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Immunogenicity of Ontario Rabies Vaccine for Small Indian Mongooses (*Herpestes auropunctatus*)

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ABSTRACT: Oral rabies vaccination is the principal strategy used to control rabies in wildlife. No oral rabies vaccine is licensed for small Indian mongooses (*Herpestes auropunctatus*). The Ontario Rabies Vaccine Bait (ONRAB) is a human adenovirus type-5 rabies glycoprotein recombinant vaccine licensed for rabies control in striped skunks (*Mephitis mephitis*) in Canada and is under experimental evaluation in the US. We evaluated varying doses of ONRAB vaccine by direct instillation into the oral cavity with three groups of 10 mongooses: Group 1 received $10^{9.5}$ $TCID_{50}$, group 2 received $10^{8.8}$ $TCID_{50}$, and group 3 received $10^{8.5}$ $TCID_{50}$ of vaccine. Six control mongooses were sham-vaccinated with culture media. We collected a serum sample prior to vaccination and on days 14 and 30 postvaccination (PV). We quantified the level of rabies virus neutralizing antibodies (RVNA) from mongoose sera and compared titers among vaccinated groups and time points PV, where values greater than or equal to 0.1 IU/mL were considered positive. On day 14 PV, 87% (26 of 30, 95% confidence interval 70–95%) of vaccinates had seroconverted, whereas all vaccinates demonstrated RVNA by day 30 PV. There was a marginal effect of vaccine dose on group means of log-transformed RVNA titers at day 14 PV (F=2.5, P=0.099), but not day 30 PV. Sham-vaccinated animals were seronegative during all time points.

Key words: *Herpestes auropunctatus*, ONRAB, oral rabies vaccination, rabies, small Indian mongoose, vaccine.

The small Indian mongoose (*Herpestes auropunctatus*) is a rabies virus (RABV) reservoir on several Caribbean islands including Grenada, Cuba, Puerto Rico, and the Dominican Republic (Seetahal et al. 2018). In Puerto Rico, mongooses comprise over 50% of reported animal rabies cases (Ma et al. 2018). Wildlife rabies control is principally accomplished through a strategy of oral rabies vaccination (WHO 2018), but oral rabies vaccination has never been attempted with mongooses. One oral rabies vaccine is licensed for use with raccoons (*Procyon lotor*) and coyotes (*Canis latrans*) in the US (RABORAL V-RG; Boehringer Ingelheim Animal Health, Athens, Georgia), but the vaccine is neither immunogenic nor efficacious in mongooses by direct instillation into the oral cavity (DIOC; Blanton et al. 2006), yet mongooses vaccinated by DIOC with an experimental recombinant RABV vaccine construct (SPBNGA-S) seroconverted and survived a lethal challenge. A related recombinant RABV vaccine is part of an oral rabies vaccine bait product (RABITEC; Ceva Santé Animale, Dessau-Rosslau, Germany) licensed in Europe for use with red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*). The RABITEC vaccine is immunogenic when delivered by DIOC to mongooses (Vos et al. 2013).

The Ontario Rabies Vaccine Bait (ONRAB; Artemis Technologies, Inc., Guelph, Ontario, Canada) is a human adenovirus type-5 rabies glycoprotein recombinant vaccine licensed in Canada for use with striped skunks (*Mephitis mephitis*; Rosatte et al. 2009, 2011). Efficacy has also been demonstrated in raccoons (*Procyon lotor*) and red foxes (Rosatte et al. 2009; Brown et al. 2014a, b; Gilbert et al. 2018b). Experimental field evaluation of ONRAB in several areas of the eastern US has been conducted since 2011 (Slate et al. 2014; Gilbert et al. 2018a; Pedersen et al. 2019). Our objective was to evaluate immunogenicity of ONRAB vaccine in mongooses by DIOC route experimentally.

We conducted this study at the US Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center field sta-

tion (Hilo, Hawaii, USA). The state of Hawaii is considered wildlife rabies-free. All animal procedures and use were compliant with the National Wildlife Research Center Institutional Animal Care and Use Committee guidelines (QA-2268). We captured mongooses in the local vicinity of the field station in cage traps (Tomahawk Trap Company, Hazelhurst, Wisconsin, USA) baited with produced sausages soaked in fish oil. We housed mongooses individually in a Biosafety Level II animal containment room in cages (60×60×40 cm) and fed them a daily ration of 50 g cat food, supplemented twice a week with raw chicken. Water was available ad libitum. Mongooses were held in captivity for a 6–8-d acclimation period prior to any treatment. Capture and housing of mongooses was approved by the State of Hawaii Department of Agriculture under permit number HI-ISL-2014-01.

We established three vaccine treatment groups of 10 mongooses each (five males and five females per group) and one control group of six mongooses (three males and three females). All vaccinates received 1.0 mL of ONRAB vaccine by DIOC route: Group 1 received $10^{9.5}$ $TCID_{50}$ (the standard production dose), group 2 received $10^{8.8}$ $TCID_{50}$, and group 3 received $10^{8.5}$ $TCID_{50}$. The control group was sham-vaccinated with 1.0 mL of vaccine diluent (minimal essential media) by DIOC route. Mongooses were lightly anesthetized by intramuscular injection of 5.0 mg/kg tiletamine-zolazepam for pretreatment blood collection and vaccination (Telazol® Zoetis, Parsipanny-Troy Hills, New Jersey, USA). We collected 1.0 mL of blood via venipuncture of the cranial vena cava as described for ferrets (Briscoe and Syring 2004). We anesthetized mongooses via inhalation of isoflurane gas for collection of day 14 and 30 postvaccination (PV) blood samples, after which all subjects were euthanized.

We separated sera and stored aliquots at –80 C until analysis. Rabies virus neutralizing antibodies (RVNA) were quantified using the rapid fluorescent focus inhibition test (Yager and Moore 2015) at the Kansas State University Rabies Laboratory (Manhattan, Kansas,

TABLE 1. Treatments and rabies virus neutralizing antibody (RVNA) response for 36 adult small Indian mongooses (*Herpestes auropunctatus*) administered varying tissue culture infective doses ($TCID_{50}$) of Ontario Rabies Vaccine (ONRAB), or sham treatment with culture media, by direct instillation into the oral cavity and monitored for 30 d postvaccination.

ID	Sex	ONRAB vaccine dose (log_{10} $TCID_{50}$)	RVNA (IU/mL) Day 0	Day 14	Day 30
9-812	Male	9.5	<0.1	1.8	12.9
2-796	Male	9.5	<0.1	2.3	10
6-355	Male	9.5	<0.1	38.0	112
8-086	Female	9.5	<0.1	4.0	17
9-015	Female	9.5	<0.1	11.5	13.5
5-268	Male	9.5	<0.1	<0.1	0.2
8-573	Male	9.5	<0.1	12.9	71
0-121	Female	9.5	<0.1	13.5	42
0-263	Male	9.5	<0.1	10.0	29
1-555	Female	9.5	<0.1	86.0	100
8-028	Female	8.8	<0.1	0.5	9.2
3-078	Female	8.8	<0.1	3.0	42
3-861	Female	8.8	<0.1	3.7	13.5
2-525	Female	8.8	<0.1	12.3	38
0-281	Female	8.8	<0.1	10.0	30
4-031	Male	8.8	<0.1	1.5	8.3
6-517	Male	8.8	<0.1	0.4	2.6
7-372	Male	8.8	<0.1	9.2	100
0-839	Male	8.8	<0.1	0.1	0.2
7-770	Male	8.8	<0.1	0.1	3.1
7-315	Female	8.5	<0.1	0.3	4.3
3-296	Female	8.5	<0.1	2.8	10.8
1-038	Female	8.5	<0.1	2.8	24
3-383	Female	8.5	<0.1	5.4	106
0-005	Male	8.5	<0.1	<0.1	0.2
4-058	Male	8.5	<0.1	2.3	11.5
7-381	Male	8.5	<0.1	11.5	93
8-598	Male	8.5	<0.1	<0.1	1.3
9-112	Female	8.5	<0.1	4.0	13.5
7-372	Male	8.5	<0.1	<0.1	1.0
6-263	Male	Sham	<0.1	<0.1	<0.1
0-627	Female	Sham	<0.1	<0.1	<0.1
6-513	Male	Sham	<0.1	<0.1	<0.1
1-594	Male	Sham	<0.1	<0.1	<0.1
8-302	Female	Sham	<0.1	<0.1	<0.1
0-274	Female	Sham	<0.1	<0.1	<0.1

TABLE 2. Rabies virus neutralizing antibody (RVNA) profiles for 12 small Indian mongoose (*Herpestes auropunctatus*) pups born to dams that were vaccinated by direct instillation into the oral cavity with varying tissue culture infective doses ($TCID_{50}$) of Ontario Rabies Vaccine or sham-treated with culture media. Dams and pups were sampled for RVNA on day 30 postvaccination.

Dam ID	Dam vaccine dose (log_{10} $TCID_{50}$)	Dam RVNA on day 30 postvaccination (IU/mL)	Pup ID	Pup RVNA (IU/mL)	Pup age on sampling (days)
8-086	9.5	17.0	8-086-1	13.5	3
9-015	9.5	13.5	9-015-1	27	5
8-028	8.8	9.2	8-028-1	<0.1	1
0-281	8.8	30	0-281-1	2.3	7
7-315	8.5	4.3	7-315-1	3.2	8
			7-315-2	5	8
3-296	8.5	10.8	3-296-1	2.8	8
			3-296-2	4	8
1-038	8.5	24	1-038-1	79	3
			1-038-2	25.8	3
9-112	8.5	13.5	9-112-1	2.8	8
0-274	Sham	<0.1	0-274-1	<0.1	2

USA). Titers were converted to international units per milliliter (IU/mL) by comparison to a positive control standard rabies immune globulin containing 2 IU/mL, and titers greater than or equal to 0.1 IU/mL were considered RVNA positive (Berentsen et al. 2015). A cutoff of greater than or equal to 0.5 IU/mL was also used to assess the response to vaccination. We transformed RVNA values by the natural logarithm to obtain normally distributed data, compared the group mean responses of vaccinates by treatment dose using analysis of variance, and interpreted the results using α=0.05. For mean calculations, values reported as less than 0.1 IU/mL were coded as 0.05 IU/mL. We present back-transformed means (SD) values in the text. We estimated proportions of seropositive mongoose vaccinates with corresponding 95% confidence intervals (CIs).

No mongooses presented evidence of RVNA prior to vaccination. On day 14 PV, 87% (26/30, 95% CI 70–95%) of vaccinates had seroconverted, and all vaccinates (30/30, 95% CI 89–100%) demonstrated RVNA seroconversion by day 30 PV (Table 1). Using the cutoff of 0.5 IU/mL, 73% (22/30, 95% CI 56–86%) of vaccinates demonstrated RVNA by day 14 PV and 90% (27/30, 95% CI 74–97%) by day 30 PV. There was a marginal effect of vaccine dose on the RVNA response among vaccinates on day 14 PV (n=30, F=2.5, P=0.099), but not on day 30 PV (n=30, F=0.7, P=0.520). The backtransformed mean RVNA responses among mongooses in Groups 1–3 on day 14 PV were 5.9 (7.0), 1.4 (5.7), and 0.8 IU/mL (7.7), respectively, and on day 30 PV they were 18.9 (5.6), 9.8 (5.5), and 7.3 (6.7) IU/mL, respectively. Sham-vaccinated animals were RVNA negative at both PV time points.

During this study, 17 pups were born in captivity to nine vaccinated and one sham-vaccinated dam (Table 2). An opportunistic blood sample was successfully collected from 12 pups via cardiac puncture immediately following euthanasia. Nine of 11 pups born to vaccinated dams were RVNA positive (Table 2), whereas one pup born to a sham-vaccinated dam was seronegative. Pups ranged in age from 1 to 8 d old at the time of sampling.

Our research suggested that ONRAB was immunogenic for small Indian mongooses at and up to one log lower than the standard production dose of $10^{9.5}$ $TCID_{50}$/mL. Based

on the RVNA titers observed PV in this study (Moore et al. 2017), we speculate that most of the vaccinates would likely survive a lethal RABV challenge, but future research should verify the immunogenicity and efficacy by bait delivery. We did not expect the birth of mongoose pups during our study, but this opportunity permitted demonstration of maternal transfer of RVNA from vaccinated dams to suckling young. Based on studies in red foxes and raccoons, we expect that maternal RVNA transferred to young may persist up until the time of weaning (Müller et al. 2002; Fry et al. 2013), which is approximately 58 d for the small Indian mongoose (Hinton and Dunn 1967), although it is not clear whether the passively transferred RVNA in young mongooses are protective against a lethal RABV challenge. Additional research might also evaluate the persistence of passively transferred maternal RVNA antibody among suckling and weaned young in relation to rabies vaccination or protection against lethal challenge.

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