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## Serological Responses of Coyotes to Two Commercial Rabies Vaccines

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**ABSTRACT:** Between August 1993 and September 1994 we documented serological responses of coyotes (*Canis latrans*) vaccinated with two commercial rabies vaccines licensed for use in domestic dogs. Serologic responses were documented by testing for rabies virus neutralizing antibodies with the rapid fluorescent focus inhibition test (RFFIT) at 30, 90, 180, 270, and 365 days post-vaccination. All coyotes vaccinated with Imrab 3® (Rhone-Merieux, Inc.), and 75% of those vaccinated with Dura-Rab 3® (Immunovet, Inc.) seroconverted, as evidenced by the presence of antirabies antibody titers  $\geq 1:5$  in one or more of the five post-vaccination samples. The percent of coyotes showing a titer  $\geq 1:5$  was generally greater and titer levels appeared higher and more persistent among animals vaccinated with Imrab 3® than Dura-Rab 3®. Presence of titers via RFFIT tests demonstrates the antibodies produced in coyotes by these rabies vaccines functionally bind and neutralize rabies virus in vitro, but these results do not constitute a demonstration of protection required for licensure for use in coyotes.

**Key words:** *Canis latrans*, coyote, immunization, rabies, vaccination.

Rabies, an infectious disease of mammals, is typically fatal once clinical symptoms are evident. Consequently, disease management concentrates on limiting exposure and immunization through vaccination (Bunn, 1991). Currently, only killed virus rabies vaccines, which are generally less immunogenic than modified live vaccines, are licensed for use in the United States. Some manufacturers incorporate adjuvants (compounds that increase the antigenicity) into vaccines to provide higher and more sustained titers (Tizard, 1996; pp. 275–277).

Susceptibility and immunologic response to rabies virus differs among species (Dreesen, 1999). As a result, rabies vaccines undergo stringent testing for ef-

ficacy and safety in each species for which they are licensed (Anonymous, 1992). Because efficacy of rabies vaccines is determined only through costly live virus challenge tests, vaccine manufacturers concentrate licensure efforts on species for which vaccination offers potential economic return (i.e., domestic pets and farm animals). Despite lack of licensed rabies vaccines for most wildlife species, many zoos, wildlife parks, and research institutions routinely vaccinate wildlife against rabies in the hope of conferring some protection for captive animals and personnel (Jenkins et al., 2001), but not in lieu of “appropriate public health activities that protect humans.”

The Logan Field Station of the National Wildlife Research Center (Logan, Utah, USA) maintains a colony of captive coyotes (*Canis latrans*) for research purposes. Health concerns for animal and personnel dictate a schedule of vaccination against common canine and zoonotic pathogens, including rabies. Coyotes in the colony receive an annual vaccination with a commercial rabies vaccine approved for use in domestic dogs with the understanding the vaccine is not licensed for use in coyotes and cannot be considered legally protective in this species. Live virus challenge studies are not within the purview of the Logan facility, but we were able to evaluate the induction and persistence of rabies virus neutralizing antibodies among captive coyotes over a 1 yr period related to use of two commercial rabies vaccines licensed for use in domestic dogs.

We started the study in August 1993 with 58 hand-reared coyotes (25 females and 33 males) about 4 mo of age from the

captive colony. They were penned in groups of three until 8-mo-old; thereafter they were housed in individual kennels or as male-female pairs in 0.1-ha enclosures. They were maintained throughout the study on a food ration prepared for the local fur industry (Furbreeders Agricultural Cooperative, Logan, Utah) and had access to water *ad libitum*. Upon completion of the study in September 1994, coyotes were returned to the colony.

We evaluated two commercial killed-rabies vaccines. One, Dura-Rab 3® (Immuno Vet, Inc., Tampa, Florida, USA; U.S. Veterinary License #302A, Serial #379, Expiration 24Jan94), was a non-adjuvanted vaccine licensed for intramuscular administration. The other, Imrab 3® (Rhone-Merieux, Inc., Athens, Georgia, USA; U.S. Veterinary License #298, Serial #12116, Expiration 10Feb96), was an adjuvanted (aluminium hydroxide) vaccine licensed for intramuscular or subcutaneous administration. Vendors donating the vaccines did not provide potency information for their respective products, although they were aware of our study intentions.

At the start of the study, coyotes were stratified by genetic background (i.e., litter) and sex and then randomly assigned to one of three treatment groups: (1) intramuscular injection of Dura-Rab 3®; (2) intramuscular injection of Imrab 3®; and (3) subcutaneous injection of Imrab 3®. They were serologically tested via rapid fluorescent focus inhibition test (RFFIT) to ensure they were negative for rabies virus neutralizing antibody. Vaccines were then administered as per manufacturers label instructions using sterile 3 ml syringes and 22 gauge  $\times$  2.5 cm needles. Intramuscular vaccination was by deep injection at a single location in caudal thigh musculature, while subcutaneous vaccination involved injection in the intrascapular region. Sera collections were conducted prior to treatment and on days 30, 90, 180, 270 and 365 post-vaccination. Coyotes were physically or chemically (i.e., intramuscular injection of 100 mg ketamine hy-

drochloride and 1 mg acepromazine maleate) restrained and blood (7 ml/animal) obtained from the cephalic vein of each subject using evacuated collection tubes. Tubes were allowed to stand for 1 to 4 hr at room temperature prior to centrifugation and aspiration of the sera. Sera samples were aliquotted into 2-ml micro-centrifuge tubes and maintained at  $-70^{\circ}\text{C}$  for 1 to 3 days prior to shipment. Samples were shipped overnight on dry ice in insulated containers to Kansas State University (Manhattan, Kansas, USA) where they were stored at  $-70^{\circ}\text{C}$  until analyzed. Rabies virus antibody titers were determined at the Veterinary Diagnostic Laboratory (Kansas State University) via RFFIT. We used analysis of variance (ANOVA) to compare overall performance of the two vaccines ( $P \leq 0.05$ ) followed by a post hoc use of Fisher's least significant difference (LSD) multiple comparison test to assess which individual comparisons were different ( $P \leq 0.05$ ). Fourteen wk after this study concluded, many of these coyotes were incorporated into another study involving an oral rabies vaccine. Anamnestic responses to re-exposure to rabies antigen in that study provided some additional information relevant to our study.

Serologic testing prior to vaccinations revealed titers of  $<1:5$  (considered negative) for all study coyotes (Table 1). Overall, 53 of the 58 coyotes (91%) are known to have seroconverted, as demonstrated by rabies specific titers  $>1:5$  via RFFIT at least once among the five sampling periods during the ensuing year (Table 1). Three additional animals, for which rabies antibodies were not detected, showed an anamnestic response to an oral rabies vaccine after this study was concluded, suggesting memory immune cells to rabies antigen were present (Van Kampen, 1999).

At 30 days post-vaccination, all 39 coyotes receiving Imrab 3®, regardless of route of administration, and 13/19 (68%) of coyotes receiving Dura-Rab 3® showed positive titers (Table 1). Absence of detectable rabies antibody in 6 coyotes in the

latter treatment group on day 30 could be related to: (1) improper administration of vaccine; (2) use of an impotent vaccine; (3) failure of the immune system; or (4) failure to detect an early immunologic response in conjunction with a rapid dissipation of antibodies. Improper vaccine administration seems unlikely since all vaccinations were given by the same individual via standard protocols, with all detection failures occurring in the same treatment. An impotent vaccine seems unlikely since it came from a common lot and was maintained under appropriate and identical storage conditions. Failure of the immune system to function (poor responders) in these individuals is possible, although seemingly unlikely. Extrinsic factors relating to antigen quantity, quality, and presentation of the vaccine could also contribute to immunologic failure but this seems unlikely.

The six coyotes in the Dura-Rab 3® treatment that were not seropositive on day 30 post-vaccination may have experienced a rise and fall in anti-rabies antibody prior to day 30. If this occurred, the immunologic response may have been sub-optimal and it is conjectural whether these animals were adequately protected in the event of subsequent exposure to rabies virus. It should be noted that four of the six gave other indications of seroconverting; one that did not have titers on days 30, 90, or 180, was seropositive on days 270 and 365, and three of four used in the oral rabies protocol after this study concluded, showed an anamnestic response, suggesting memory immune cells to rabies antigen were present. At 365 days post-vaccination, 20 of 20 (100%) coyotes receiving Imrab 3® intramuscularly, 16 of 19 (84%) coyotes receiving Imrab 3® subcutaneously, and eight of 19 (42%) coyotes receiving Dura-Rab 3® intramuscularly were seropositive for anti-rabies antibody (Table 1).

Overall, ANOVA revealed a significant difference in mean titers among the three treatments ( $F = 11.64$ ,  $df = 55$ ,  $P < 0.001$ ). Post hoc use of Fisher's LSD test

suggests titers associated with intramuscular injections of Imrab 3® were consistently higher than Durab-3® injected similarly. Initially, differences among the treatments were substantive, but they waned with advancing time (Table 1). A decrease in circulating antibody can be expected as the protective function of the immune system shifts from production of circulating antibody to reliance on memory immune cells to combat exposures to rabies antigen. Superficially, coyotes receiving Imrab 3® appeared to maintain higher and more persistent titers throughout the study (Table 1). This likely reflects presence of an adjuvant in Imrab 3®, (Tizard, 1996) but might also relate to other aspects of vaccine composition.

Although differences were not statistically significant ( $P > 0.05$ ), coyotes vaccinated with Imrab 3® intramuscularly appeared to have higher titers compared to those vaccinated subcutaneously (Table 1). Similarly, while males seemed to have (1) a greater percent of subjects with rabies antibody titers; (2) higher average titers; and (3) the highest individual titers (Table 1) compared to females, differences were not significant ( $P > 0.05$ ).

Maintenance of high, persistent titers may provide a measure of protective assurance, but does not necessarily identify vaccines as superior products because protection against disease also includes the ability to produce memory immune cells (Artois et al., 1993). These could result in anamnestic responses, as evidenced here by 4 animals that showed titers  $<1:5$  (considered negative) during one sampling but had substantial titers during a subsequent sampling, and at least eight animals that more than doubled antibody levels between post-vaccination samples. In addition, three animals for which we never detected a titer showed an anamnestic response following exposure to an oral rabies vaccine after this study was over. Although some animals may be protected at low or non-detectable titer levels, Bunn et al. (1984) showed a correlation between high-

TABLE 1. Percent seropositive, mean titers, and standard errors (SE) of coyote sera at five sampling periods after being vaccinated with rabies vaccines licensed for use in domestic dogs.

Days post-vaccination (Initial <i>n</i> )	Dura-Rab 3® (intramuscular) <sup>a</sup>			Inmrab 3® (intramuscular) <sup>b</sup>			Inmrab 3® (subcutaneous) <sup>b</sup>		
	Females (8)	Males (11)	All (19)	Females (9)	Males (11)	All (20)	Females (8)	Males (11)	All (19)
% seropositive:									
Pre-vaccination	None	None	None	None	None	None	None	None	None
30	38	91	68	100	100	100	100	100	100
90	25	36	32	100	100	100	100	82	84
180	13	45	32	100	100	100	100	82	84
270	38	45	42	100	100	100	100	82	84
365	38	45	42	100	100	100	100	82	84
Mean titer ± SE <sup>c</sup>									
Pre-vaccination	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
30	93 ± 44	108 ± 26	104 ± 21	697 ± 122	755 ± 218	729 ± 129	216 ± 45	591 ± 149	433 ± 97
90	31 ± 6	88 ± 28	69 ± 21	243 ± 57	333 ± 121	293 ± 70	169 ± 69	184 ± 33	178 ± 34
180	50 ± NA <sup>d</sup>	52 ± 4	52 ± 3	346 ± 135	382 ± 148	366 ± 99	109 ± 35	182 ± 39	147 ± 27
270	43 ± 7	70 ± 19	60 ± 13	369 ± 131	408 ± 152	391 ± 100	206 ± 71	267 ± 109	238 ± 65
365	45 ± 5	66 ± 15	58 ± 10	365 ± 132	384 ± 147	376 ± 98	256 ± 115	236 ± 112	245 ± 78

<sup>a</sup> Immunovet, Inc.  
<sup>b</sup> Rhone-Merieux, Inc. (now Merial Limited).  
<sup>c</sup> Titer values expressed as 1:xx, with only ≥ 5 used to calculate the mean.  
<sup>d</sup> Only one animal in group had titer ≥ 5.

er antibody titers and higher survival rates of dogs during live virus challenge. The level of antibody necessary to confer protection in the coyote is not known and can only be determined through controlled challenge studies utilizing a virulent rabies virus.

Efforts to compare titers in coyotes and dogs receiving the same vaccine are subjective because titer information collected by manufacturers is proprietary and not readily available. In addition, there are significant differences in species susceptibility to various strains of rabies virus, with titers that are protective in one species sometimes failing to protect another. Data on dogs provided by manufacturers suggests the titers among coyotes immunized with Dura-Rab 3® were lower and less persistent than among dogs vaccinated with the same product. However, titers appeared similar between coyotes and dogs vaccinated with Imrab 3®.

One goal of vaccination is to protect animals from disease by producing antibodies that effectively bind and neutralize a pathogen. The use of RFFIT in this study essentially demonstrates this function. RFFIT titers are determined by incubating sera dilutions with live rabies virus with the antibody binding and neutralizing the virus providing a quantitative assay of the sample. The resulting titers demonstrate the antibody produced was functional in vitro.

Our study demonstrated that coyotes can produce rabies specific antibody in response to vaccination with two commercial rabies vaccines licensed for use in domestic dogs. Furthermore, the antibodies produced were capable of binding and neutralizing live rabies virus in vitro. While it is reasonable to infer a measure of protection was conferred to coyotes receiving these vaccines, confirmation of such protection can only be obtained through live rabies challenge tests. Research on other

species suggests that high, persistent titers are usually protective for most individuals.

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