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Recombinant rabies vaccines: efficacy assessment in free-ranging animals

C.E. RUPPRECHT[1,2], C.A. HANLON[2], M. NIEZGODA[2], J.R. BUCHANAN[2], D. DIEHL[2] and H. KOPROWSKI[2]

ABSTRACT

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With the advancement of recombinant DNA techniques, a number of potent biologicals are available for the oral vaccination of free-ranging animals. Once oral immunogenicity and vaccine safety have been demonstrated, efficacy then becomes of paramount importance. Classical assessment of efficacy is conducted under carefully controlled laboratory conditions, whereas efficacy of oral wildlife rabies vaccination programs, to date, have been assessed by the lack (or occurrence) of field cases of rabies in a vaccinated area. This communication describes an intermediate vaccine efficacy strategy in which self-vaccinated, free-ranging animals from a study site were captured seven months after vaccine-laden bait distribution for laboratory rabies challenge. This technique is specifically reviewed in the context of available recombinant products for the consideration of extension towards dog rabies control.

INTRODUCTION

Since its inception several decades ago, significant theoretical and applied progress has been made in the concept of oral vaccination against rabies, both in the laboratory and field. Several combined vaccine and bait strategies, currently at initial stages, are ready to be seriously extended towards considerations of implementation for vaccination of free-ranging dogs. Historical reservations over vaccine safety, specifically concerning viral latency, establishment of new reservoir hosts or other unforeseen concerns, have not been substantiated. Earlier, first generation modified-live vaccines, based upon a few viral prototypes, are gradually being replaced by apathogenic or recombinant rabies vaccines, with either a reduction or inability to cause vaccine-induced rabies. In the evaluation of a rabies biological, vaccine efficacy, rather than mere immunogenicity, generally involves severe challenge of a primary host animal under laboratory conditions with a relevant strain, dose, and route of street rabies virus, in which a significant number of vaccinates survive compared to control animals. Without initial oral efficacy results, it is imprudent to embark seriously into laborious, expensive and time-consuming animal safety models, especially that encompass perceived, rather than apparent risks. At present, there are a number of promising vaccine candidates in various stages of laboratory and field testing, for both safety and efficacy in primary target

[1] Centres for Disease Control, Division of Viral and Rickettsial Diseases, Viral and Rickettsial Zoonoses Branch, 1600 Clifton Road, Atlanta, Georgia 30333, United States of America

[2] Thomas Jefferson University, Centre for Neurovirology, Department of Microbiology and Immunology, 1020 Locust Street, Phila, PA 19107, United States of America

and notable non-target host species, principally wildlife. The objectives of this communication are:

- to evaluate the efficacy of a vaccinia-rabies vaccine (Rupprecht, Wiktor, Johnston, Hamir, Dietzschold, Wunner, Glickman & Koprowski 1986) con tained within a bait, for an important free-ranging species, the raccoon (*Procyon lotor*); and
- to briefly review the currently available recombinant rabies biologicals as they relate to potential suitability for the oral vaccination of domestic or feral dogs.

METHODS AND MATERIALS

Study site description

The first North American release of a vaccinia-rabies glycoprotein (V-RG) recombinant vaccine occurred on Parramore Island, USA (37°11'N, 75°38'W), the largest (3 440 ha) and most biologically diverse barrier island off the eastern shore of Virginia, USA, during August 1990. More specific details have been previously communicated (Hanlon, Hayes, Hamir, Snyder, Jenkins, Hable & Rupprecht 1989; Hanlon, Buchanan, Nelson, Niu, Diehl & Rupprecht 1993). Briefly, Parramore Island is 12,8 km long, 1,2–2,0 km wide, and 7,7 km from the mainland. On the bayside of the southern third of Parramore Island is Revel's Island, separated from Parramore by a tidal gut less than 0,3 km wide and 2,0–4,0 m deep at mean low tide.

Vaccination and surveillance areas

A roughly rectangular (1,5 km x 2,0 km) study area was designated on the central upland forest region of the island. This 300 ha 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; three on distant areas of Parramore Island and a surveillance site (no vaccine distribution) on upland sections of Revel's Island.

Vaccine, bait, and biomarkers

Approximately 1,0 mℓ of the V-RG recombinant virus vaccine (10^8 pfu/mℓ) was inserted into parrafin ampules placed into fishmeal polymer, cylindrical, baits with 100 mg of tetracycline, as a calciphillic biomarker, as described (Hanlon *et al.* 1989). Immediately prior to field distribution, each vaccine-laden bait was placed in an individual polyethylene bag carrying a descriptive label. Approximately 50 mℓ of a shellfish slurry was added to the bag to enhance bait attractiveness to raccoons. In addition to tetracycline in the bait matrix, a commercial formulation of sulfadimethoxine (SDM), used in the treatment of dog/cat gastrointestinal disorders, was included in the slurry at a dose of 250 mg, as a second biomarker, for serological detection, as described (Hanlon *et al.* 1993). Three-thousand vaccine-laden baits were hand-placed approximately 12–30 m apart on linear transects to achieve a baiting density of 10 baits/ha.

Live-trapping

Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, Wisconsin 54487, USA) were placed in pairs at permanent stations 100 m apart on transects throughout the vaccination and surveillance areas. Traps were baited, were set for four continuous nights and were checked daily, shortly after sunrise. Live-trapped furbearers were sedated with a mixture of 10 mg/kg ketamine (Veterinary Products, Bristol Laboratories, Division of Bristol-Meyers Co., Syracuse, New York 13220, USA) and 0,4 mg/kg xylazine (Haver, Bayvet Division, Miles Laboratory, Inc., Shawnee, Kansas 66203, USA) administered intramuscularly. After recording the sex, age, and mass, the animals were ear-tagged (National Band and Tag Co., 721 York St., Newport, Kentucky 41072, USA), blood samples were collected, as described below, and the animals were released.

Biomarker analysis

SDM

A rapid commercial card test (Environmental Diagnostics, Inc., Burlington, N.C. 27215, USA) was used to screen routinely-collected large mammal sera within the first two weeks of baiting for the presence of SDM, as an indication of bait contact.

Tetracycline

Bait acceptance was also assessed by examination of raccoon mandibular bone samples (post-mortem samples), viewed under a Leitz ultraviolet illumination microscope for tetracycline deposition within cementum and dentine.

Rabies antibody determination

Blood samples were collected from live-trapped raccoons while they were sedated for physical examination and ear-tagging. Serum samples were removed from clotted blood and were frozen at −20 °C for subsequent determination of rabies virus-neutralizing antibody (VNA) titers, by a modification of the rapid fluorescent focus inhibition test (Reagan, Wunner, Wiktor & Koprowski 1983). Rabies VNA titers were expressed as the reciprocal of the highest dilution that caused a 50 % reduction in the number of rabies infected cells, converted into international units (IU/mℓ) by comparison to US Standard Rabies Immune Globulin (Office of Biologics Research and Review, FDA, Bethesda, MD 20205, USA) reference sera (lot R-3) as standard.

Rabies laboratory challenge

Salivary glands were obtained from a naturally infected rabies-positive raccoon from Pennsylvania; these were collected and a pooled salivary gland suspension was prepared. A titration of the pooled salivary gland suspension was made by intracerebral inoculation of 4-week-old ICR mice yielding a concentration of $10^{4,5}$ $MICLD_{50}/m\ell$ of street rabies virus.

Six months after initial bait distribution, raccoons were retrapped upon Parramore and Revel's Islands for transport to the laboratory. Captured raccoons were housed in individual stainless steel squeeze cages and given commercial dry cat food (Purina Cat Chow, etc.) and water *ad libitum*. Animals were acclimated to holding conditions for a minimum of one month prior to inoculation.

At seven months after original bait distribution on Parramore Island, the raccoons were inoculated with street rabies virus in the right masseter muscle and observed daily for clinical signs of rabies for three months following challenge. Upon the first clinical signs of rabies, raccoons were sedated and euthanized by intravenous administration of Beuthanasia-D Special (Schering Corporation, Kenilworth, NJ 07033, USA). Brainstem samples were collected for rabies diagnosis by the fluorescent antibody test. Survivors were similarly treated until euthanasia at 90 days post-challenge.

RESULTS

Twenty-nine raccoons were live-trapped for the efficacy experiment: 18 from Parramore and 11 (controls) from Revel's Island. Parramore raccoons had been handled between one and 27 times in the previous six months; control raccoons were captured on one to eight different occasions. Six of seven Parramore raccoons available for sampling during the first week of the field trial, were positive for the serum marker SDM. All 11 control raccoons were negative for rabies VNA; of the Parramore raccoons, seven had rabies VNA > 0,5 IU/mℓ (range 0,6–54,0 IU/mℓ) on the day of laboratory challenge (Table 1). Ten of the 11 control raccoons succumbed within 30 days of experimental rabies virus challenge. In comparison, 14 of the 18 Parramore raccoons survived; of the four that succumbed, only one was negative for the tetracycline biomarker post-mortem, whereas all other Parramore raccoons in the experiment were tetracycline positive, including the single SDM-negative raccoon. All animals that succumbed had immunofluorescent inclusions indicative of rabies infection, while all surviving raccoons remained healthy and were negative for rabies virus immunofluorescence at necropsy.

TABLE 1 Experimental rabies challenge of Parramore Island raccoons[a]

Animal #	Sex	Times captured	SDM[b]	Tetracycline	VNA[c]	R[d]
186	M	1	ND[e]	+	0,60	S[f]
222	F	7	+	+	1,20	S
6759	M	3	ND	+	6,00	S
6807	M	3	ND	+	0,20	S
8040	M	8	ND	+	0,07	S
8109	F	5	+	+	3,50	S
8156	M	27	+	+	0,10	S
8317	M	5	–	+	0,20	S
8428	M	2	ND	+	0,70	S
8703	M	5	ND	+	54,00	S
7236	M	1	ND	+	1,10	S
6503	M	2	ND	+	0,20	S
8874	M	3	ND	+	0,20	S
8044	F	2	ND	+	0,10	S
464	F	4	+	+	0,07	D
8303	F	9	+	+	0,20	D
8219	F	8	+	+	0,07	D
8812	M	16	–	–	0,07	D

[a] All control raccoons originated from Revel's Island, had been captured between one and eight times, were biomarker and antibody negative. Of 11 controls, ten succumbed to experimental raccoon rabies virus inoculum (0,5 mℓ intramasseter, $10^{4,5}$ $MICLD_{50}/m\ell$)

[b] SDM = sulfadimethoxine

[c] Rabies virus neutralizing antibody (VNA), expressed in IU/mℓ, on the day of challenge

[d] R = Response

[e] ND = Not done, either because serum was not available or it was sampled after day 10

[f] S = survived; D = died

DISCUSSION

The efficacy of oral rabies vaccine has usually been measured in one of two different ways: either directly, by the challenge of appropriate mammalian hosts given vaccine in the laboratory, or indirectly by surveillance for naturally occurring rabies in the vector populations following field distribution of vaccine-laden baits. The experiment reported herein is intermediate to the above two, in that animals were allowed free-choice access to baits under natural conditions until the time of capture and laboratory challenge. When feasible, this method should be a more reliable indicator of vaccine efficacy because animals in the field may consume baits and respond to vaccine differently than in the laboratory environment. A potential drawback to this proposal is the lack of precise control of the actual delivered vaccine dose for minimum efficacy, but this can be alleviated by various combinations of efficacy testing. Although the number, and temporal/spatial distribution, of baits should reflect relative population density, home range and habitat preferences of the intended hosts, there

is little other control over the multiple variables affecting the degree of bait ingestion. Animals may contact none, partially consume one, or eat multiple baits. Bait biomarkers and seroconversion can serve as crude indicators for presumed vaccine exposure.

Only one (# 8812) of 18 Parramore raccoons in the present report lacked evidence of either bait consumption or vaccine contact. The three tetracycline-positive, antibody-negative raccoons that succumbed apparently consumed sufficient portions of the biomarker-laden portion of the bait, in lieu of significant contact with vaccine in the interior chamber. Conversely, the SDM marker, while somewhat useful as a measure of bait contact, was indicative of contact with only the outermost, superficial, portion of the bait-attractant-slurry complex. Moreover, a SDM-negative raccoon may have consumed the bait and vaccine, in lieu of a sufficient amount of the SDM-containing slurry for detection (e.g. animal # 8317). Of the 17 biomarker-positive animals, 14 (82 %) survived a severe raccoon rabies virus challenge in which 91 % of control animals succumbed. None of these three biomarker-positive raccoons that succumbed had detectable rabies VNA at the time of challenge. However, absolute VNA titer alone may not necessarily be indicative of protection, because at least seven of the surviving raccoons had equivalent or lower VNA titers than those which succumbed.

Likewise, there is no minimum VNA titer which is "protective" *per se*, that is, predictive with certainty whether an animal will assuredly survive single or multiple rabies virus exposures. Rather, an anamnestic VNA response may be a better predictor of adequate immune status (Rupprecht & Dietzschold 1987); of the 14 surviving Parramore raccoons, all had a greater than four-fold rise in VNA titer within seven days of virus challenge, whereas none of the four others nor the control raccoons responded in kind (data not shown). Clearly, similar approaches may yield better approximations of overall efficacy for the intended target species, utilizing different vaccines, regimens, etc., bearing in mind that any vaccination campaign may not succeed simply on the basis of a highly potent, safe, inexpensive, and efficacious vaccine alone, but rather on a number of related program parameters.

The V-RG vaccine, an orthopoxvirus, was the first recombinant rabies virus constructed, having the ERA rabies strain glycoprotein gene inserted into the TK region of the vaccinia viral genome (Kieny, Lathe, Drillien, Spehner, Skory, Schmitt, Wiktor, Koprowski & Lecocq 1984). When inoculated parenterally (Wiktor, MacFarlan, Reagan, Dietzschold, Curtis, Wunner, Kieny, Lathe, Lecocq, Mackett, Moss & Koprowski 1984) or orally (Wiktor, MacFarlan, Dietzschold, Rupprecht & Wunner 1985), the V-RG vaccine led to the rapid induction of rabies VNA and protected animals against severe rabies challenge. Laboratory efficacy of the V-RG vaccine *per os* has been demonstrated for multiple reservoir hosts, including raccoons (Rupprecht, Hamir, Johnston & Koprowski 1988), foxes (Blancou, Kieny, Lathe, Lecocq, Pastoret, Soulebot & Desmettre 1986), skunks (Tolson, Charlton, Stewart, Campbell & Wiktor 1987) and dogs (Chappuis, in press). The safety of the V-RG vaccine was also shown for an extensive array of non-target species (Rupprecht & Kieny 1988; Rupprecht, Hanlon, Hamir & Koprowski 1992a; Rupprecht, Hanlon, Cummins & Koprowski 1992b), including primates. Since 1987, millions of doses of V-RG vaccine have been used safely to control fox rabies in Europe, including Belgium (Brochier, Kieny, Costy, Coppens, Bauduin, Lecocq, Languet, Chappuis, Desmettre, Blancou & Artois 1991) and portions of France (M. Aubert 1993, personal communication), demonstrating efficacy under field conditions by the elimination of enzootic fox rabies from large contiguous areas.

Additional vaccinia-rabies recombinants, utilizing different sources of parental vaccinia virus, rabies cDNA, viral promoters, etc., have also been produced (Esposito, Brechling, Baer & Moss 1987). Novel improvements in safety have included the deletion of virulence and host range gene segments (Tartaglia, Perkus, Taylor, Norton, Audonnet, Cox, Davis, Van Der Hoeven, Meignier, Riviere, Languet & Paoletti 1992) without apparent loss of potency by parenteral administration, but the efficacy of these new preparations by the oral route has not been thoroughly investigated.

Another orthopoxvirus, raccoon poxvirus, genetically distinct from vaccinia virus, has also been used as a vector for lyssavirus genes (Esposito, Knight, Shaddock, Novembre & Baer 1988; Fekadu, Shaddock, Sumner, Sanderlin, Knight, Esposito & Baer 1991; Esposito, Sumner, Brown, Ebert, Shaddock, He, Dobbins & Fekadu 1992). When administered orally, this recombinant has also been shown to protect a number of carnivores, including dogs, against lethal rabies virus challenge. No field trials have been reported to date.

Alternatively, avipoxviruses represent novel vectors for viral genes because they are replication-incompetent in mammalian cells, but can express foreign genes and stimulate protective immunity to rabies (Taylor, Weinberg, Languet, Desmettre & Paoletti 1988; Taylor, Trimarchi, Weinberg, Languet, Guillemin, Desmettre & Paoletti 1991; Cadoz, Strady, Meignier, Taylor, Tartaglia, Paoletti & Plotkin 1992). Unfortunately, appropriate oral efficacy studies for rabies have not been published; it is unlikely that the current generation of avian poxviruses will be effective when administered via baits suitable for free-ranging animals, but this line of investigation should be pursued, considering the advantage that avipoxviruses

represent with regard to relative risk associated with environmental release.

Besides the Poxviridae, the only other recombinant viruses that have been communicated as being tested specifically as live oral immunogens against rabies for mammalian carnivores belong to the Adenoviridae. A human adenovirus type 5 (HAV5) rabies recombinant has been reported (Prevec, Campbell, Christie, Belbeck & Graham 1990) that appears safe, thermostable, and efficacious under laboratory conditions, with abortive replication *in vivo*. Relevant species evaluated include raccoons, skunks, and dogs, among others (Charlton, Artois, Prevec, Campbell, Casey, Wandeler & Armstrong 1992). Utility of extending the HAV concept to canine adenoviruses has been discussed (Sumner, Shaddock, Wu & Baer 1988; Baer, Brooks & Foggin 1989), although no candidate recombinant microorganisms are entirely exempt from practical environmental assessment (Hamir, Raju & Rupprecht 1992). In addition, the widespread distribution of carnivore adenoviruses (as opposed to orthopoxviruses) may present certain problems related to pre-existing host immunity, especially in canids.

Seeming concentration upon the overall safety of rabies vaccines is due in part to the acknowledgment of the threat of improper biological use in human, domestic animal or wildlife hosts having altered immunocompetence. In each specific case, this degree of compromise is usually relative and rarely absolute. The particular causation for such insults is frequently multiple. For some of these conditions, all affected hosts may be severely threatened, whereas in others, the disease patho-physiology and staging will determine the outcome. Examples of severe immunocompromise include neoplasia (e.g. lymphoma, leukemia, generalized malignancy, etc.) therapeutic or environmental exposures (e.g. radiation, anti-metabolites, alkylating agents, etc.) and congenital factors, such as inherited severe combined immunodeficiency (SCID) syndromes. Intermediate to severe events may be iatrogenic (e.g. corticosteroid imbalance, wildlife capture stress, etc.), infectious (e.g. HIV, FIV, FELV, CDV, ICH, etc.) or be limited temporally to periods of systemic illness (e.g. renal disease, cirrhosis, diabetes, etc.). With the focus on rabies control via oral vaccines in developing countries, and with the recognition that many systemic insults may be synergistic in nature (e.g. overt parasitism, marginal nutritional balance, etc.), the use of self-replicating viral vaccines should be carefully weighed when the opportunity of unintentional exposure to severely immunocompromised human or animal populations is deemed high. Under such conditions, the chances for the vaccinal agent to pose certain safety risks may be increased, due to the theoretical possibility of alterations in tropism, replication enhancement or other untoward events related to pathogenesis. Inactivated vaccines do not generally represent a danger to the immunocompromised patient (Rupprecht, Dietzschold, Campbell, Charlton & Koprowski 1992c). Higher vaccine doses or more frequent booster regimens may be required to avoid suboptimal responses, again depending upon the nature of the immunocompromization. It is conceivable that inactivated vaccines may eventually replace live biologicals as the development of new expression systems, such as the baculovirus system (Fu, Rupprecht, Dietzschold, Saikumar, Niu, Babka, Wunner & Koprowski 1994), are maximized.

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