

*Impact of rabies vaccination history on attainment of an adequate antibody titre among dogs tested for International Travel Certification, Israel - 2010-2014*

Article

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

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14 **Impacts**

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

25

## 26 **Summary**

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

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

## 42 **Introduction**

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

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

56         During the early 1990s, many countries converted from a strict quarantine  
57 requirement for domestic dog entry to requiring serological evidence of immunity (Cliquet *et*  
58 *al.*, 2003). These changes were driven by both increasing interest in free-circulation of people  
59 and animals among countries and improved scientific understanding of the relationship  
60 between rabies antibody titre levels in dogs and cats and resistance to infection upon  
61 exposure (Aubert 1992, WHO 1992, Cliquet *et al.*, 2003). In challenge experiments, a rabies  
62 virus neutralizing antibody (RVNA) titre of  $\geq 0.5$  international units (IU)/ml correlated best  
63 with protection from rabies virus infection on exposure (Aubert, 1992). The World Health  
64 Organization (WHO) designated RVNA titres of  $\geq 0.5$  IU/ml in an actively immunized dog  
65  $>16$  weeks of age as the standard for certifying protection against rabies infection (WHO,  
66 1992). Since 1993, the World Organization for Animal Health (OIE) has recommended  
67 requiring serologic evidence of immunity by quantification of RVNA whenever dogs or cats  
68 are imported from countries with endemic rabies virus circulation to areas that are considered  
69 rabies free (OIE, 1996). Many countries require demonstration of an adequate RVNA titre  
70 ( $\geq 0.5$  IU/ml) for international movement of pets (in the European Union for example: EU,  
71 2003). Dogs with lower titres or even without detectable antibodies have survived virulent  
72 rabies challenge (Sikes *et al.*, 1971; Brown *et al.*, 1973; Barth and Jaeger, 1977; Ganiere *et*  
73 *al.*, 1989; Aubert, 1992).

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

76 Agriculture and Rural Development, 1934). Since 1956, domestic dogs in Israel must be  
77 vaccinated against rabies by law, first at three months old and then annually (Israel Ministry  
78 of Agriculture and Rural Development, 2015). Legally mandated vaccination of dogs  
79 substantially shifted the dominant rabies reservoir. While dogs were the most commonly  
80 affected through the mid-1950s (Nobel & Neumann, 1962; Yakobson *et al.*, 2004), red foxes  
81 (*Vulpes vulpes*) and to a lesser extent golden jackals (*Canis aureus*) became the primary  
82 rabies reservoirs after 1956. During the mid-1970s, sylvatic fox rabies virus variant surpassed  
83 the canine variant (Yakobson *et al.*, 1998). Since 1998 wildlife rabies has been controlled  
84 through the use of oral rabies vaccines (Yakobson *et al.*, 2006). However, despite mandatory  
85 dog vaccination, canine rabies has re-emerged in northern Israel, resulting in rabies cases in  
86 unvaccinated dogs and other species (David *et al.*, 2009; David, Bellaiche, and Yakobson,  
87 2010; David and Yakobson, 2011).

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

## 93 **Materials and methods**

### 94 *Data source*

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

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

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

### 109 ***Study design***

110 Data were extracted from the IVSAH national dog registration database held by including a  
111 study population of dogs presented for travel certification RVNA titres from 3rd January  
112 2010 to 19th May 2014. The following variables were extracted from the national registry for  
113 each dog as explanatory variables (i.e., putative risk factors): sex; age at most recent rabies  
114 vaccination prior to blood draw for the pre-travel titre (in months; hereafter “age at most  
115 recent vaccination”); number of rabies vaccinations prior to blood draw for the pre-travel titre  
116 (hereafter “number of previous vaccinations”), and time between the most recent rabies  
117 vaccination and blood draw for a pre-travel titre (in days; hereafter “gap between vaccination  
118 and titre”). These records were linked to the date and outcome of the pre-travel titre reported  
119 by the Kimron Veterinary Institute by microchip identification number. Microchip numbers  
120 were subsequently removed to protect owner privacy.

121 We then performed a retrospective case-control analysis, where *cases* were defined as  
122 dogs presented for testing that *did not* achieve an adequate RVNA titre for travel, and  
123 *controls* were those presented for testing that *did* achieve an adequate RVNA titre for travel.  
124 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 ( $\leq 15$  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 ( $\leq 60$  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  $\leq 0.1$ .



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

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

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

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

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

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

## 178 **Results**

### 179 *Sample description*

180 From 3rd January 2010 to 19th May 2014, 4,949 dogs presented for travel certification,  
181 evenly distributed across years (range of 1,000-1,200 dog/year). Of these, 395 (8.0%) did not  
182 have an adequate RVNA titre for travel but many of these did have detectable RVNA below  
183 0.5 IU/ml (for these, median titre=0.18 IU, range=0.02-0.48 IU). Forty nine of these 395  
184 cases were excluded due to incomplete records for one or more necessary variables.  
185 Therefore 346 cases and 346 controls (692 dogs total) were selected for analysis.

### 186 *Univariate and stratified analysis*

187 Approximately half of both cases (49%) and controls (47%) were male (Table 1a). A  
188 significantly larger portion of cases received only one vaccination prior to presentation for  
189 pre-travel titre (85% versus 35.3% of controls), had received the most recent vaccination at  $\geq$   
190 15 months old (62.4% versus 27.5% of controls), and had a gap of  $>60$  days between  
191 vaccination and titre (80.3% versus 60.1% of controls; Table 1a).

192 Mean gap between vaccination and titre did not differ significantly between cases  
193 (173 days) and controls (160 days; T-test: p-value=0.3896). However, despite similar means,  
194 the distribution of gap between vaccination and titre was different between cases and controls  
195 (Figure 1). Specifically, 40% of test dates for the controls fell within 60 days of the most  
196 recent vaccination compared to only 20% for the cases ( $X^2$  test: p-value $<0.0001$ ).

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

199 vaccination. Both variables differed significantly between cases and controls as both binary  
200 categories (Table 1a) and in the original continuous variable (Spearman rank coefficient  
201 =0.6854, p-value<0.0001; Table 2). The mean age at most recent vaccination for dogs having  
202 received only one previous vaccination was 15.5 months, compared with 53.3 months for  
203 dogs that had received more than one previous vaccination (T-test: p-value <0.0001; Table  
204 2).

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

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

#### 214 ***Logistic regression modelling***

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

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

### 228 *Scenario risk estimation*

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

### 235 *Evaluation of study representativeness*

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

### 243 **Discussion**

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

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

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

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

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

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

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

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

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

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

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

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

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#### 438 **Figure Legends**

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

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

444 **Tables**

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

**(a) INITIAL UNIVARIATE ANALYSES**

Variable (exposure factor)		n	% 'exposed'	Odds ratio (95% confidence interval) & Yates' Corrected Chi <sup>2</sup> p-value (2 tail)	
Gender (male)	Cases	276 <sup>‡</sup>	49.3%	1.12 (0.81 to 1.53)	p=0.546
	Controls	344 <sup>‡</sup>	46.5%		
Number of previous vaccinations (Only one)	Cases	346	85.0%	10.38 (7.18 to 15.00)	p<0.0001 <sup>§</sup>
	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 <sup>§</sup>
	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 <sup>§</sup>
	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*, <sup>§</sup>
		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 <sup>§</sup>
		Controls 251	14.7%		

447 <sup>‡</sup>some cases did not have gender recorded448 \*Fisher exact p-value used here because conditions not met to use X<sup>2</sup>449 <sup>§</sup>Significant at α<0.05.

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

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

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

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

454 <sup>§</sup> Significant at  $\alpha < 0.05$ .

455

456 Table 3: Parameter estimates of exploratory multivariable logistic regression models for  
 457 likelihood of failing to achieve an adequate RVNA titre in dogs presented for travel  
 458 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 ( $\leq 15$ mo old)	-2.14831 (0.72174)	- -	0.0029*
	Adult ( $> 15$ mo old)	-1.95515 (0.26658)	- -	<0.0001*
Age at most recent vaccination (exposure: $\leq 15$ mo old)*				
	'Base'	- -	- -	-
	Age forced	0.07971 (0.23289)	1.08 (0.69-1.71)	0.7322
	Young ( $\leq 15$ 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 ( $\leq 15$ 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 ( $\leq 15$ 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*

459 \*Coefficients are deviation of "exposure" level listed from the alternative referent level for each  
 460 binomial variable ( $\leq 15$  mo old:  $> 15$  mo old; only one vaccination:  $> 1$  vaccination; gap  $\leq 60$  d: gap  
 461  $> 60$  d)

462 \*\* Significant at  $\alpha < 0.05$ .

463

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

<b>Variable and level</b>	<b>Coefficient (SE)</b>	<b>Adjusted OR (95% CI)</b>	<b>p-value</b>
Intercept	-1.93271 (0.26137)	-	<0.0001**
Age at most recent vaccination ("Age")			
Young ( $\leq 15$ 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 ( $\leq 60$ days)	Ref.	1.0	
Interaction: Age x Gap	0.99766 (0.39562)	2.71 (1.25-5.89)	0.0117**

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

469 \*\* Significant at  $\alpha < 0.05$ .

470