EXPLORING CHARACTERISTICS OF VACCINATED DOGS THAT FAIL TO ACHIEVE AN ADEQUATE LEVEL OF RABIES VIRUS NEUTRALIZING ANTIBODIES

By

ANNA M. PEES

B.S., University of Arizona, 2004
D.V.M., Kansas State University, 2014

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Approved by:

Major Professor
Susan Moore

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Introduction

Rabies is caused by a group of related viruses, Lyssaviruses, of which there are now 14 recognized species (International Committee on Taxonomy of Viruses, ICTV). Among the Lyssavirus species, Rabies Virus, the one with the greatest public health impact, is further divided into numerous virus variants which are maintained in specific reservoir populations. The canine rabies virus variant, responsible for dog-to-dog transmission of rabies, is a threat to human health and responsible for an estimated 59,000 deaths each year (Hampson et al 2015). In many regions including most of North America and Europe, the canine rabies virus variant was pushed to extinction through mandatory dog vaccination and stray dog control (WHO Expert Consultation on Rabies, 2013).

Although preventative control measures have minimized the incidence of canine rabies in developed countries, recommendations on rabies vaccination for domestic dogs vary among countries and even within states and provinces. The National Association of State Public Health Veterinarians recommends that regardless of the age of the animal at initial vaccination, a booster vaccination should be administered one year later. All licensed rabies vaccine manufacturers have approved an initial vaccination can occur as early as three months of age (Rabies Compendium 2016). However, state and local governments regulate the administration of rabies vaccinations whereby the age at initial vaccination and required frequency can vary (AVMA Sate Laws 2016). Most states require administration of the initial vaccine by 3-4 months of age and subsequent booster vaccination annually or every 3 years; however, there is no epidemiologic or laboratory data available to support the annual or biennial administration of 3- or 4-year vaccines after the initial series (Rabies Compendium 2016).

Vaccination against rabies is not assumed to be 100% protective. Rabies neutralizing antibody titers ≥ 0.5 IU/mL has been defined by the WHO and OIE as the minimum post vaccination antibody level (OIE 2011, WHO 2013). However, neutralizing antibodies levels decline over time in vaccinated animals and are dependent on the timing of the blood sampling for antibody measurement in relation to vaccination. Therefore, if the timing of antibody measurement is delayed months or even years after vaccination, the level of circulating antibody may not be

detectable. This does not mean that the animal did not have an adequate response immediately post-vaccination and it does not mean that the animal is susceptible to rabies challenge (Aubert 1993, Moore and Hanlon 2010). While the majority of animals tend to achieve the recommended titer threshold 7-14 days post-vaccination (Sage et al., 1993; Sihoven et al., 1995; Cliquet et al., 2003; Mansfield et al., 2004; Kennedy et al.; 2008), some animals may fail to mount an immunological response to rabies vaccination and therefore protection against rabies challenge cannot be conferred from a history of vaccination alone.

Countries that have eliminated the canine rabies virus variant often require proof of vaccination and/or proof of adequate rabies antibody titer for pet import. For example, dogs importing to New Zealand, a rabies free country, are required to have received a rabies vaccine given no less than six months and not more than one year prior to the date of shipment. In addition, a proof of a rabies antibody test with a result of at least 0.5 IU/ml is required whereby the blood sample was collected not less than three months and not more than 24 months prior to the date of entry. Depending on the jurisdiction, dogs without proof of vaccination may be, turned away, euthanized, or in special occasions granted access through special monitoring programs (USDA, Pet Travel 2016).

For many canine rabies-free countries, imported dogs must have documentation of a rabies antibody titer value of ≥ 0.5 IU/ml for entry. However, multiple factors likely play a role in whether of not an animal is likely to achieve this value, including biological factors, vaccination schedules, and the timing of the blood draw for the antibody test. The purpose of this study is to assess the risk factors that may contribute to the failure of an animal to respond adequately after primary rabies vaccination.

Materials and Methods

Serum samples with accompanying dog-demographic data from 162,739 animals were submitted to the Kansas State University Rabies Laboratory between 2006 and 2010 for the detection of rabies antibody titer levels for the purpose of pet travel. Samples were tested via Fluorescent Antibody Virus Neutralization (FAVN) testing and data from submission forms were entered

into Microsoft Access and the Universal Veterinary Information System. Submission forms included the name and address of the submitting clinic or laboratory and the animal's information (species, name, identification number, birthdate, sex, breed, vaccination history, and serum draw date).

This study was conducted to evaluate the detection of rabies antibody in naïve dogs after primary vaccination. Therefore, the dataset was further limited to dogs under one year of age (n = 13,061) and with no documented history of prior rabies vaccination (n = 8,571). Sample data associated with submission forms without an identification number, birth date, serum draw date, and vaccination history were omitted from the study.

All qualifying dogs were categorized into early vaccination (<12 weeks), on-time vaccination (12-16 weeks), and late vaccination (>16 weeks). Data from a subset of dogs (10%) from each category were compared to their original submission form for accuracy of dataset information. Systematic errors were found in a small subset of dogs, which has shorter intervals between vaccination blood draws and biologically implausible rabies titer values. These observations were assessed for errors primarily associated with erroneous dates. Failure to record accurate information either on the original submission or upon transfer into the database was identified to the source of error regarding a higher than expected titer result for these observations. Correction of all records in the dataset with the appropriate information on the submission form was completed, and the records remained in the dataset for analysis. Records that could not be verified and corrected were assumed to be biologically implausible and removed from the dataset. The total eligible sample size was 8,367 dogs.

The sera were analyzed using the Fluorescent Antibody Virus Neutralization (FAVN) test (Cliquet et al 1998). Sera with a titer less than 0.5 IU/ml were considered to have a failed test and a titer greater than or equal to 0.5 IU/ml was considered to have a passing test (WHO). Canine samples were categorized into the following cut-off titer groups: 0 - <0.5 IU/ml (Low responders), ≥0.5 - <1.5 IU/ml (Moderate responders), ≥1.5 - 2.62 IU/m (High responders). The categories were chosen to further delineate the failed and passing groups.

Canine samples less than 1 year of age at the time of vaccination were categorized into three age groups: less than 12 weeks of age, 12-16 weeks of age, and greater than 16 weeks of age. Recommendations from the National Association of State Public Health Veterinarians (NASPHV) state a one-year killed rabies vaccination should be administered not earlier than 12 weeks of age with revaccinations one year following the initial vaccinations (Compendium of Animal Rabies Prevention and Control, 2016). Therefore all canine samples with an initial vaccination prior to 12 weeks were grouped and considered early vaccinations. Most state regulations require that a dog be vaccinated prior to 16 weeks of age, therefore, defining the second age category and considered on schedule. Dogs receiving rabies vaccination later than 16 weeks were considered late immunizations.

The time of initial vaccination to sample draw date was calculated based on dates provided on submission forms and defined as the draw-delay period. Eight draw-delay periods were defined as follows: 1) $\leq$ 3 days, 2) 4 days to $\leq$ 7 days, 3) 8 days to $\leq$ 15 days, 4) 16 days to $\leq$ 35 days, 5) 36 days to $\leq$ 77 days, 6) 78 days to $\leq$ 154 days, 7) 155 days to $\leq$ 224 days, and 8) > 224 days.

The submission form accompanying the sample included an entry titled breed. If the breed of the animal was known then that breed was entered in the space provided. If the breed was unknown then "mixed" was entered into the space provided. If the animal was designated a breed and mixed then the animal was entered into the database as the primary breed of the animal. The analysis did not delineate between different breeds but rather assessed the variables of pure breed verse mixed breed.

Breed was used to categorize dogs into 5 size groups: toy, small, medium, large, and giant. The American Kennel Club (AKC) has defined each size group with a weight range and the breeds included in each size group. The AKC groupings were used to define the size groups in this study.

Statistical Analysis

Statistical analyses were performed with SAS software. Comparison of the frequency for these variables was analyzed using the Cochran-Mantel-Haensel test of significance. Geometric mean titers were calculated by different groups mentioned previously and Analysis of Variance (ANOVA) was used to determine if these means significantly differed. Serological responses were plotted by the delay in drawing the blood to check the titer (draw-delay) and viewed in two ways; proportions failing to reach 0.5 IU.ml and GMT (Figure 1 and 2). A polynomial trend line with 3 orders was determined to provide the best fit for the data.

Results

The number of dog samples less than 1 year of age with no documented history of prior vaccinations before the vaccination given to qualify for pet travel was 8,571. A total of 204 (2.4%) dogs were removed from analysis due to missing or inconsistent data, for a final study sample size of 8,367 dogs (table 1). The number of dog samples that failed the FAVN test (<0.5 IU/ml) included 1,002 (11.7%) samples and the number of dog samples that passed the FAVN test (>0.5 IU/ml) included 7,365 (85.9%) samples. A lower proportion of dogs failed when they were vaccinated at an age greater than 16 weeks compared to dogs vaccinated early or on-time (10.4% compared to 16.3% and 15.5%) (Table 1)

The geometric mean titer (GMT) for all dogs sampled was 1.49 IU/mL (Table 2) with a standard deviation of 1.49.

Further delineation of the sample numbers into three age groups at which the primary vaccination was received is displayed in Table 2. The influence of age on antibody titer response was significant for all three age groups with dogs vaccinated age greater than 16 weeks having the highest GMT (1.6 IU/ml) (Table 2). There is no statistical difference in GMT between dog vaccinated early and dogs vaccinated on-time (1.27 IU/ml compared to 1.27 IU/ml) (Table 2).

Table 1: Rabies Vaccination Data- Dogs less than 1 year of age given only 1 Rabies vaccine

	Samples	%
Total Dogs < 1 year	13,061	
Total Dogs < 1 year & 1 vaccine	8,571	65.6%
Total plausible < 1 year & 1 vaccine	8,367	97.6%
Geometric Mean Titer Value	1.33 IU/ml	
Total Dogs Failed to Reach 0.5 IU/ml	1,002	11.7%
Total Dogs Reached or Surpasses 0.5 IU/ml	7,365	85.9%
Age at Primary Vaccination < 12 weeks	301	3.6%
Passed	252	83.7%*
Failed	49	16.3%*
Age at Primary Vaccination 12-16 weeks	2,291	17.5%
Passed	1,936	84.5%**
Failed	355	15.4%**
Age at Primary Vaccination > 16 weeks	5,775	44.2%
Passed	5,177	89.6%***
Failed	598	10.3%***

*Percentage out of <12 weeks group (301)
*Percentage out of 12-16 weeks group (2,291)
***Percentage out of >16 weeks group (5,77

Table 2: Number of dog samples by titer range and age at primary vaccination

	Rabies Serological Titer Range of Values								
Age at Primary Vaccination	0-<0.5	0.5-≤02.6	>2.62	Total	CMH	GMT	Lower Limit	Upper Limit	ANOVA
< 12 weeks	40 (3.9%)	124 (3.3%)	128 (3.4%)	301 (3.5%)		1.27	1.08	1.49	
12-16 weeks	355 (35.4%)	1,081 (29.5%)	855 (23%)	2,291 (27.3%)		1.27	1.20	1.34	
>16 weeks	598 (59.6%)	2,453 (67%)	2,724 (73.4%)	5,775 (69%)		1.60	1.55	1.66	
Total	**1,002**	**3,658**	**3,707**	**8,367 (100%)**	**<0.01**	**1.49**			**<0.01**

Table 3: Dogs Less than 12 Weeks of Age

	RABIES ANTIBODY TITER VALUES						
		LOW RESPONDERS	**MODERATE RESPONDERS**	**HIGH RESPONDERS**	**CMH**	**Geometric Mean Titer**	**LN ANOVA**
Age at vaccination: <12 Weeks	***TOTAL***	***49 (16.3%)***	***124 (41.1%)***	***128 (42.5%)***		***1.27***	
	Sex [a]				**0.51**		**0.93**
	Male	25 (15.9%)	77 (42.6%)	65 (41.4%)		1.26	
	Female	24 (17.2%)	56 (40.2%)	59 (42.45%)		1.25	
	Unspecified	0	1 (20%)	4 (80%)		1.72	
	Time from Vaccine Administration to Titer Check				**<0.01**		**<0.01**
	≤ 3 days	0	0	0		0	
	4 to ≤ 7 days	1 (25%)	1 (25%)	2 (50%)		1.32	
	8 to ≤ 15 days	0	0	4 (100%)		3.45	
	16 to ≤ 35 days	5 (6.1%)	29 (35.8%)	47 (58%)		1.99	
	36 to ≤ 77 days	15(21.1%)	26 (36.6%)	30 (42.2%)		1.15	
	78 to ≤ 154 days	19 (25%)	37 (48.6%)	20 (26.3%)		0.77	
	155 to ≤ 224 days	5 (15.6%)	14 (43.8%)	13 (40.6%)		1.18	
	≥ 225 days	4 (12.1%)	17 (51.5)	12 (36.3%)		1.49	
	Type of Dog				**0.39**		**0.95**
	Mixed Breed	10 (20.4%)	17 (34.6%)	22 (44.9%)		1.27	
	Pure Breed	32 (14.4%)	97 (44.2%)	90 (41.1%)		1.29	
	Dog Size				**0.77**		**0.46**
	Toy	4 (8%)	25 (50%)	21 (42%)		1.73	
	Small	9 (15.8%)	23 (40.4%)	25 (43.9%)		1.41	
	Medium	7 (18.4%)	15 (29.5%)	16 (42.1%)		1.18	
	Large	11 (16.9%)	30 (46.2%)	24 (36.9%)		1.10	
	Giant	1 (14.3%)	3 (42.9%)	3 (42.9%)		0.83	
	Unspecified	17 (20.2%)	28 (33.3%)	39 (46.4%)		1.13	

Table 4: Dogs 12-16 Weeks of Age

	RABIES ANTIBODY TITER VALUES						
		LOW RESPONDERS	**MODERATE RESPONDERS**	**HIGH RESPONDERS**	**CMH**	**Geometric Mean Titer**	**LN ANOVA**
Age at vaccination: 12-16 Weeks	***TOTAL***	***355 (15.4%)***	***1,081 (47.1%)***	***855 (37.3%)***		***1.27***	
	Sex				**0.10**		**0.25**
	Male	189 (16.7%)	530 (47%)	408 (36.2%)		1.23	
	Female	164 (14.2%)	542 (47%)	445 (38.6%)		1.30	
	Unspecified	2 (15.3%)	9 (69.2%)	2 (15.3%)		0.68	
	Time from Vaccine Administration to Titer Check				**<0.01**		**<0.01**
	≤ 3 days	7 (100%)	0	0		0.02	
	4 to ≤ 7 days	1 (25%)	1 (25%)	2 (50%)		0.43	
	8 to ≤ 15 days	5 (8.4%)	27 (45.7%)	27 (45. 7%)		1.52	
	16 to ≤ 35 days	46 (5.5%)	380 (45.4%)	410 (49.0%)		1.93	
	36 to ≤ 77 days	65 (12.9%)	248 (49.3%)	190 (37.7%)		1.35	
	78 to ≤ 154 days	124 (23.7%)	259 (49.5%)	140 (26.7%)		0.66	
	155 to ≤ 224 days	65 (27.9%)	113 (48.5%)	55 (23.6%)		0.65	
	≥ 225 days	42 (33.3%)	53 (42%)	31 (24.6%)		0.69	
	Type of Dog				**0.19**		**0.34**
	Mixed Breed	34 (13.3%)	133 (52.1%)	88 (34.5%)		1.35	
	Pure Breed	298 (16.2%)	853 (46.3%)	688 (37.4%)		1.24	
	Dog Size				**<0.01**		**<0.01**
	Toy	52 (12.2%)	199 (46.7%)	175 (41.1%)		1.47	
	Small	74 (14.6%)	236 (46.5%)	198 (39%)		1.37	
	Medium	44 (16.7%)	118 (44.9%)	101 (38.4%)		1.25	
	Large	112 (21%)	247 (46.3%)	175 (32.8%)		1.02	
	Giant	11 (11.5%)	49 (51%)	36 (37.5%)		1.44	
	Unspecified	62 (13.4%)	232 (50%)	170 (36.6%		1.25	

Table 5: Dogs Greater Than 16 Weeks of Age

		RABIES ANTIBODY TITER VALUES					
		LOW RESPONDERS	**MODERATE RESPONDERS**	**HIGH RESPONDERS**	**CMH**	**Geometric Mean Titer**	**LN ANOVA**
Age at vaccination: >16 Weeks	***TOTAL***	***598 (10.3%)***	***2,453 (42.4%)***	***2,724 (47.1%)***		***1.60***	
	Sex				**0.47**		**0.44**
	Male	291 (9.8%)	1263 (42.9%)	1386 (47.1%)		1.62	
	Female	301 (10.8%)	1161 (41.7%)	1316 (46.3%)		1.58	
	Unspecified	6 (8.9%)	29 (51.7%)	22 (39.2%)		1.34	
	Time from Vaccine Administration to Titer Check				**<0.01**		**<0.01**
	≤ 3 days	40 (100%)	0	0		0.05	
	4 to ≤ 7 days	11 (8.5%)	35 (37.2%)	51 (54.2%)		1.65	
	8 to ≤ 15 days	19 (5.25%)	116 (32.%)	227 (62.7%)		2.22	
	16 to ≤ 35 days	66 (2.9%)	826 (36.8%)	1351 (60.2%)		2.30	
	36 to ≤ 77 days	119 (8.5%)	655 (47%)	618 (44.4%)		1.63	
	78 to ≤ 154 days	214 (19.7%)	544 (50.2%)	324 (29.9%)		1.04	
	155 to ≤ 224 days	113 (23.2%)	240 (49.4%)	132 (27.2%)		0.84	
	≥ 225 days	19 (24.6%)	37 (48%)	21 (27.2%)		0.76	
	Type of Dog				**<0.01**		**<0.01**
	Mixed Breed	66 (8%)	343 (41.8%)	410 (50%)		1.77	
	Pure Breed	498 (10.8%)	1944 (42.4%)	2138 (46.6%)		1.57	
	Dog Size				**<0.01**		**<0.01**
	Toy	124 (9.2%)	563 (41.7%)	664 (49.1%)		1.67	
	Small	142 (10.6%)	577 (43.1%)	620 (46.3%)		1.60	
	Medium	56 (10%)	241 (43.2%)	261 (46.8%)		1.71	
	Large	144 (12.9%)	480 (42.9%)	495 (44.2%)		1.42	
	Giant	29 (14.9%)	74 (37.9%)	92 (47.2%)		1.44	
	Unspecified	102 (8.4%)	518 (42.7%)	592 (48.8%)		1.61	

Among dogs vaccinated yearly, sex and breed-type had no statistically significant influence on the measured antibody response (CMH p=0.51 and 0.39, ANOVA p=0.93 and 0.95). The influence of the draw-delay period on titer response was statistically significant (CMH, ANOVA p= <0.01). The time between vaccination and blood draw was significantly associated with titer value, with the highest GMT seen between days 8-35 (3.45 and 1.99 IU/ml). A greater proportion of dogs were classified as "high responder" when their titer was checked 8-77 days after vaccination.

Among dogs vaccinated on-schedule, sex and breed did not influence their antibody response (CMH p=0.1, 0.19, ANOVA p=0.25, 0.34). Dog size did influence antibody response (CMH, ANOVA p<0.01) and large breed dogs had the highest failure rate (21%) and lowest GMT (1.02). The influence of the draw-delay period on titer response was statistically significant (CMH, ANOVA p= <0.01). The overall GMT was 1.27 and 15.4% of dogs failed. Failure rates were higher when titers were checked within 7 days and after 78 days of vaccination. The highest GMT occurred when titers were checked 4-35 days post-vaccination. After day 36-post vaccination an inverse relationship between was observed between titer response and delay in titer check with titer response decreasing as the delay period increased.

Dogs vaccinated after 16 weeks of age has no significant association between rabies antibody titer and the dog's sex (CMH p=0.47, ANOVA p=0.44). The influence of breed on antibody response was statistically significant both in terms of titer group and GMT (CMH, ANOVA p= <0.01), mixed breed dogs had a higher GMT (1.77 vs 1.57) and lower failure rate (8.0% vs 10.8%) compared to pure breed dogs. The influence of size on antibody response was statistically significant for both titer group and GMT (CMH, ANOVA p= <0.01), toy, small and medium dogs had a higher GMT than large and giant dogs. The influence of the draw-delay period on titer response is was statistically significant (CMH, ANOVA p<0.01). The highest GMT was observed when titers were drawn between days 4-36 days post vaccination. After day 36-post vaccination there was an inverse relationship between titer response and the draw-delay period with titer response decreasing as the draw-delay period increasing The highest rate of failure is at day 3 or less and greater than 78 days. The failure rate was higher when the titer was checked 78 days or greater after vaccination. The failure rate was 8 times higher among dogs that had a titer draw-delay of 225 days or greater compared to dogs sampled at 16-35 days post-vaccination.

When serological data were plotted by the delay between vaccination and titer draw, all vaccination age groups showed a polynomial relationship (Figure 1). Rates of failure were highest when titers were drawn shortly after vaccination with the failure rate dropping below 20% when titers were drawn approximately 10 days after vaccination. According to the polynomial trend lines, failure rates were relatively low when titer was checked between 12 and 27 days post vaccination. Failure rates rose when titers were checked approximately 25 days post-vaccination. A similar relationship was noted among GMTs, with titers low within the first five days post-vaccination, raising to their peaks 15 days post-vaccination, and declining steadily after (Figure 2). Dogs vaccinated on time and late have higher-squared values (0.4, 0.79). Model fit was poor for dogs vaccinated early (r-square = 0.04 and 0.1).

Figure 1: Proportion of dogs failing the antibody titer test

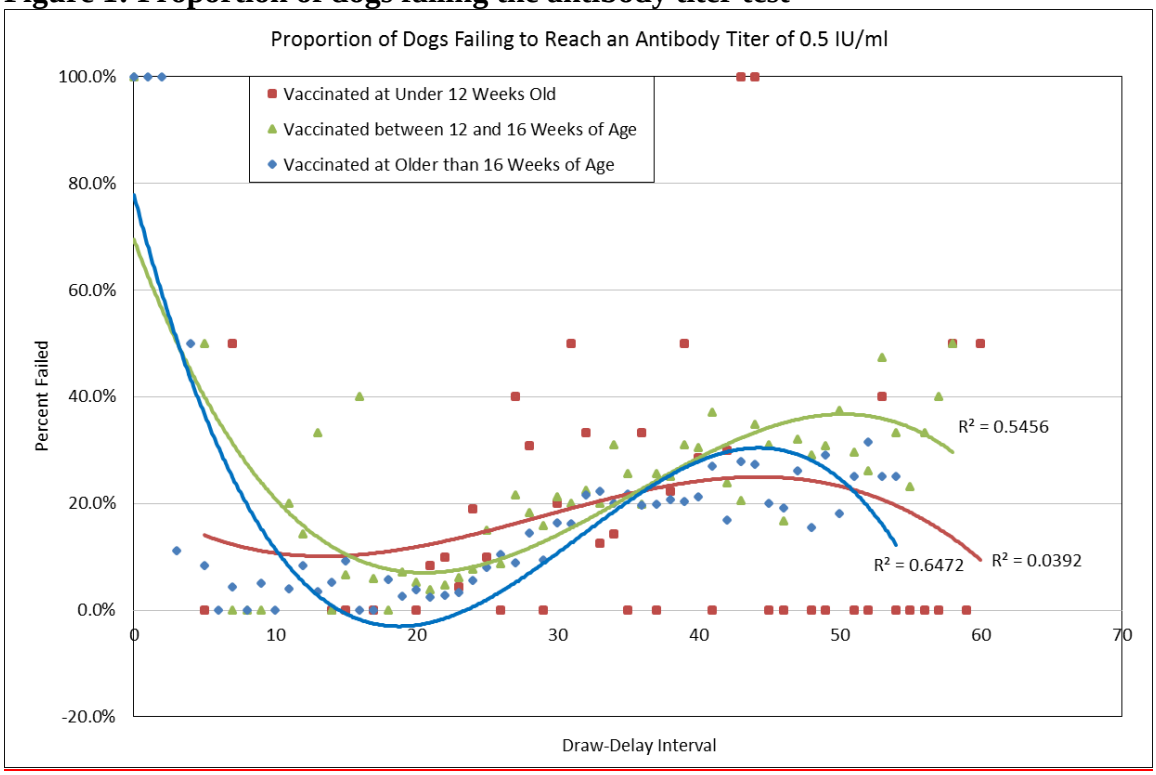
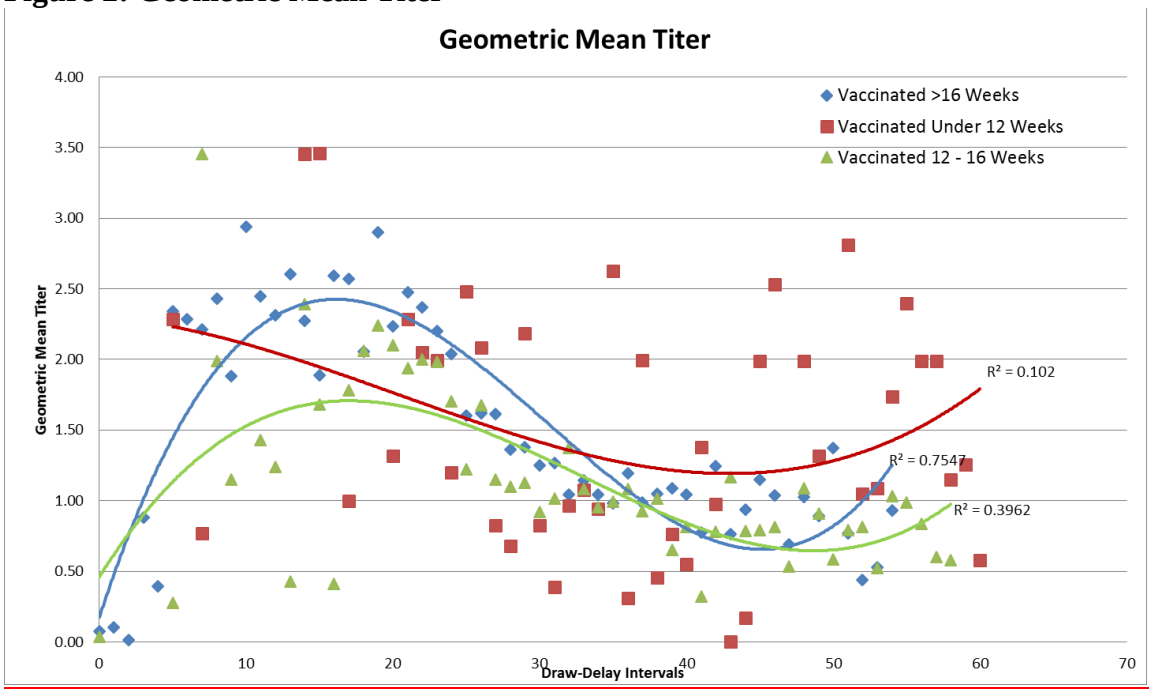


Figure 2: Geometric Mean Titer



Discussion

This study conducted the largest retrospective review of rabies vaccine naïve dog's response to primary vaccination. Dogs vaccinated later than 16 weeks of age had a higher GMT; however, early vaccination was not associated with a higher rate of failure nor was it associated with lower GMT when compared to dogs vaccinated on time. Some demographic factors may influence serological responses to vaccination such as breed-type and dogs size but their influence appears minimal and needs further, targeted exploration. In this study, the greatest risk for a dog failing to achieve an adequate immune response to rabies vaccination was associated with the timing of the blood draw following vaccination. Sample collection occurring too early or too late resulted in a higher failure rate.

In today's globalized world, people and their pets can now traverse continents in a matter of hours. While this progress has eased international trade and communications, a pathway for infectious diseases to travel rapidly across vast distances has emerged. Countries that have undergone the financial and physical responsibility to eliminate canine rabies virus have a high priority when it comes to protecting that status. Most canine rabies-free countries require evidence of vaccination for the purpose of pet travel. Serological testing for rabies antibodies is intended to validate that an animal has achieved an appropriate immunologic response following vaccination. If achieved, the animal is assumed to pose no rabies importation risk to the country. While many rabies-free countries require proof of a titer test prior to entry, each individual country may have additional time sensitive deadlines for time of vaccination and sample collection for testing. This study, consistent with literature from Tepsumethanon et al 1991, Sage et al 1993, Sihvonen et al 1995, Cliquet 2003, Mansfield et al 2004, Kennedy et al 2007, Jakel et al 2008, and Berndtsson et al 2011) showed a high risk of failure to document serological conversion when titers were drawn prior to 8-days and after 77 days from the time of vaccination. Failure to document serological conversion may result in added expense for the owner to repeat the test, additional vaccine booster doses for the dog, of the denial of entry of a pet into a country, or costly in-country quarantine. For pet owners, coordinating travel plans in alignment with meeting vaccination and titer requirement need to be well calculated to ensure successful entry into the importing country.

Previous studies have shown that dogs less than 1 year of age have a higher risk of failure to achieve an adequate rabies titer after vaccination (Mansfield et al 2004, Kennedy et al 2007,

Berndtsson et al 2011, Seghaier et al 1999). Two reasons likely explain this finding. First, dogs under one year of age are likely to be receiving their first rabies dose and a primary response is being measured. The second reason is that older dogs potentially have two advantages; they have a more mature immune response compared to very young dogs (Day 2007), and they may have had more than one vaccine and are displaying an anamnestic response to vaccination (Seghaier et al., 1999). This study exclusively looked at characteristics of dogs less than one year of age with a history of only one rabies vaccination to ensure that only factors associated with failure after primary vaccination was measured. Consistent with other studies, this analysis showed that vaccination at older age was associated with a significant decrease in probability of failure. This retrospective study was not designed to explain why older age was associated with higher GMT; however, it is likely due to the maturation of the functional immune system as animal's age or the absence of maternal antibody interference.

The World Health Organization (WHO) recently revised recommendations for canine rabies endemic countries to vaccinate dogs of any age that present to a mass vaccination clinic (WHO Expert Consultation). The decision was based primarily on the need to eliminate time wasted at vaccination clinics to verify animal age and to maximize the campaign's ability to vaccinate a dog that would otherwise not be vaccinated. The results of this study indicate that vaccinating dogs at an earlier age (less than 12 weeks) is likely to be just as successful as vaccination of dogs at the ages recommended by nation and manufacturer recommendations. This study did not evaluate the effect of very early-age vaccination and age at vaccination was categorical, but the findings support the WHO stance on vaccination of all dogs presented to a mass vaccination campaign regardless of age.

The association between dog breed or size and the probability of mounting an adequate serological response has also been evaluated by; Berndtsson 2011, Kennedy 2007, and Zanoni 2010. In these papers, large breed dogs appear to fail at a higher rate compared to small breed dogs. A similar association was found in this study with significant differences in failure rate and GMT between both breed type and dog size. Various theories attempt to explain the effect of this variable, primarily breed-specific genetic traits and the delivered antigenic load in a vaccine dose. The antigenic load is proportionally larger to a small breed dog and may stimulate a more robust response in the small and toy breeds. This study is not able to further explain the effect of breed and size as the associations are not strong and possible attributable to a multitude of factors.

Studies designed to specifically address this relationship should be carried out to differentiate the roles of genetic factors, antigenic load, and likely other factors at play.

The recent revisions in national guidelines for managing animals with potential rabies exposures recommend checking titers for animals with questionable vaccination history for evidence of an anamnestic response (significant rise in titer and a value of at least 0.5 IU/ml) (NASPHV Compendium of Animal Rabies Prevention and Control, 2016, Part I B.5(4b)). The recommendation is based on the theory that only animals with previous history of vaccination will show a robust immune response within seven days of a rabies booster vaccination. In this study, a large proportion of rabies vaccine-naïve dogs showed a robust immune response within only seven days; 61% achieved a serological response above 0.5 IU/ml within only seven days. The high percentage of dogs in this study reaching 0.5 IU/ml RVNA level at day 7 could be attributed to the presence of maternal antibodies (especially at less than 12 weeks of age) (Day 2007) of undocumented previous vaccination (especially in dogs vaccinated at greater than 16 weeks of age). Evidence of a robust immune response to vaccination likely confers immunity against developing rabies; however, this can never be guaranteed. The findings from this study suggest that detection of an immune response at 0.5 IU/ml within seven days of rabies vaccination cannot be used to establish a history of vaccination.

Limitations

This retrospective study was conducted using a large dataset of information and was subject to error from two major sources; the recorded submission forms at the submitting clinics and data entry at the receiving laboratory. Additional areas within this study that could lead to misinterpretation of the data include; distribution of data into variable categories, the handling of missing data, variance of laboratory methods, and a significance bias given the statistical tests chosen. While the analysis performed was intended to be preliminary, repeating the analysis using a logistic regression would allow the strength of association to be better quantified.

Conclusion

This study provides additional findings that could potentially have implications for current and future guidelines as they pertain to pet travel, vaccination campaigns, and exposure recommendations. Furthermore, the study provides insight into additional research needed within

the area of rabies serology specifically as it pertains to the influence of breed or dog size, primary versus anamnestic responses to vaccination, and smaller sampling intervals to determine the initial detection of immunocompetence.

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