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Evaluation of Immune Responses in Dogs to Oral Rabies Vaccine under Field Conditions*

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Abstract

During the 20th century parenteral vaccination of dogs at central-point locations was the foundation of successful canine rabies elimination programs in numerous countries. However, countries that remain enzootic for canine rabies have lower infrastructural development compared to countries that have achieved elimination, which may make traditional vaccination methods less successful. Alternative vaccination methods for dogs must be considered, such as oral rabies vaccine (ORV). In 2016, a traditional mass dog vaccination campaign in Haiti was supplemented with ORV to improve vaccination coverage and to evaluate the use of ORV in dogs. Blisters containing live-attenuated, vaccine strain SPBNGAS-GAS were placed in intestine bait and distributed to dogs by hand. Serum was collected from 107 dogs, aged 3 – 12 months with no reported prior rabies vaccination, pre-vaccination and from 78/107 dogs (72.9%) 17 days post-vaccination. The rapid florescent focus inhibition test (RFFIT) was used to detect neutralizing antibodies and an ELISA to detect rabies binding antibodies. Post-vaccination, 38/41 (92.7%) dogs that received parenteral vaccine had detectable antibody (RFFIT >0.05 IU/mL), compared to 16/27 (59.3%, $p < 0.01$) dogs that received ORV or 21/27 (77.8%) as measured by ELISA (>40% blocking, $p < 0.05$). The fate of 291 oral vaccines was recorded; 283 dogs (97.2%) consumed the

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Conflicts of Interest

Ad Vos is full-time employee of a company that manufactures oral rabies vaccine bait.

bait; 272 dogs (93.4%) were observed to puncture the blister, and only 14 blisters (4.8%) could not be retrieved by vaccinators and were potentially left in the environment. Pre-vaccination antibodies (RFFIT >0.05 IU/mL) were detected in 10/107 reportedly vaccine-naïve dogs (9.3%). Parenteral vaccination remains the most reliable method for ensuring adequate immune response in dogs, however ORV represents a viable strategy to supplement existing parental vaccination campaigns in hard-to-reach dog populations. The hand-out model reduces the risk of unintended contact with ORV through minimizing vaccine blisters left in the community.

Keywords

Rabies Virus; Canine Rabies Vaccine; Oral Rabies Vaccine; Immune Response; Serology

Introduction

Globally, canine rabies is responsible for more human deaths than any other zoonotic disease[1], and can still be found in 122 primarily low-resource countries[2]. Provision of post-exposure prophylaxis to persons with bites from suspected rabid animals is integral to reducing human burden; however, vaccination of the reservoir species (most often dogs) is recognized as the most cost-effective and permanent solution to rabies prevention[3–5]. To interrupt transmission of canine rabies the World Health Organization (WHO) recommends ≥70% of dogs need to be vaccinated, and this level of herd immunity must be maintained for 3 – 7 years for elimination[6]. Elimination has been achieved in numerous high-income countries through parenteral vaccination at central point locations. However, there have been few low and middle income countries that have achieved this goal nationally.

Many multifaceted barriers prohibit effective vaccination through parenteral routes in low-income countries including lack of funding, infrastructure and political will, poorly organized campaigns, owner's inability to control their dogs, vaccinator's inability to reach dogs without extraordinary effort, and large proportions of free-roaming dogs[3, 7–10]. Oral rabies vaccine (ORV) for free roaming dogs has been proposed to reach dog populations inaccessible via parenteral vaccination routes[6]. While ORV for dogs has been utilized in numerous settings[11–15], it is not yet widely integrated into existing vaccination campaigns. For this study, a highly attenuated rabies virus, SPBNGAS-GAS, was selected for ORV. SPBNGAS-GAS is derived from SAD L16, a cDNA clone of the ORV strain SAD B19, two mutations at amino acid positions 194 and 333 of the glycoprotein and an additional identically modified glycoprotein gene are incorporated to enhance its safety profile compared to conventional attenuated oral rabies virus vaccines[16, 17].

In Haiti, canine rabies remains enzootic with current estimates of 130 annual human rabies deaths due to bites from dogs[2, 18]. Barriers to canine rabies elimination persist in Haiti including economic, educational, and cultural factors that make vaccine delivery and rabies surveillance difficult[19]. Furthermore, an estimated 80% of Haiti's dog population is allowed to roam freely, which negatively impacts the success of central point, parenteral vaccination efforts[19]. The Haiti Ministry of Agriculture, Natural Resources, and Rural Development (MARNDR) has steadily increased the canine rabies vaccination coverage

through parenteral vaccination campaigns conducted at central point locations, yet national vaccination coverage has not surpassed 50% of dogs. In 2016, the US Centers for Disease Control and Prevention (CDC) evaluated a dog vaccination campaign organized by MARNDR in partnership with Christian Veterinary Mission, Humane Society International, and IDT-Biologics in Croix-des-Bouquets, Haiti to understand how new methods for vaccination realize national rabies elimination. Methods evaluated included central point, door-to-door, ORV, and capture-vaccinate-release. Here we report the serologic response of previously unvaccinated dogs that received ORV or parenteral rabies vaccine to determine if ORV is a viable strategy for improving vaccination coverage in Haiti.

Materials and Methods

Study Design

This study was conducted during a two-week, dog vaccination campaign in Croix-des-Bouquets, Haiti in 2016, in which 10,000 dogs received parenteral vaccine and 590 dogs ORV. Dogs fitting inclusion criteria (aged 3 – 12 months, owner-reported rabies vaccine-naïve, and owner consent) were enrolled in a sero-survey to compare primary immune response to parenteral and oral vaccination. Veterinary care is not routinely available in this community, access to rabies vaccine is typically only available through annual government sponsored campaigns, making previous history of rabies vaccination unlikely for dogs fitting the inclusion criteria.

Dogs were restrained within 5 minutes of primary vaccination and blood was collected (1.5 – 3 mL). Owners were contacted 17–20 days post-vaccination, and post-vaccination serum was obtained. Sample size was determined by the Fleiss method[20], with alpha = 0.05, beta = 80%, failure in parenteral = 5%, and failure in oral = 25%. The sample size was calculated at 49 dogs for both parenteral and oral. This study was conducted in compliance with approved CDC animal care and use protocol 2757DOTMULX.

Vaccines

7,500 doses of Rabvac 1 (lot: 4130242A, expiration: 08 Sep 2017, Boehringer Ingelheim Vetmedica, Saint Joseph, MO, USA) were procured by CDC. Rabvac 1 was depleted by day 11 of the 14 day campaign, so MARNDR opted to use 3,000 doses of vaccine that was five-months expired, Rabisin R (lot: L399308, expiration: 20 Mar 2016, Merial, Leon, France).

590 doses of ORV was provided by IDT-Biologika, Dessau-Rosslau, Germany (Vaccine strain: SPBNGAS-GAS[21], lot: 0010716, expiration: Oct 2016, experimental batch). A PVC-blister sealed with aluminum foil containing ORV was placed inside 7 – 10 cm of boiled beef or swine intestine. Swine was superior due to the size of the blister.

Oral Vaccination

Prior to distribution of the oral vaccine a thorough safety review was conducted by CDC, MARNDR, and IDT Biologika as per WHO recommendations[6]. During the campaign, consent was obtained from the dog owner before ORV use, and flyers with a phone number to report adverse events or exposures to persons through vaccine contact or the dog’s saliva

within 48 hours post-vaccination were provided to the owner (Fig. S1). A hand-out and retrieval model was conducted[6], to ensure that very few vaccine blisters were left in the environment. An enhanced bite and bait contact surveillance system was operated during the campaign and for 1 week post-campaign to improve detection of bites from orally vaccinated dogs or bait contact events[6, 22]. Data were collected on the dog's response to the ORV bait, puncture of the blister, and ability of the vaccinator to retrieve the blister when not ingested by the dog. Owners of dogs orally vaccinated were not given vaccination certificates; while, owners of dogs vaccinated parenterally, regardless of product, received a rabies vaccination certificate.

Vaccine Conditions

Rabvac 1 arrived in two shipments (3,750 vaccines each); one shipment maintained cold-chain (4–8°C), the second shipment arrived at ambient temperature (presumed duration of 2 – 4 days) and was then properly-stored (4–8°C) during the 1-week prior to the campaign. Before use, the antigen content in improperly-stored vaccine was measured using an antigen capture assay as previously described[23] and was indistinguishable from properly-stored vaccine. Expired vaccine was evaluated post-hoc, but aluminum adjuvant in its formulation interfered with accurate antigen counts.

Serology

Dogs selected for parenteral vaccination were split between three groups: properly-stored (n = 27), improperly-stored (n = 27), and expired (n = 18). Rabies virus neutralizing antibody (rVNA) was measured in serum samples by the rapid fluorescent focus inhibition test (RFFIT) according to a standard protocol[24]. For RFFIT >0.05 IU/mL was considered positive. Serum samples from dogs which received ORV or had pre-vaccination rVNA were also tested using a blocking ELISA (O.K. Servis BioPro, Prague, Czech Republic) according to the manufacturer's instructions. For ELISA >40% blocking was considered positive. Seroconversion is used to indicate the number of samples that meet the specified cut-off.

Case-Control Follow-up Survey

A semi-structured interview was conducted with owners of dogs that had rVNA pre-vaccination. Dogs without pre-vaccination rVNA and matched based on location of residence, were selected randomly as controls from the pool of dogs for which blood had been collected (i.e. fit inclusion criteria). Information regarding vaccination history, known fights with other dogs, unexplainable wounds, and time spent roaming freely were captured (Fig. S2).

Data Analysis

Results were compared between oral and parenteral at 0.05 IU/mL and within the parenteral group for improperly-stored, properly-stored, and expired vaccines. Geometric mean titers were calculated for each group. Mid-p exact 1-tailed p-values, odds ratios, and 95% confidence intervals were calculated using OpenEpi.

Results

Of 590 oral baits distributed using a hand-out method, the final disposition of 291 were tracked as part of this study (Table 1). 235 baits (80.8%) were distributed to dogs on private property, 50 (17.2%) were distributed to dogs in the street, and for six (2%) the location was not recorded; all had discrete owners. Overall, 283 dogs (97.2%) accepted the bait, and vaccinators reported 272 dogs (93.4%) perforated the blister, suggesting vaccine exposure. Of 291 baits, 277 blisters (95.2%) were ingested or recovered, only 14 baits (4.8%) were not recovered by the vaccinators (Table 2).

Vaccinators collected pre-vaccination serum from 107 dogs which fit the inclusion criteria: 72 parenterally and 35 orally vaccinated dogs. Parenterally vaccinated dogs were further sub-divided by properly-stored (n = 27), improperly-stored (n = 27), and expired vaccines (n = 18). Of the 107 enrolled dogs, 78 (72.9%) were located 17 days post-vaccination for serum collection: 49/72 dogs (68.0%) parenterally and 29/35 dogs (82.8%) orally vaccinated. Due to poor ability to follow-up on many dogs, the power is 47.9% (goal was 80%).

Ten dogs had rVNA pre-vaccination and were not included in aggregate analysis: eight parenterally and two orally vaccinated dogs. Thus, 38/41 (92.7%) parenterally vaccinated dogs in aggregate had detectable rVNA (>0.05 IU/mL) with a GMT of 1.3 IU/mL, and 16/27 (59.3%) orally vaccinated dogs had detectable rVNA (>0.05 IU/mL, $p < 0.01$) with a GMT of 0.5 IU/mL (Fig. 1). When binding antibodies for the orally vaccinated dogs were measured using a blocking ELISA, 21 dogs (77.8%) had detectable antibodies (>40% blocking, $p < 0.05$). Seroconversion was not statistically different for dogs that punctured and ingested the blister compared to the group that punctured then expectorated the blister (data not shown).

Dogs receiving properly-stored and improperly-stored parenteral vaccine did not have significantly different antibody responses; 100% and 81.3% respectively (>0.05 IU/mL, $p > 0.05$, Fig. 1). The GMT for dogs that received properly-stored, improperly-stored, and expired vaccine (with adjuvant) was 1.2 IU/mL, 0.8 IU/mL, and 5.5 IU/mL respectively. Similar to Lankester, et. al. this finding supports that high-quality veterinary vaccines can break cold-chain and maintain immunogenicity[25]. However, each vaccine product and cold-chain disruption event should be critically evaluated. Vaccines that undergo >37°C or >6 month excursions outside of cold-chain were not assessed by Lankester, et. al. or this study.

Ten dogs (9.3%) enrolled in the study had >0.05 IU/mL rVNA pre-vaccination (Table 3). Seven of these samples were also positive for binding antibodies using the ELISA (Table 3). The GMT for these sero-positive dogs was 0.25 IU/mL pre-vaccination and 5.34 IU/mL post-vaccination (21.6-fold increase) compared to 1.29 IU/mL (4.1-fold lower than cases) for matched controls post-vaccination.

Six dog owners for dogs with pre-vaccination rVNA and 6 dog owners for dogs without pre-vaccination rVNA (matched controls) participated in a follow-up survey to evaluate risk factors for rabies exposure in their dogs (Table 4). Lack of previous vaccination was confirmed in all 12 dogs. Dogs with pre-vaccination rVNA were more likely to always be

free roaming, have unexplained wounds, have a history of fighting with other dogs, or have both wounds and history of fighting; and less likely to receive veterinary care (Table 4). One owner of a dog with pre-vaccination rVNA reported that the dog had eaten the remains of a dead dog. In a previous study, 10% of found-dead dogs in Haiti were confirmed rabies-positive[22].

Discussion

Recently numerous programs have achieved rabies control in low-resource settings, yet these programs have not yet achieved sufficient, sustainable coverage to eliminate canine rabies nationally[4, 26, 27]. Expansion of rabies elimination programs in low-resource countries has been constrained by logistics of vaccine procurement, distribution, and trained personnel[3]. Many enzootic countries have high proportions of free-roaming dogs which can further hamper vaccination efforts[9, 19]. Reliance upon central-point, parenteral vaccination may be inadequate in settings with logistical constraints and inaccessible dog populations [8, 28, 29]. Oral vaccination of dogs may overcome some of these limitations[30].

Post-Vaccination Safety Evaluation

No deaths were reported among the 590 baited dogs, and no calls were received regarding human exposure to the baits or vaccine. Furthermore, of 32 dog bites reported in the vaccination zone during the campaign and 2-weeks post-campaign, none were from dogs that had been orally baited. The hand-out model was crucial to reducing ORV exposure among community members, as only 14 (4.8%) vaccine blisters were left in the community. Even this residual rate may be inflated since some baits were likely ingested after the vaccinators left the dog’s territory. These data support that in Haiti, using a hand-out method with SPBNGAS-GAS vaccine in an intestine bait, the likelihood of ORV exposure to people and non-target animals is low.

Performance of ORV

Successful oral vaccination is complex and requires a series of critical steps, including that the animal is attracted to the bait, the blister is punctured, and the vaccine has adequate contact time with the oral mucosa. Any breakdown in these steps can result in vaccination failure. The SPBNGAS-GAS vaccine, packed inside a boiled intestine bait, was highly effective at attracting dogs (97.2% acceptance). Recent ORV studies in dogs and wolves, focusing on this critical step, found acceptance ranging from 47% to 93%[15, 31, 32]. Bait acceptance for dogs in the Philippines using the identical bait and blister was similar (96.1%)[30]. Furthermore, vaccinators noted that 93.4% of dogs punctured the blister, indicating vaccine exposure. Food scarcity for dogs in Haiti likely contributed to bait uptake[19], and in Haiti and other countries with similar dog populations, oral vaccines in an intestine bait are likely to be well-accepted and vaccine exposure in dogs is likely to occur at a high rate.

Overall, 77.8% of orally-vaccinated dogs had evidence of rabies antibodies post-vaccination, which is consistent with reported conversion rates in orally vaccinated wildlife[33–36]. The

study design may have unintentionally influenced antibody production. As all dogs selected for this study were free-roaming, vaccinators had to judiciously plan the post-prandial capture of the dog. Although not quantified, supervisors noted that vaccinators captured some dogs before the bait was fully consumed, which could have led to decreased oral mucosal contact and negatively impacted seroconversion. The high rate of antibody production among orally vaccinated dogs in Haiti suggests SPBNGAS-GAS vaccine could be used to improve vaccination coverage among inaccessible dog populations where parenteral vaccination has failed to reach desired coverage levels.

Serial serum sampling was not feasible in this study due to logistical and political constraints: the presidential election occurred 35 days post-vaccination and a low follow-up rate made identification of a meaningful sample unlikely after the election. Sampling 17 days post-vaccination is well supported from previous literature, in which dogs orally vaccinated had peak rVNA detection between 14 and 30 days post-vaccination[30, 31, 37] and 15 days post-vaccination for parenterally vaccinated dogs[38]. While serial sampling was not feasible for this study, another evaluation of SAD B19 ORV in dogs in the Philippines, found 43% of dogs seroconverted (RFFIT ≥ 0.5 IU/mL) by day 15 and that the rate increased to 71% by 29 days post-bait consumption[30]. Thus, our results likely represent a minimum expected seroconversion rate for SPBNGAS-GAS ORV in Haiti.

This study only evaluated the primary immune responses in vaccine-naïve dogs < 1 year old, which results in lower antibody production compared to previously vaccinated or older dogs[39, 40]. Numerous studies have shown higher rates of seroconversion after primary vaccination than we detected[25, 41]. The health status of dogs in this study may have led to reduced responses to both oral and parenteral vaccination. Schildecker et.al. described a 30% annual death rate for dogs in Haiti, and an average body condition score of 3 out of 10, indicating that dogs in Haiti are overall malnourished and potentially immunosuppressed[19]. While seroconversion rates as measured at the >0.5 IU/mL level were lower than expected (Table S1), the most critical measure of successful vaccination is evidence of antibody production[42], and at >0.05 IU/mL, the vast majority of dogs in this study succeeded (Fig. 1). Even animals orally vaccinated with rVNA titers <0.05 IU/mL may be protected from rabies if an anamnestic response is induced[37]. For this reason, we choose to use a blocking ELISA which may provide a better correlate of protection for ORV than RFFIT [43].

Evidence of Rabies Exposures

Rabies is referred to as universally fatal, and once signs appear in animals this is typically true[1]. However, not every rabies exposure leads to infection and disease, with some studies showing that as few as 18% of rabies exposures result in development of disease and death[44, 45]. Wildlife surveys have detected that 1% – 40% of reservoir species may have rVNA in the absence of any vaccination programs[46–49]. This likely reflects prior rabies exposure and aborted infection, rather than recovery from acute disease. In Tanzania and Sri Lanka, 1.3% and 1.6% of dogs, respectively, had >0.5 IU/mL rVNA pre-vaccination[25, 41]. Similarly, we found 2/107 dogs (1.8%) had >0.5 IU/mL pre-vaccination (Table 3); while, 10/107 dogs (9.3%) had >0.05 IU/mL pre-vaccination. A follow-up survey conducted with

owners confirmed that these dogs were still alive, and had never received rabies vaccine. The survey also identified elevated risk factors for rabies exposure, including fighting with other dogs and presence of wounds. While the sample size was too small to determine if any of the factors were significant, the direction of the associations indicates higher risk for rabies exposure in dogs with pre-vaccination rVNA compared to dogs without, and leads to speculation that these dogs had past rabies exposure resulting in rVNA. This finding that 9.3% of dogs surveyed may have been exposed to rabies virus suggests a high rabies exposure rate in Haiti. A conclusion supported by previous studies in Haiti[22]. The majority of dogs with pre-vaccination rVNA had robust anamnestic response to vaccination, possibly representing a mode of non-vaccine acquired immunity in Haiti's dog population.

In many settings, inaccessible dogs cannot be reached through parenteral methods. Therefore, the finding that using a hand-out model, an intestine bait, and SPBNGAS-GAS ORV in Haiti was safe and highly effective for vaccinating dogs could improve vaccination coverage in communities with large populations of inaccessible dogs, and thereby decrease canine-mediated human rabies deaths.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CDC	US Centers for Disease Control and Prevention
GMT	geometric mean titer
IU	international unit
MARNDR	Haiti Ministry of Agriculture, Natural Resources, and Rural Development
ORV	oral rabies vaccine
rVNA	rabies virus neutralizing antibodies
RFFIT	rapid fluorescent focus inhibition test
WHO	World Health Organization

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Highlights

- Dogs in Croix-des-Bouquets, Haiti received oral rabies vaccine by a hand-out method
- 78 dogs 3–12 months old were tested pre- and post-vaccination for rabies antibodies
- 77.8% of orally vaccinated dogs had evidence of rabies antibodies after 17 days
- 95.2% of oral vaccine blisters were ingested by the dog or retrieved by vaccinators
- 9.3% of dogs with no reported rabies vaccination had pre-existing rabies antibodies

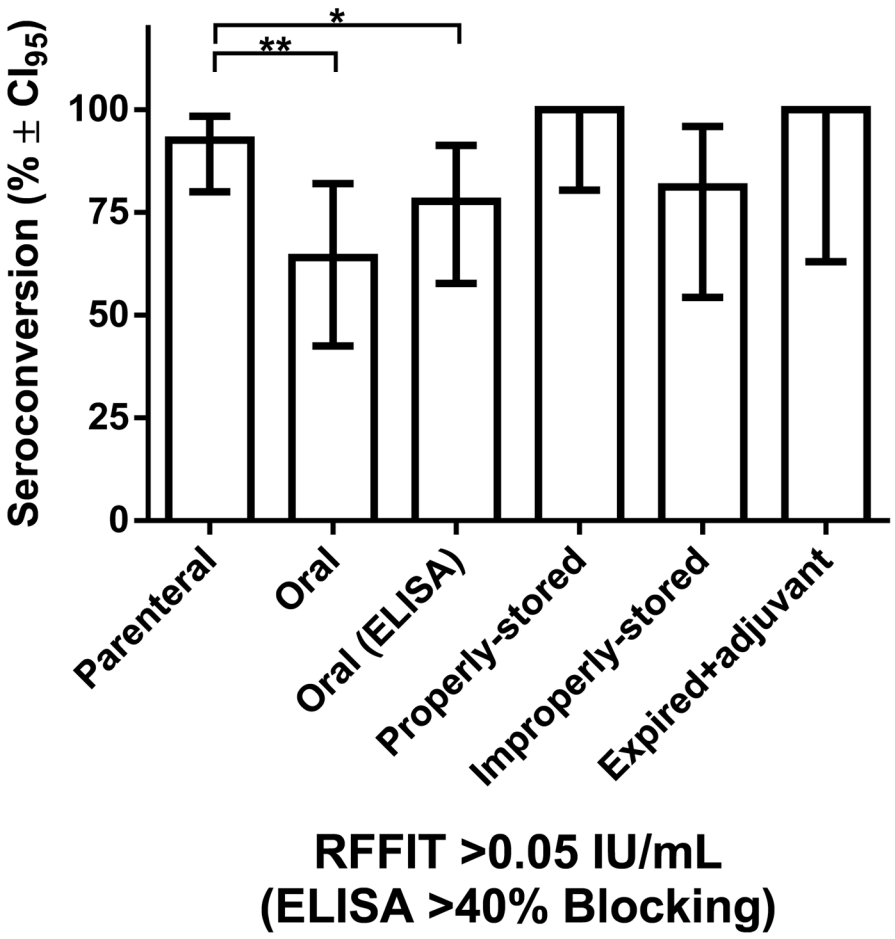

Figure 1. Comparison of Rabies Antibodies in Rabies Vaccine-naïve Dogs by Vaccine Type and Assay

Serum collected from 78 dogs, aged 3 – 12 months with no reported prior rabies vaccination, 17 days after rabies vaccination was tested by RFFIT or ELISA. Dogs received either parenteral or oral vaccine. The group that received parenteral vaccine was further sub-divided into dogs that received properly-stored, improperly-stored or expired (with adjuvant) vaccines. Only dogs without detectable rVNA (<0.05 IU/mL) pre-vaccination by RFFIT were included in the analysis. The percentage of dogs with >0.05 IU/mL rVNA by RFFIT or >40% blocking by ELISA and the 95% confidence interval is shown. Using the mid-p exact (1-tailed) test the difference between parenteral and oral vaccination was significant by RFFIT (** p <0.01) and ELISA (* p <0.05). No significant differences were found based on the storage conditions of parenteral vaccine. See Table S1 for complete results.

TABLE 1

Dog's Reaction to the Bait and ORV Blister

Bait Acceptance	Bait not Accepted		Bait Accepted									
Blister Exposure	None		Blister perforated, not ingested		Blister perforated and ingested		Blister not perforated		Blister fate unknown		TOTAL	
Location of Bait Offering	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
On private property[a]	8	3.4%	120	51.1%	100	42.6%	7	3.0%	0	0.0%	**235**	**80.8%**
On the Street	0	0.0%	29	58.0%	17	34.0%	0	0.0%	4	8.0%	**50**	**17.2%**
Not Recorded	0	0.0%	3	50.0%	3	50.0%	0	0.0%	0	0.0%	**6**	**2.0%**
TOTAL	**8**	**2.7%**	**152**	**52.2%**	**120**	**41.2%**	**7**	**2.4%**	**4**	**1.4%**	**291**	

[a] does not imply confined or accessible dogs

TABLE 2

Recovery of Vaccine Blister Using a Hand-out Method for Oral Rabies Vaccination of Dogs

ORV Team (total ORV baits)	Blisters Left with the Dog	Blisters Taken by Dog, Fate Unknown	Total Unrecovered Blisters[a]	Total Blister Recovered or Ingested by Dog
A (84 ORV baits)	2 (2.4%)	0 (0.0%)	2 (2.4%)	82 (97.6%)
B (85 ORV baits)	4 (4.7%)	0 (0.0%)	4 (4.7%)	81 (95.3%)
C (80 ORV baits)	3 (3.8%)	0 (0.0%)	3 (3.8%)	77 (96.2%)
D (42 ORV baits)	1 (2.4%)	4 (9.5%)	5 (11.9%)	37 (88.1%)
TOTAL (291 ORV baits)	**10 (3.4%)**	**4 (1.4%)**	**14 (4.8%)**	**277 (95.2%)**

[a]Refers to ORV blisters that were not ingested and could not be recovered by the vaccination team.

Table 3

Rabies antibody titers for vaccine-naïve dogs with pre-vaccination RFFIT titer >0.05 IU/mL and matched controls (pre-vaccination RFFIT <0.05 IU/mL), before and after primary rabies vaccination

	Pre-Rabies Vaccination						Post-Rabies Vaccination		
	Group[a]	RFFIT Titer	IU/mL	RFFIT Interpretation	ELISA[b]	ELISA Interpretation	RFFIT Titer	IU/mL	Interpretation
Pre-Vaccination rVNA	PI	1:25	0.18	Positive	38%	Negative	1:1400	10	Positive
	PE	1:56	0.45	Positive	99%	Positive	1:1300	10	Positive
	PP	1:33	0.24	Positive	88%	Positive	1:200	1.5	Positive
	PP	1:7	0.052	Positive	78%	Positive	1:250	1.8	Positive
	PI	1:180	1.3	Positive	75%	Positive	1:22652	170	Positive
	O	1:29	0.21	Positive	13%	Negative	1:1100	8.1	Positive
	PI	1:7	0.052	Positive	10%	Negative	1:1400	10	Positive
	O	1:250	1.8	Positive	89%	Positive	1:65	0.48	Positive
	PE	1:11	0.081	Positive	80%	Positive	1:270	2	Positive
	PI	1:54	0.43	Positive	77%	Positive	*Not available for follow-up*		
Controls	O	<1:5	<0.04	Negative	11%	Negative	1:900	6.7	Positive
	O	<1:5	<0.04	Negative	1%	Negative	<1:5	<0.04	Negative
	O	<1:5	<0.04	Negative	12%	Negative	<1:5	<0.04	Negative
	PE	<1:5	<0.04	Negative	−8%	Negative	1:85	0.63	Positive
	PE	<1:5	<0.04	Negative	20%	Negative	1:3125	23	Positive
	PP	<1:5	<0.04	Negative	3%	Negative	1:50	0.36	Positive
	PP	<1:5	<0.04	Negative	−7%	Negative	1:625	4.6	Positive
	PP	<1:5	<0.04	Negative	−17%	Negative	1:1000	7.4	Positive
	PP	<1:5	<0.04	Negative	−2%	Negative	1:1300	9.6	Positive
	PI	<1:5	<0.04	Negative	8%	Negative	1:95	0.7	Positive

[a] Vaccine received: PI, parenteral improperly-stored; PE, parenteral expired; PP, parenteral properly-stored; O, oral.

[b] ELISA values represent percent blocking (positive >40% blocking).

Table 4

Comparison of Rabies Exposure Factors for Dogs with Pre-vaccination Rabies Antibodies (Cases) Compared to Dogs without Pre-vaccination Rabies Antibodies (Controls)

Exposure Variable	Response	Cases	Controls	Odds Ratio (95% CI)[a]	P-value[b]
History of Rabies Vaccination	Yes	0 (0%)	0 (0%)	NA	NA
	No	6 (100%)	6 (100%)		
Free Roaming	Always	5 (83%)	3 (50%)	4.0 (0.2 – 88.7)	0.25
	Sometimes	1 (17%)	3 (50%)		
History of Wounds	Yes	4 (67%)	2 (33%)	3.0 (0.3 – 28.8)	0.38
	No	2 (33%)	4 (67%)		
History of Dog Fighting	Yes	4 (67%)	3 (50%)	2.0 (0.2 – 22.1)	0.63
	No	2 (33%)	3 (50%)		
History of Wounds and Fighting	Yes	5 (83%)	3 (50%)	3.0 (0.3 – 28.8)	0.38
	No	1 (17%)	3 (50%)		
Veterinary Care	Yes	1 (17%)	2 (33%)	0.5 (0.02 – 14.9)	0.5
	No	5 (83%)	4 (67%)		

[a] Matched odds-ratio and 95% confidence interval.

[b] Although sample size is too small (follow-up with owners is difficult in Haiti) to determine significant associations, the direction of the odds-ratio measures are consistently supportive of higher-risk rabies exposure factors for dogs that had pre-vaccination rVNA.