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Impact of rabies vaccination history on attainment of an adequate antibody titre among dogs tested for international travel certification, Israel – 2010-2014

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Impacts

- Many countries require demonstration of an adequate level of anti-rabies antibodies in the blood (i.e., rabies titre of 0.5 IU/ml) to permit entry of dogs traveling internationally.
- We analysed rabies titres of dogs seeking travel certification in Israel to assess demographic and vaccine history factors associated with not having an adequate rabies virus neutralizing antibody (RVNA) titre for travel certification.
- Only having received one previous rabies vaccination and a longer time since the last vaccination was received were associated with not achieving an adequate RVNA titre for travel certification.
- These findings reiterate the importance of the first booster vaccination for ensuring dog populations are protected against rabies.

Summary

Rabies is endemic in wildlife or domestic carnivore populations globally. Infection of domestic dogs is of particular concern in many areas. In regions where domestic animals are at risk of exposure to rabies virus, dogs should be routinely vaccinated against rabies to protect both pet and human populations. Many countries require demonstration of an adequate level of serum rabies neutralizing antibodies to permit entry of dogs during international travel. We analysed rabies titres of dogs seeking travel certification in Israel to assess demographic and vaccine history factors associated with antibody titres below the acceptable threshold for travel certification. Having received only one previous rabies vaccination and a longer duration since the most recent vaccination was received were primary risk factors for not achieving an adequate RVNA titre for travel certification. These risk factors had stronger effects in younger animals, but were consistent for dogs of all ages. In particular, these findings reiterate the importance of administering at least two rabies vaccinations (the primo vaccination and subsequent booster) to ensure population-level protection against rabies in dogs globally.

Key words: dogs, global travel, immunity, Israel, prevention, rabies, serology, vaccination

Introduction

Rabies is endemic in wildlife and domestic carnivore populations globally. In regions where domestic animals are at risk of rabies virus exposure, dogs should be routinely vaccinated against rabies to protect both pet and human populations from this nearly invariably fatal infection (WHO, 2015). In many countries rabies vaccination protocols are legally prescribed. Proof of rabies vaccination is typically required as a condition for international pet travel, both due to the risk of rabies virus exposure in endemic destinations and the risk of rabies virus introduction to rabies-free areas by unimmunized animals during travel (reviewed in Lankau *et al.*, 2014). Countries vary in their dog entry regulations, which may

include a combination of age and identification method (e.g., microchipping) requirements, documentation of having received rabies vaccine a sufficient duration prior to travel to mount an immune response (i.e., proof of vaccination), serologic demonstration of immunity prior to travel, or a quarantine period before or after arrival (examples of different country requirements may be located at USDA, 2015).

During the early 1990s, many countries converted from a strict quarantine requirement for domestic dog entry to requiring serological evidence of immunity (Cliquet *et al.*, 2003). These changes were driven by both increasing interest in free-circulation of people and animals among countries and improved scientific understanding of the relationship between rabies antibody titre levels in dogs and cats and resistance to infection upon exposure (Aubert 1992, WHO 1992, Cliquet *et al.*, 2003). In challenge experiments, a rabies virus neutralizing antibody (RVNA) titre of ≥0.5 international units (IU)/ml correlated best with protection from rabies virus infection on exposure (Aubert, 1992). The World Health Organization (WHO) designated RVNA titres of ≥ 0.5 IU/ml in an actively immunized dog >16 weeks of age as the standard for certifying protection against rabies infection (WHO, 1992). Since 1993, the World Organization for Animal Health (OIE) has recommended requiring serologic evidence of immunity by quantification of RVNA whenever dogs or cats are imported from countries with endemic rabies virus circulation to areas that are considered rabies free (OIE, 1996). Many countries require demonstration of an adequate RVNA titre (≥0.5 IU/ml) for international movement of pets (in the European Union for example: EU, 2003). Dogs with lower titres or even without detectable antibodies have survived virulent rabies challenge (Sikes *et al.*, 1971; Brown *et al.*, 1973; Barth and Jaeger, 1977; Ganiere *et al.*, 1989; Aubert, 1992).

In Israel, rabies is a notifiable disease according to the Animal Disease Ordinance (New Version) of 1985 and the Rabies Ordinance of 1934 (FAO, 2001; Israel Ministry of

Agriculture and Rural Development, 1934). Since 1956, domestic dogs in Israel must be vaccinated against rabies by law, first at three months old and then annually (Israel Ministry of Agriculture and Rural Development, 2015). Legally mandated vaccination of dogs substantially shifted the dominant rabies reservoir. While dogs were the most commonly affected through the mid-1950s (Nobel & Neumann, 1962; Yakobson *et al.*, 2004), red foxes (*Vulpes vulpes*) and to a lesser extent golden jackals (*Canis aureus*) became the primary rabies reservoirs after 1956. During the mid-1970s, sylvatic fox rabies virus variant surpassed the canine variant (Yakobson *et al.*, 1998). Since 1998 wildlife rabies has been controlled through the use of oral rabies vaccines (Yakobson *et al.*, 2006). However, despite mandatory dog vaccination, canine rabies has re-emerged in northern Israel, resulting in rabies cases in unvaccinated dogs and other species (David *et al*., 2009; David, Bellaiche, and Yakobson, 2010; David and Yakobson, 2011).

Given continued rabies virus transmission in Israel, dogs must be tested to ensure adequate RNVA titres (≥0.5 IU/ml) for travel certification. This study used data obtained from routine pre-travel testing of dogs to explore factors associated with failure to achieve adequate RNVA titres for travel in vaccinated dogs. We consider how these findings may inform broader discussions about vaccination strategies for domestic pets.

Materials and methods

Data source

Dogs travelling to certain countries outside of Israel are required to have an RVNA titre ≥0.5 IU/ml (hereafter referred to as an adequate RVNA titre for travel). The National Rabies Laboratory at the Kimron Veterinary Institute, part of the Israeli Veterinary Services and Animal Health (IVSAH), has performed travel certification serology (hereafter referred to as a pre-travel titre) since 2004.The laboratory is accredited by the National Laboratory Accreditation Authority and annually meets the requirements of inter-laboratory testing

organized by the EU-designated Institute AFSSA-Nancy (France). Serum RVNA were measured using the rapid fluorescent focus inhibition test (RFFIT; Smith *et al.*, 1973, modified by Zalan *et al.*, 1979).

Dog licensure is mandatory in Israel and requires identification by microchip, registration in a central database and having recorded vaccination against rabies during the last year. Annual re-vaccination is required to maintain validity. The IVSAH is responsible for management of the national computerized dog registration database, which includes each animal's age, sex and vaccination history.

Study design

Data were extracted from the IVSAH national dog registration database held by including a study population of dogs presented for travel certification RVNA titres from 3rd January 2010 to 19th May 2014. The following variables were extracted from the national registry for each dog as explanatory variables (i.e., putative risk factors): sex; age at most recent rabies vaccination prior to blood draw for the pre-travel titre (in months; hereafter "age at most recent vaccination"); number of rabies vaccinations prior to blood draw for the pre-travel titre (hereafter "number of previous vaccinations"), and time between the most recent rabies vaccination and blood draw for a pre-travel titre (in days; hereafter "gap between vaccination and titre"). These records were linked to the date and outcome of the pre-travel titre reported by the Kimron Veterinary Institute by microchip identification number. Microchip numbers were subsequently removed to protect owner privacy.

We then performed a retrospective case-control analysis, where ***cases*** were defined as dogs presented for testing that ***did not*** achieve an adequate RVNA titre for travel, and ***controls*** were those presented for testing that ***did*** achieve an adequate RVNA titre for travel. Controls were randomly selected stratified by year with a 1:1 case-to-control ratio using the

125 random number function in Microsoft Excel (v. 2010, Microsoft Corporation, Washington,
126 USA).

127 ***Data analysis***

128 Associations among putative risk factors and between these factors and titre status (case or
129 control) were assessed using a Spearman's rho rank correlation for associations between two
130 continuous variables, a t-test between continuous and binary variables, or the X^2 or Fisher's
131 exact test between two binary variables. Strength of associations was expressed as an odds
132 ratio (OR) with 95% confidence interval (CI; Taylor series, Dean, Sullivan and Soe, 2015).
133 An odds ratio that is significantly greater than one indicates that the risk factor is associated
134 with increased likelihood of failing to achieve an adequate RVNA titre for travel. Where
135 significant associations between risk factors were detected, stratified analyses were
136 performed to consider the effects of confounding on univariate results.

137 Since the incremental impact of continuous factors may not necessarily be linear, risk
138 factors were transformed into binary categories for some analyses. Categories were defined
139 by visual examination of each variable's distribution for natural breaks or based on pertinent
140 biological information (e.g., 15 months is the age at which dogs would typically receive a
141 second rabies vaccination). Continuous variables converted to binary categories were age at
142 most recent vaccination (≤15 month old or >15 months old), number of previous vaccinations
143 (only one vaccination or >1 vaccination received), and the gap between vaccination and titre
144 (≤60 days or >60 days).

145 Multivariate logistic regression modelling was then performed to provide adjusted
146 ORs for each risk factor. Logistic regression with forwards and backwards stepwise model
147 selection was performed, with the criteria for entry and exit of parameters being a significant
148 change in the model deviance as judged by a p-value of ≤0.1.

First, a "base" model was constructed for model selection using all putative risk factors (sex, age at most recent vaccination, number of previous vaccinations, and the gap between vaccination and titre). Different variables were offered as starting variables in repeated runs to assure that the final model was not dependent on the order of factor entry and exit. This base model had no restrictions on variable entry or exit from the model.

We then constructed additional multivariable logistic regression models to consider potential confounding between age at most recent vaccination and other putative risk factors before arriving at a final model. Due to concern that effects of age at most recent vaccination could be confounded by associations with other variables, a second model was constructed in which the age at most recent vaccination variable was forced to remain in all models through the model selection process ("age forced" model). Next, two age-stratified models were constructed by model selection, one using only the data for young animals (≤15 months at most recent vaccination; "young" model) and one using only the data for adult animals (>15 months at most recent vaccination; "adult" model). Then, a final model was built guided by the findings of these exploratory models and including biologically relevant interaction terms.

This final logistic regression model produced OR estimates adjusted for complex associations among multiple factors and failure to achieve an adequate RVNA titre for travel that were then used to estimate the odds of failing to achieve an adequate RVNA titre for travel (i.e., scenario risk assessments), given specific combinations of factors (scenarios) for variables included in the model (e.g., for a young dog, having had only one vaccination within 60 days of the test). Odds was converted to probability (risk) of failing to achieve an adequate RVNA titre for travel using the equation: probability = odds/(1+odds).

Finally, to assess representativeness of findings for the broader registered dog population, a sample was extracted from Israel's national dog registration database to serve as a snapshot of the overall registered dog population's vaccination history. Demographic and

vaccine history of the registered dog population during August 2013 was qualitatively compared to the population of dogs presented for travel certification during 2013.

All statistical tests and regression modelling were carried out using the statistical package Statistix version 10 (© 1985-2013 Analytical Software, Tallahassee, FL, USA).

Results

Sample description

From 3rd January 2010 to 19th May 2014, 4,949 dogs presented for travel certification, evenly distributed across years (range of 1,000-1,200 dog/year). Of these, 395 (8.0%) did not have an adequate RVNA titre for travel but many of these did have detectable RVNA below 0.5 IU/ml (for these, median titre=0.18 IU, range=0.02-0.48 IU). Forty nine of these 395 cases were excluded due to incomplete records for one or more necessary variables. Therefore 346 cases and 346 controls (692 dogs total) were selected for analysis.

Univariate and stratified analysis

Approximately half of both cases (49%) and controls (47%) were male (Table 1a). A significantly larger portion of cases received only one vaccination prior to presentation for pre-travel titre (85% versus 35.3% of controls), had received the most recent vaccination at ≥ 15 months old (62.4% versus 27.5% of controls), and had a gap of >60 days between vaccination and titre (80.3% versus 60.1% of controls; Table 1a).

Mean gap between vaccination and titre did not differ significantly between cases (173 days) and controls (160 days; T-test: p-value=0.3896). However, despite similar means, the distribution of gap between vaccination and titre was different between cases and controls (Figure 1). Specifically, 40% of test dates for the controls fell within 60 days of the most recent vaccination compared to only 20% for the cases (X^2 test: p-value<0.0001).

Assessment of associations among these putative risk factors revealed a notable potential confound between the number of previous vaccinations and the age at most recent

vaccination. Both variables differed significantly between cases and controls as both binary categories (Table 1a) and in the original continuous variable (Spearman rank coefficient =0.6854, p-value<0.0001; Table 2). The mean age at most recent vaccination for dogs having received only one previous vaccination was 15.5 months, compared with 53.3 months for dogs that had received more than one previous vaccination (T-test: p-value <0.0001; Table 2).

Given this association between age at most recent vaccination and number of previous vaccinations, two stratified analyses were performed. When stratified by the number of previous vaccinations, age at most recent vaccination was not significantly associated with not having an adequate RVNA titre for travel (i.e., being a case; Table 2b), yet when stratified by age at most recent vaccination, the number of previous vaccinations was significantly associated with the case outcome and with a similar OR for both age groups (Table 2c).

Significant associations were not noted among other putative risk factors; for this reason, additional bivariate analyses were not performed.

Logistic regression modelling

The base logistic regression model retained two significant factors: having only one previous rabies vaccination and having a > 60 day gap between vaccination and titre (Table 3). When age at most recent vaccination was forced to remain in the model (age forced), age was not significant and the model was otherwise similar to the base model, indicating no significant direct influence of age. In the stratified models for either young or adult dogs, the ORs for number of previous vaccinations and gap between vaccination and titre differed from that in the base model (although with wider 95% CIs), suggesting that age may have some modifying effect on the influence on these factors (Table 3). Finally, when interaction terms (age at most recent vaccination x number of previous vaccinations, age at most recent

vaccination x gap between vaccination and titre, and number of previous vaccinations x gap between vaccination and titre) were included in the final model selection, the interaction between age at vaccination and gap between vaccination and titre was significant and both variables were retained in the final model (Table 4).

Scenario risk estimation

The highest estimated risk of failure to achieve an adequate RVNA titre for travel was for dogs tested > 60 days after receiving their first vaccination (81% for young dogs;73% for adults; Figure 2). In contrast, estimate risk of failure to achieve an adequate RVNA titre for travel was lowest for dogs that had received one or more booster vaccination and were tested within 60 days of receiving the most recent vaccination (8% in young dogs; 13% is adults; Figure 2).

Evaluation of study representativeness

The snapshot of 367,388 registered dogs in the national dog registration database during August 2013 was compared to dogs in the study population during 2013. The registered dog population sex ratio (50% male) was similar to that of travelling dogs (48%). The proportion of young animals (≤15 months) was less in the registered population (7%) than for dogs presented for pre-travel testing (24%). The difference in the proportion of dogs with only one vaccination was smaller: 29% of the registered dogs had only one rabies vaccination compared with 36% of the travelling dogs.

Discussion

Failure to achieve adequate RVNA titre for travel occurred in approximately 8% of the study population of dogs presenting for travel certification in Israel during January 2010-May 2014. However, many dogs failing to reach the threshold for travel certification (0.5 IU/ml) did have a detectable RVNA titre and may or may not have had sufficient protection against rabies virus if exposed.

Case-control analysis suggested higher odds of failure to achieve an adequate RVNA titre for travel (cases in this analysis) in primo vaccinates or dogs vaccinated >60 days prior to blood collection for titre. Effects of age (measured in this study as the age at most recent vaccination) were confounded by correlation of this variable with the number of previous vaccinations received. This association is not unexpected, as young animals will more typically have only received a single documented vaccination when dogs are receiving rabies vaccination on the recommended schedule. Stratified analysis suggested that the number of previous vaccinations was the driving variable in the observed relationship, with fewer dogs having received more than one vaccination in cases compared to controls in both the young (≤15 mo) and older (>15 mo) groups. In contrast, age group proportions did not differ between cases and controls when stratified by the number of previous vaccinations, a finding supported during exploratory multivariate analysis by the negligible impacts of forcing retention of the age at most recent vaccination variable during model selection.

The strongest explanatory variables in the final logistic model was the number of previous vaccinations, followed by the gap between vaccination and titre. In this model, age at most recent vaccination was not itself a significant effect but did significantly interact with the gap between vaccination and titre, with a higher odds of failure to achieve an adequate RVNA titre for travel in young animals with a >60 day gap. The estimated odds of failure to achieve an adequate RVNA titre for travel for dogs with only one previous rabies vaccination was approximately 3x higher than those with more than one previous vaccination if tested within 60 days and was 5x higher if tested after 60 days.

The sub-population of dogs presented for travel certification contained more young dogs and more dogs with only one previous rabies vaccination compared to the registered dog population in Israel. This suggests that the 8% of dogs that failed to achieve an adequate titre for travel in the study population may be an overestimate for the general dog population in

Israel. Of dogs in the study population that failed to achieve an adequate titre, 36% had received only one vaccination when blood was drawn for pre-travel titre, whereas 29% of the general population had only one rabies vaccination.

These findings agree with previous work in assessing travel titre levels in dogs which generally find that age, time since vaccination, and in particular booster vaccination are critical factors for a reasonable assurance of protection against rabies during travel, as measured by adequacy of RVNA titre levels (Cliquet et al., 2003; Zanoni et al., 2010; Berdtsson et al., 2011; Klevar et al., 2015). While dogs with titre values below the 0.5 IU threshold accepted for travel could be protected, assurance of protection is less certain below this accepted titre value (Aubert, 1992). The practical implication of these results is that dogs should not be considered to have strong assurance of being protected from rabies virus infection until they have received at least two vaccinations. While risk of failing to achieve an adequate titre for travel certification is highest in young dogs, who under current vaccination schedules in many countries will not receive a rabies booster vaccination until over one year of age, our study suggests that the risk for adult primo vaccinates is also elevated. Rescue animals in particular may be a particularly high-risk group for failure to achieve sufficient antibodies for assurance of protection due not only to being primo vaccinates but other health issues that my reduce vaccine efficacy in these populations (Klevar et al., 2015).

Dog rabies vaccination protocols are well established and largely agreed upon by public health advisory bodies and vaccine manufacturers (WHO, 1992; Brown *et al.*, 2011; OIE, 2013). A single dose of rabies vaccine is generally sufficient to immunise, due to the potent glycoprotein G antigen included along with a powerful adjuvant (Petrovsky and Aguilar, 2004). Available canine rabies vaccines are licensed as providing either a one or three year duration of immunity (DOI) (Brown *et al.*, 2011) and when required in regulations, the timing of subsequent doses is typically determined by this licensed DOI except for the

timing of the first booster dose. To ensure adequate population-level protection, a second dose at up to a year after the first dose is strongly recommended to improve titres in individuals with insufficient primary antibody response (Brown *et al.*, 2011). Low antibody production on initial vaccination is of particular concern in puppies due to potential interference from maternally derived antibodies.

In countries where dog vaccination is routine and obligatory, most puppies are born with protective levels of maternally derived antibodies that will gradually decline to a level that allows successful active immunization at between six and 12 weeks of age (Aghomo *et al.*, 1990; Mitmoonpitak & Tepsumethanon, 1998). In the period of waning of maternal antibodies prior to development of active immunity young animals may not be protected (Mitmoonpitak & Tepsumethanon, 1998; Clark & Wilson, 1996). Maternally-derived antibody levels and rate of decline vary such that some puppies may respond poorly to vaccination up to 12 weeks of age or older.

A common protocol for rabies vaccination specifies initial vaccination of puppies at eight to twelve weeks of age then a second vaccination one year later, followed by booster vaccinations at one or three year intervals, depending on the licensed DOI of the vaccine used and country regulations (Brown *et al.*, 2011). In contrast, vaccination programs in canine rabies endemic areas assume that many puppies will not have maternal antibodies to interfere with primary vaccination. However, poor responders to primo vaccination will occur in all dog populations, resulting in a low but real risk for rabies in these animals if exposed to rabies virus.

The World Small Animal Veterinary Association recently recommended that a second dose of vaccine should be given two to four weeks after the first dose in high-risk regions, if permitted by law (Day *et al.*, 2010). Similarly, the European Food Safety Authority has suggested that more proximate booster vaccination (within 4-6 weeks) would reduce risk of

rabies translocation by insufficiently protected primo vaccinates even more effectively than monitoring for a serologic threshold prior to travel (ESFA, 2006). However, compliance with a shortened booster schedule for rabies vaccination could be poor if recommendations are not aligned with other vaccination schedules. Further study would be beneficial to determine the ideal timing of the first booster vaccination to reduce the risk period during which titre levels may have fallen below the desired protection threshold in low-responders at primo vaccination. After receiving the first booster, providing additional booster vaccinations on the schedule determined by the vaccine's licensed DOI and local regulation is important to ensure sustained immunity. However, in order to maximise rabies protection in the general dog population, the first priority should be to ensure as many dogs as possible have received ***at least two*** vaccinations.

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Figure Legends

Figure 1: Distribution of gap between most recent rabies vaccination and presentation for pre-travel titre.

Figure 2: Estimated risk of failure to have an adequate RVNA titre for different scenarios, estimated using the logistic regression model. “Gap” refers to the time passed between the most recent rabies vaccination and presentation for pre-travel titre.

Tables

Table 1. Preliminary analysis of variables associated with adequacy of rabies neutralizing antibody titre in dogs presenting for travel certification, Israel – Jan. 2010 to May 2014

(a) INITIAL UNIVARIATE ANALYSES					
Variable (exposure factor)		**n**	**%'exposed'**	**Odds ratio (95% confidence interval) &Yates' Corrected Chi^2**	**p-value (2 tail)**
Gender (male)	Cases	276‡	49.3%	1.12 (0.81 to 1.53)	p=0.546
	Controls	344‡	46.5%		
Number of previous vaccinations (Only one)	Cases	346	85.0%	10.38 (7.18 to 15.00)	p<0.0001$
	Controls	346	35.3%		
Age at most recent vaccination (≤15 months)	Cases	346	62.4%	4.39 (3.18 to 6.05)	p<0.0001$
	Controls	346	27.5%		
Gap between vaccination and titre (>60 days)	Cases	346	80.3%	2.71 (1.93 to 3.82)	p<0.0001$
	Controls	346	60.1%		
(b) STRATIFIED ANALYSES: effect of age at most recent vaccination for dogs with a different number of previous vaccinations.					
Variable (exposure factor)		**n**	**%'exposed'**		
STRATUM: only one vaccination — Age at most recent vaccination ≤15 months	Cases	294	72.4%	1.14 (0.72 to 1.82)	p=0.6509
	Controls	122	69.7%		
STRATUM: >1 vaccination — Age at most recent vaccination ≤15 months	Cases	52	5.8%	1.31 (0.35 to 4.94)	p=0.9138*
	Controls	224	4.5%		
(c) STRATIFIED ANALYSES: effect of number of previous vaccinations for dogs most recently vaccinated at different ages.					
Variable (exposure factor)		**n**	**%'exposed'**		
STRATUM: vaccination at ≤15 months — Only one vaccination received	Cases	216	98.6%	8.35 (2.24 to 31.09)	p=0.0012*,$
	Controls	95	89.5%		
STRATUM: vaccination at >15 months — Only one vaccination received	Cases	130	62.3%	9.56 (5.81 to 15.72)	p<0.0001$
	Controls	251	14.7%		

‡some cases did not have gender recorded

**Fisher exact p-value used here because conditions not met to use X^2*

$Significant at α<0.05.

Table 2: Comparison of mean age of cases and controls for the whole dataset and, separately, for dogs with only one and dogs with more than one previous rabies vaccination.

Number of previous vaccinations	Group	Mean age* in months (±SE)	p-value**
≥1 vaccinations (all dogs in study)	Cases (n=346)	20.5 ±1.6 mo	<0.0001$
	Controls (n=346)	40.7 ±2.0 mo	
	Cases & controls (n=692)	**30.6 ±1.3 mo**	
Only 1 vaccination (60% of all dogs in study)	Cases (n=294)	16.0 ±1.4 mo	0.4399
	Controls (n=122)	14.2 ±1.8 mo	
	Cases & controls (n=416)	**15.5 ±1.1 mo**	
>1 vaccination (40% of all dog in study)	Cases (n=52)	45.8 ±5.5 mo	0.1273
	Controls (n=224)	55.1 ±2.4 mo	
	Cases & controls (n=276)	**53.2 ±2.2 mo**	

** Age=age at most recent vaccination in months*

*** P-value represents a two-tailed t-test for cases versus controls.*

$ Significant at α<0.05.

Table 3: Parameter estimates of exploratory multivariable logistic regression models for likelihood of failing to achieve an adequate RVNA titre in dogs presented for travel certification

Variable	Model	Coefficient (SE)		Adj. OR (95% c.i.)		p-value
Intercept						
	'Base'	-2.33245	(0.22778)	-	-	<0.0001*
	Age forced	-2.33232	(0.22759)	-	-	<0.0001*
	Young (≤15 mo old)	-2.14831	(0.72174)	-	-	0.0029*
	Adult (>15 mo old)	-1.95515	(0.26658)	-	-	<0.0001*
Age at most recent vaccination (exposure: ≤15 mo old)*						
	'Base'	-	-	-	-	-
	Age forced	0.07971	(0.23289)	1.08	(0.69-1.71)	0.7322
	Young (≤15 mo old)	-	-	-	-	-
	Adult (>15 mo old)	-	-	-	-	-
Number of previous vaccinations (exposure: only one vaccination)						
	'Base'	2.41769	(0.19518)	11.22	(7.65-16.45)	<0.0001*
	Age forced	2.36395	(0.24984)	10.68	(6.52-17.35)	<0.0001*
	Young (≤15 mo old)	1.92167	(0.70891)	6.83	(1.70-27.42)	0.0067*
	Adult (>15 mo old)	2.31752	(0.25989)	10.15	(6.1-16.89)	<0.0001*
Gap between vaccination and titre (exposure: gap >60 d)						
	'Base'	1.16636	(0.2019)	3.21	(2.16-4.77)	<0.0001*
	Age forced	1.16217	(0.20215)	3.20	(2.15-4.75)	<0.0001*
	Young (≤15 mo old)	1.65068	(0.28236)	5.21	(3.00-9.06)	<0.0001*
	Adult (>15 mo old)	0.6634	(0.27817)	1.94	(1.13-3.35)	0.0171*

**Coefficients are deviation of "exposure" level listed from the alternative referent level for each binomial variable (≤15 mo old:>15 mo old; only one vaccination: >1 vaccination; gap ≤60 d: gap >60 d)*

*** Significant at α<0.05.*

Table 4: Parameter estimates of final multivariable logistic regression model for likelihood of failing to achieve an adequate RVNA titre in dogs presented for travel certification

Variable and level	Coefficient (SE)	Adjusted OR (95% CI)	p-value
Intercept	-1.93271 (0.26137)	-	<0.0001**
Age at most recent vaccination ("Age")			
Young (≤15 months)	-0.55717 (0.34353)	0.57 (0.29-1.12)	0.1048
Adult (>15 months)	Ref.	1.0	
Number of previous vaccinations			
Only one vaccination	2.2738 (0.24489)	9.72 (6.01-15.7)	<0.0001**
>1 vaccination	Ref.	1.0	
Gap between vaccination and titre			
Long gap (>60 days)	0.65557 (0.2762)	1.93 (1.12-3.31)	0.0176**
Short gap (≤60 days)	Ref.	1.0	
Interaction: Age x Gap	0.99766 (0.39562)	2.71 (1.25-5.89)	0.0117**

*Overall model: Deviance =729.12; p-value = 0.1289; Degrees of freedom=687. As deviance reduces the better the correspondence between the observed and fitted values, a non-significant p-value indicates no gross deficiencies with the overall model fit.

** Significant at $\alpha<0.05$.