rabies world-wide, wildlife rabies reservoirs such as the raccoon (Procyon lotor), red fox (Vulpes vulpes), skunk (Mephitis mephitis) and several species of insectivorous bat (Chiroptera) maintain the risk of transmission to humans in Europe and North America (31). Contrary to some mathematical models (2), European investigators have successfully demonstrated not only control but, in some cases, elimination of terrestrial wildlife rabies from rabies-endemic areas through oral immunization of the red fox (16, 25, 26, 29, 30). In the United States of America (USA), raccoon rabies has been present in epizootic proportions in the mid-Atlantic region for nearly fifteen years (8). Although the initial concept of oral rabies vaccination of wildlife originated in the USA (3), implementation has been delayed due to several factors, including the relative refractoriness of raccoons to oral immunization with traditional modified live rabies vaccines used for red fox vaccination (22) and the potential pathogenicity of these vaccines in some North American fauna (23). A vaccinia-rabies glycoprotein (V-RG)
recombinant virus vaccine developed nearly a decade ago (14, 31) was found to be orally efficacious in raccoons (18) and has since undergone extensive safety and efficacy evaluation in the laboratory (1, 4, 5, 6, 7, 19, 20, 21, 28, 33).

Following laboratory evaluation, a limited trial focusing on the field safety of V-RG virus was considered a logical prerequisite to widespread application in rabies epizootic areas in the USA. The study was conducted on Parramore Island, Virginia, due to the geographical isolation of the island and the relatively large, stable, and well-characterized raccoon population (11). The primary objective was to evaluate mammalian fauna at risk of contact with vaccine-laden baits, through extended live-trapping efforts, for evidence of adverse effects attributable to the vaccine.

Vaccine safety evaluations in this population ranged from evidence of overt field morbidity or mortality to screening for subtler potential adverse effects which may result, for example, in a decrease in recapture rate, mean body weight, etc. To properly assess vaccine safety, an estimate of the proportion of animals liable to come into contact with vaccine was critical, as well as identification of individuals which had actually contacted vaccine-laden baits. A biomarker is generally used to identify members of a free-ranging population contacting placebo or vaccine-laden baits. Tetracycline, which is incorporated in calciphilic hard tissue (bone and teeth) and usually evaluated post-mortem, is the most commonly used biomarker (13). The novel feature of this intensive vaccine field trial was that animals under study were readily live-captured, facilitating marking and release for further prospective study (most rabies vaccine field trials to date have relied on examination after harvesting). An ante-mortem biomarker was thus needed to identify animals for prospective, rather than retrospective study. For this purpose, sulfadimethoxine (SDM), a broad-spectrum antimicrobial (27), was employed as a short-term ante-mortem seromarker.

**MATERIALS AND METHODS**

**Laboratory trials**

A total dose of 250 mg or 500 mg per raccoon (approximately 35 mg/kg or 70 mg/kg respectively) of commercial SDM, used in the treatment of dog and cat gastrointestinal disorders, was orally administered via needle-less syringe to two groups of four raccoons under sedation. All raccoons were sedated and bled (11) on days 4, 7, 10 and 14 post-administration.

In a related experiment, two groups of ten raccoons each were offered approximately 50 ml of an attractant slurry containing 100 mg (15 mg/kg) or 250 mg (35 mg/kg) of SDM. The slurry consisted of equal parts of 20% sucrose, whole chicken eggs, vegetable oil and crushed shellfish. All raccoons from each group were sedated and bled daily for 10 days (11).

Sera were collected from clotted blood samples and evaluated for residual SDM with a commercially available rapid card test. The card test is a qualitative enzyme immunoassay for the detection of concentrations greater than 0.16 ppm of SDM in sera.

**Parramore Island field trial**

**Study site description**

Parramore Island (37°11'N, 75°38'W) is the largest (3,440 ha) and most biologically diverse barrier island off the eastern shore of Virginia (12). It is 12.8 km long,
1.2-2.0 km wide and 7.7 km from the mainland. The island is bounded on its eastern shore by the Atlantic Ocean and on its western edge by salt marsh ranging in width from 0.2-2.0 km, terminating against Swash Bay or broad tidal channels. On the bayside of the southern third of Parramore Island is Revel’s Island, which is separated from Parramore by a tidal gut less than 0.3 km wide and 2.0-4.0 m deep at mean low tide.

Mammalian species commonly found on Parramore include raccoon, red fox, white-tailed deer (*Odocoileus virginianus*), rice rat (*Oryzomys palustris*), meadow vole (*Microtus pennsylvanicus*), house mouse (*Mus musculus*) and Norwegian rat (*Rattus norvegicus*) (11).

**Vaccination and surveillance areas**

A roughly rectangular 300 ha (1.5 km × 2.0 km) study area was designated on the central upland forest region of the island. The vaccination area was the only part of the island where vaccine-laden baits were distributed. In addition, four major control areas of approximately 60 ha each were established. The surveillance sites (no vaccine distribution) on Parramore Island were approximately 1.0 km north of the vaccination area, and 1.5 km and 3.0 km south of the vaccination area; on Revel’s Island, surveillance was conducted approximately 7.0 km south-east of the Parramore Island vaccination area.

**Vaccine, bait and biomarker preparation**

Approximately 1.0 ml of the V-RG recombinant virus vaccine (10⁸ plaque-forming units/ml) was inserted into parrafin ampoules. The sealed ampoules were placed into fish-meal polymer cylinders (length 4.0 cm, diameter 2.8 cm) consisting of fish oil, fish meal, a synthetic polymer binder and 100 mg tetracycline hydrochloride (11). Fish-meal polymer plugs were inserted into both ends of the cylinder and sealed with paraffin wax. Immediately prior to field distribution, each vaccine-laden bait was placed in an individual polyethylene bag with a descriptive label. Approximately 50 ml of a slurry (previously described), was added to the bag to enhance bait attractiveness to raccoons and repugnance to potentially trespassing humans. SDM was included in the slurry at a dose of 250 mg per bait-package, as a second biomarker.

**Bait distribution**

On 20 August 1990, 3,000 vaccine-laden baits were hand-placed approximately 12-30 m apart on linear transects, to achieve a baiting density of roughly 10 baits/ha (1,000 baits/km²) (24).

**Live-trapping**

Live traps were placed in pairs at permanent stations 100 m apart on transects throughout the vaccination and surveillance areas. Live-trapped furbearers were sedated. In the course of handling, individually numbered ear-tags were applied, as previously described (11).

Small mammal live-trapping was conducted in parallel with tomahawk live-trapping (11).

**Biomarker analysis**

**Sulfadimethoxine**

As described for the laboratory trials, a rapid commercial card test was used to screen routinely-collected sera, within the first two weeks after bait distribution, for the presence of SDM as an indication of contact with the slurry surrounding the bait.
Bait acceptance was assessed by examination of mandibular bone samples collected from captured, sedated and subsequently euthanized animals, under a Leitz ultraviolet illumination microscope for tetracycline deposition (13). Additionally, bone samples were collected for tetracycline analysis from any carcasses found in the vaccination and surveillance areas.

**RESULTS**

**Laboratory trials**

In the preliminary study, all raccoons from both experimental groups were SDM-positive on day 4. In the 250 mg group, only one of four raccoons was still positive by day 7, and all four were negative by day 10. In the 500 mg group, all four were SDM-positive on days 7 and 10, but were SDM-negative by day 14.

In the second trial, all sera (n = 10 per group) collected on days 1 to 4 had detectable SDM residues. On days 5 and 6, 90% of the 250 mg group were SDM-positive, while the 100 mg group declined from 80% to 60% positive. By day 7, only 20% of raccoons offered 250 mg of SDM and none of the 100 mg group gave positive SDM card tests. All card tests on days 8 to 10 were negative.

**Parramore Island field trial**

**Biomarker analysis**

**Sulfadimethoxine**

On the first two days of the field trial, 19 of 20 raccoons captured in the vaccination area were SDM-positive. By day 4, 40 raccoons had been captured and 90% (36/40) were SDM-positive. The cumulative SDM results from raccoons captured for the first time during the first six days of the field trial were 77.5% positive (38 of 49 sera). The frequency of positives was slightly higher (79%, or 42 of 53 sera) when positive samples from recaptured animals already known to be SDM-positive were included in the cumulative six-day rate. Thereafter, only four additional serum samples from 110 (3.6%) examined from the vaccination area on days 6-14 of the field trial were found to be positive for SDM. All sera collected from raccoons in the surveillance areas during the first six days of the field trial (n = 12) were negative for SDM. During the first two weeks of the trial, none of 32 raccoon nor 4 red fox sera collected from surveillance areas were positive for SDM.

**Tetracycline**

From 31 August 1991 onwards, 47 of 56 (83.9%) raccoons collected from the vaccination area were tetracycline-positive. Conversely, all 34 raccoons from the surveillance areas were tetracycline-negative. Of the 47 positive raccoons from the vaccination area, six were live-trapped during the first six days of the field trial and known to be SDM-positive.

**DISCUSSION**

In the field safety evaluation of a vaccine offered *ad libitum* to wildlife, two factors weigh heavily on follow-up field data:
the relative ease of obtaining live-trapped individuals for examination and
recapture over time

the ability to identify those animals liable to come into contact with vaccines.

Based on a preliminary study on Parramore Island (11), raccoons were known to be
relatively abundant and easily live-trapped. To identify the proportion of raccoons
liable to come into contact with vaccines in the vaccination area, two biomarkers were
used. As in previous placebo baiting trials, tetracycline assayed in post-mortem bone
samples was sufficient to determine the proportion of free-ranging animals consuming
baits. However, in order to assess vaccine safety over time, an ante-mortem biomarker
was desirable so that biomarker status could be determined in animals remaining alive
for further study in the field.

Biomarkers may act as physical markers, seromarkers and tissue markers. An ideal
ante-mortem biomarker would be reliable, inexpensive and readily assessed by non-
invasive techniques, as well as having a low likelihood of pre-existing background levels in
the population under study. Although relatively inexpensive and easily assessed, rhodamine B (15, 17) was demonstrated to be unreliable in raccoons either as a systemic
marker of vibrissae or as an external marker, due to variable duration of skin and fur
discoloration (9, 11). Conversely, iophenoxic acid appears to be a promising, relatively
long-lived (6-12 weeks) seromarker (10), but the expense of analysis is prohibitive
(> 20 US$ per sample) for most wildlife studies. In contrast, one of the major advantages
of SDM is that a card test is commercially available and suitable for field application. A
distinct disadvantage of SDM is its relatively short duration; in the laboratory, only
20-25% of raccoons receiving 250 mg still had positive card tests by day 7. Similar results
were observed during the Parramore Island field trial, with the median number of
positives (n = 23) occurring by day 3 and 93% (43 of 46) of all positives detected by day 7.
Nevertheless, SDM status provided an immediate, daily assessment of bait contact during
the first critical week of the Parramore Island field trial with V-RG vaccine and correlated
well with tetracycline results from animals collected throughout the year-long study.

Despite greater than 90% bait disturbance rates by day 5 (24) and high bait contact
rates of approximately 80% based on SDM and tetracycline, no gross lesions compatible
with an Orthopoxvirus aetiology were observed in 887 live-trapped raccoons and nearly
600 live-trapped small mammals, nor were there any gross or histopathological lesions
suggestive of Orthopoxvirus infection in 54 necropsied raccoons (24). Moreover, there
were no differences between biomarker-positive raccoons, vaccination area resident
raccoons and surveillance/control populations with regard to trap success, recapture
rates, age and sex distributions and mean body weight (12).

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Résumé : Lors de l'évaluation sur le terrain de l'innocuité d'un vaccin destiné à la faune sauvage, préparé à partir d'un virus recombinant Vaccine-glycoprotéine rabique, deux marqueurs biologiques ont été employés pour identifier les contacts éventuels avec les appâts contenant le vaccin. La tétracycline, marqueur tissulaire calciphile fiable et couramment utilisé, a été incluse dans une matrice préparée avec un polymère et une farine de poisson, puis recherchée dans les prélèvements osseux obtenus à l'autopsie. Un marqueur ante-mortem a par ailleurs été nécessaire pour identifier, lors des études prospectives, les rats laveurs qui avaient été en contact avec les appâts et, éventuellement, ingéré le vaccin. De la sulfadaméthoxine (SDM), nouveau marqueur sérique de courte durée, a été incluse dans un mélange attractif, disposé près de l'appât. Les études de laboratoire préliminaires chez des rats laveurs ont montré la présence de résidus de SDM pendant une semaine après l'ingestion d'une dose unique de 250 mg. Au cours des six premiers jours suivant la mise en place des appâts, 49 rats laveurs ont été capturés vivants dans la zone de vaccination et la SDM a été décelée dans 38 des 49 échantillons sériques (77,5 %). De même, sur 56 prélèvements osseux de rats laveurs provenant de la zone de vaccination au cours des 12 mois de l'étude, 47 (83,9 %) contenaient de la tétracycline. Inversement, aucun des échantillons sériques analysés lors des six premiers jours de l'essai (n = 12) ni aucun prélèvement osseux provenant de la zone de surveillance (n = 34) n'ont donné de résultat positif.


Resumen: Durante la evaluación en el terreno de la inocuidad de una vacuna destinada a la fauna salvaje, preparada a partir de un virus recombinante vaccinia glucoproteína rábica, se usaron dos marcadores biológicos para identificar posibles contactos con cebos que contenían la vacuna. La tetraciclina, marcador tisular calcifilo fiable y usado corrientemente, se incluyó en una matriz preparada con un polímero y harina de pescado y luego se buscó en las muestras óseas obtenidas en la autopsia. Fue necesario por otra parte un marcador ante-mortem para identificar, en los estudios prospectivos, los mapaches que habían estado en contacto con los cebos e ingerido eventualmente la vacuna. Se incluyó sulfadimetoxina (SDM), un nuevo marcador sérico de corta duración, en una mezcla atractiva dispuesta cerca del cebo. Los estudios de laboratorio preliminares en mapaches mostraron la presencia de residuos de SDM durante una semana después de la ingestión de una dosis única de 250 mg. En los seis primeros días siguientes al depósito de
los cebos, se capturaron vivos 49 mapaches en la zona de vacunación y se descubrió la SDM en 38 de las 49 muestras de suero (77,5%). Asimismo, de 56 muestras óseas de mapaches provenientes de la zona de vacunación durante los 12 meses de la investigación, 47 (83,9%) contenían tetraciclina. Por el contrario, ninguna de las muestras de suero analizadas en los seis primeros días del ensayo (n=12) ni ninguna muestra ósea proveniente de la zona de vigilancia (n=34) dieron resultados positivos.


REFERENCES


