ORIGINAL ARTICLE

Rabies Vaccination: Higher Failure Rates in Imported Dogs than in those Vaccinated in Italy

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Impacts

- Dogs entering Italy had a higher vaccine failure rate than dogs vaccinated in Italy, raising doubts as to the real compliance with vaccination.
- The choice of vaccine and time of sampling may influence the individual's titre level post-vaccination and should be considered when interpreting serological test results.
- The legislation regulating the free movement of pets needs to be readdressed if the risk of spreading rabies has to be minimized.

Keywords:
Rabies; dogs; post-vaccination assessment; vaccination

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Summary

The current European Union (EU) legislation decrees that pets entering the EU from a rabies-infected third country have to obtain a satisfactory virus-neutralizing antibody level, while those moving within the EU require only rabies vaccination as the risk of moving a rabid pet within the EU is considered negligible. A number of factors driving individual variations in dog vaccine response have been previously reported, including a high rate of vaccine failure in puppies, especially those subject to commercial transport. A total of 21 001 observations collected from dogs (2006–2012) vaccinated in compliance with the current EU regulations were statistically analysed to assess the effect of different risk factors related to rabies vaccine efficacy. Within this framework, we were able to compare the vaccination failure rate in a group of dogs entering the Italian border from EU and non-EU countries to those vaccinated in Italy prior to international travel. Our analysis identified that cross-breeds and two breed categories showed high vaccine success rates, while Beagles and Boxers were the least likely to show a successful response to vaccination (88.82% and 90.32%, respectively). Our analysis revealed diverse performances among the commercially available vaccines, in terms of serological peak windows, and marked differences according to geographical area. Of note, we found a higher vaccine failure rate in imported dogs (13.15%) than in those vaccinated in Italy (5.89%). Our findings suggest that the choice of vaccine may influence the likelihood of an animal achieving a protective serological level and that time from vaccination to sampling should be considered when interpreting serological results. A higher vaccine failure in imported compared to Italian dogs highlights the key role that border controls still have in assessing the full compliance of pet movements with EU legislation to minimize the risk of rabies being reintroduced into a disease-free area.

Introduction

Terrestrial rabies in carnivores has been eliminated in most industrialized countries through the implementation of effective control measures. The risk of rabies introduction into a free area has long been recognized as linked to either free-roaming wildlife across borders or human-dependent movement of domestic animals, mainly dogs (Cliquet et al., 2014). At an international level, quarantine and vaccination (alone or coupled with serological testing) are the two most common approaches to prevent reintroduction of infection into a rabies-free area.

In Europe, although the risk of moving a rabid pet within the EU territories is considered negligible (European Food Safety Authority 2006), the current epidemiological situation shows an uneven distribution of the disease incidence, especially in wildlife, which highlights the risk of any country losing its rabies-free status.

The current EU legislation (European Commission 2013) states that pets entering the EU from a third country have to be accompanied by an international certificate confirming a satisfactory virus-neutralizing antibody level. A serum titre ≥0.5 IU/ml of rabies-neutralizing antibodies is considered adequately protective against the disease (OIE World Organization for Animal Health 2013). A titre below this cut-off is indicative of vaccination failure and could mean that the vaccination guidelines have not been complied with (such as failure with respect to the minimum vaccination age, improper vaccine storage and/or administration). As noted in the previous literature, primo-vaccination may not induce an adequate serological response in dogs, depending on risk factors such as the time lapse between vaccination and testing, the breed, the size, the age, the type of vaccine and the route of administration (Cliquet et al., 2003; Mansfield et al., 2004; Kennedy et al., 2007; Jakel et al., 2008; Zanoni et al., 2010; Berndtsson et al., 2011). Although individual variations in vaccine response may partly be due to genotypic differences, such as those between breeds (Sage et al., 1993; Cliquet et al., 2003; Kennedy et al., 2007), a high rate of vaccine failure has been shown in those puppies subject to commercial transport (Fevre et al., 2006; Tietjen et al., 2011). The Italian Reference Centre for Rabies (De Benedictis et al., 2008) has reported a 37% vaccination failure rate in dogs crossing the Italian border from Eastern European countries.

The aim of this study was to assess the effect of different risk factors on rabies vaccine efficacy, with special attention to a group of dogs subject to international transport. To achieve this, we performed a retrospective analysis of serological response to rabies vaccination in dogs and then compared the results of analyses performed on dogs subject to pre-travel tests departing from Italy to those obtained during import control testing at the border.

Materials and Methods

Source of sampling and sample selection

Samples were collected between January 2006 and December 2012 on dogs vaccinated against rabies and tested at the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) for post-vaccination efficacy.

Information relating to the risk factors under investigation was retrieved from the submission forms and exported to the survey database (MICROSOFT OFFICE® EXCEL worksheet, Microsoft, Redmond, WA, USA).

For the purposes of our study, only samples with a minimum database of the following information were retained: date of vaccination, vaccine used, date of sampling and rabies-neutralizing serum titre. Only those reporting vaccination with the four most represented vaccines in the database were included, to allow reliable comparison studies. Observations displaying entry errors, inconsistent or incomplete information were not considered for the analysis. After such omissions, a total of 21 001 observations were included in the study.

Diagnostic test

For each sample, the serum titre was calculated using the fluorescent antibody virus neutralization (FAVN) test, as previously described (Cliquet et al., 1998). A serum titre ≥0.5 IU/ml is considered indicative of protection against rabies infection (positive outcome) (OIE World Organization for Animal Health 2013).

Data sets and statistical analyses

Analyses were conducted using two different data sets, namely data sets A and B. Data set A included observations from dogs living in the Italian territory (including the Republic of San Marino) and sampled before the intended international travel (n = 20 119; henceforth referred to as group Italy). Data set B included data set A plus additional observations from dogs travelling to Italy (n = 882; henceforth referred to as group Eastern Europe) and sampled at the Italian border during routine border control activity. Dogs in group Eastern Europe came from the following Eastern European countries: Albania, the Czech Republic, Hungary, Montenegro, Poland, Romania, Slovakia, including Russia, Serbia and Ukraine. None of these countries, except for the Czech Republic, were free from terrestrial rabies at the time of sampling.

Variables collated in data set A were as follows: vaccine used, time from vaccination to sampling (VtS), age at vaccination, sex, breed, size of the animal and rabies-neutralizing serum titre (test outcome). Less information was available in terms of associated risk factors for group

Eastern Europe; thus, in data set B, the variables under consideration were vaccine used, VtS and rabies-neutralizing serum titre.

All variables, except for 'age', were treated as categorical. The variables 'titre' and 'sex' were analysed considering their outcomes as binary, < or ≥0.5 IU/ml and male or female, respectively. The variable 'size' was categorized into four groups: small (≤10 kg), medium (11–25 kg), big (26–44 kg) or giant (≥45 kg). Individuals were allocated to a specific size category dependent on the average size of the breed or on the predominant breed in case of cross-breeds, when reported; otherwise, the information was classified as 'missing'. Considering that the database included observations from more than 200 different breeds, the variable 'breed' was recategorized following the classification criteria of the International Canine Federation (FCI-*Fédération Cynologique Internationale*). Besides cross-breeds, ten breed categories with similar characteristics were considered and classified according to the FCI guidelines (Table 1). In addition, eight breeds of interest (English Setter, Pinscher, Chihuahua, Maltese, West Highland White Terrier, German Shepherd, Boxer and Beagle) were selected from data set A to evaluate their response to vaccination in comparison with cross-breeds. These breeds were selected as 'of interest' due to either their size or previous evidence of poor response to vaccination.

The variable 'VtS' was classified into six categories (Table 1). The variable 'vaccine' contained four categories corresponding to four commercially available formulations. The variable 'age' was organized into 11 categories (1-year categories, up to 10 years), but was included in the model as a continuous variable.

Descriptive statistics were run to describe the characteristics of the data sets.

Univariate analysis was performed to investigate the association between each of the variables of interest and the outcome (titre) using the chi-square test. The strength of the association was measured through the Cramer *V*. Cramer *V* values range between 0 (no association) and 1 (perfect association) (Table 1).

Stepwise logistic regression analysis was used to build up the multivariate models adding one by one the variables associated with the outcome ($P < 0.05$ and Cramer's $V > 0.10$). Whenever relevant, interactions between variables were explored and included in the model. Possible colinearity among breed and size variables was contended by using separate models to explore the effect of each of these variables on the outcome. *P*-values <0.05 in the multivariate analysis were considered as significant. Likelihood ratio and the Akaike's Information Criterion (AIC) were used to compare nested and non-nested models' fit, respectively.

Overall, four different multivariate models were built to assess the effect of risk factors in the two data sets (Table 2).

Statistical analyses were performed using STATA 11 (College Station, TX, USA: StataCorp LP).

Results

Table 1 reports the descriptive statistics of the overall data set (group Italy and Eastern Europe) for each variable included in the study and the results of the univariate analysis, expressed as Cramer's V. The majority of observations in group Italy reported the use of vaccine I (62.51%), while vaccine IV was the least frequently used (2.06%). The latter was not reported among the observations outside Italy (group Eastern Europe), where vaccine II was the most commonly used (80.38%). Overall, the proportion of successful vaccinations (titre ≥ 0.5 IU/ml) was 93.81% (94.12% and 86.85% in group Italy and Eastern Europe, respectively) with the majority of animals tested more than 15 days post-vaccination (p.v.). The percentage of vaccination failure was lower in the observations from group Italy (5.89%) than in those from group Eastern Europe (13.15%). When comparing the vaccination success rates in imported dogs (88.19%) to Italians dogs (91.38%) sampled >75 days after vaccination, the difference in success rate was reduced, although still significant ($P < 0.001$), decreasing from 7.26% to 3.19%.

Univariate analysis

The univariate analysis showed that vaccine protection was independent from sex ($P = 0.399$), while all the other variables were found to be associated with the outcome ($P < 0.001$).

The vaccine's ability to induce a protective titre differed considerably between the four vaccines in group Italy, with vaccine II displaying the highest percentage failure (11.13%) and vaccine IV the lowest (3.13%); vaccines I and III failed in 3.82% and 5.72% of cases, respectively (Table 1). The chi-square test demonstrated that the association between type of vaccine and titre was statistically significant ($P < 0.001$), confirming different performances among vaccines.

In group Eastern Europe, the performance also differed between vaccines ($P < 0.001$); however, vaccine II had the second highest performance with 89% of the observations showing a titre ≥0.5 IU/ml and was the most represented vaccine in the group (Table 1). Vaccine III, which had only 11 observations, all with a positive outcome, was the most successfully performing vaccine in group Eastern Europe.

As for VtS, the highest percentage of vaccination success was observed in dogs sampled from two weeks to two months following vaccination.

Of note, the number of protected dogs was greater in cross-breeds (96.29%) than in pure breeds (93.07%). A more detailed analysis looking at specific FCI categories

Table 1. Description of the sub-data sets and results of the univariate analysis. Group Italy: observations collected from dogs living in Italy and sampled before the intended travel. Group Eastern Europe: dogs entering Italy from an Eastern European country and subject to frontier control. Missing data in the database mean that the total number of observations was slightly different among variables

Variables	Categories	Observations [*n* (%)]	Positive observations*[*n* (%)]	Cramer's *V*
Group Italy				
Vaccine	I	12 577 (62.51)	12 096 (96.18)	0.1340
	II	5222 (25.96)	4641 (88.87)	
	III	1905 (9.47)	1796 (94.28)	
	IV	415 (2.06)	402 (96.87)	
	Total	**20 119 (100)**	**18 935 (94.12)**	
VtS[a]	≤15	314 (1.56)	293 (93.31)	0.1043
	16–30	3131 (15.57)	3011 (96.17)	
	31–45	5177 (25.74)	5001 (96.60)	
	46–60	1729 (8.60)	1669 (96.53)	
	61–75	1074 (5.34)	1018 (94.79)	
	>75	8685 (43.19)	7936 (91.38)	
	Total	**20 110 (100)**	**18 928 (94.12)**	
Breed (FCI)[b]	Cross-breed	3235 (16.14)	3115 (96.29)	0.0611
	FCI 1	813 (4.06)	756 (92.99)	
	FCI 2	1456 (7.27)	1347 (92.51)	
	FCI 3	1423 (7.10)	1360 (95.57)	
	FCI 4	386 (1.93)	365 (94.56)	
	FCI 5	452 (2.26)	404 (89.38)	
	FCI 6	433 (2.16)	396 (91.45)	
	FCI 7	8005 (39.95)	7514 (93.87)	
	FCI 8	1467 (7.32)	1363 (92.91)	
	FCI 9	2182 (10.89)	2073 (95.00)	
	FCI 10	186 (0.93)	172 (92.47)	
	Total	**20 038 (100)**	**18 865 (94.15)**	
Size[c]	Small	4958 (26.32)	4734 (95.48)	0.0372
	Medium	3503 (18.60)	3285 (93.78)	
	Big	9823 (52.15)	9180 (93.45)	
	Giant	552 (2.93)	513 (92.93)	
	Total	**18 836 (100)**	**17 712 (94.03)**	
Sex	Female	9859 (49.15)	9297 (94.30)	(−0.0060)
	Male	10 201 (50.85)	9591 (94.02)	
	Total	**20 060 (100)**	**18 888 (94.16)**	
Age[d]	<1	6411 (32.47)	5791 (90.33)	0.1156
	1	3737 (18.93)	3556 (95.16)	
	2	2341 (11.86)	2250 (96.11)	
	3	1785 (9.04)	1703 (95.41)	
	4	1358 (6.88)	1308 (96.32)	
	5	1007 (5.10)	970 (96.33)	
	6	862 (4.37)	839 (97.33)	
	7	671 (3.40)	650 (96.87)	
	8	519 (2.63)	502 (96.72)	
	9	368 (1.86)	357 (97.01)	
	≥10	686 (3.47)	664 (96.79)	
	Total	**19 745 (100)**	**18 590 (94.15)**	
Breed[e]	Cross-breed	3235 (30.87)	3115 (96.29)	0.0772
	Beagle	155 (1.48)	140 (90.32)	
	German Shepherd	315 (3.01)	290 (92.06)	
	Boxer	152 (1.45)	135 (88.82)	
	Chihuahua	332 (3.17)	322 (96.99)	
	Pinscher	298 (2.84)	287 (96.31)	
	English Setter	5506 (52.53)	5144 (93.43)	
	West Highland White Terrier	171 (1.63)	164 (95.91)	
	Maltese	317 (3.02)	309 (97.48)	
	Total	**10 481 (100)**	**9906 (93.72)**	

Table 1. (Continued)

Variables	Categories	Observations [*n* (%)]	Positive observations*[*n* (%)]	Cramer's *V*
Group Eastern Europe				
Vaccine	I	162 (18.37)	124 (76.54)	0.1491
	II	709 (80.38)	631 (89.00)	
	III	11 (1.25)	11 (100.00)	
	IV	0 (0)	0 (0)	
	Total	**882 (100)**	**766 (86.85)**	
VtS[a]	≤15	1 (0.11)	1 (100)	0.2023
	16–30	29 (3.29)	18 (62.07)	
	31–45	49 (5.56)	34 (69.39)	
	46–60	35 (3.97)	35 (100)	
	61–75	5 (0.57)	5 (100)	
	>75	762 (86.50)	672 (88.19)	
	Total	**881 (100)**	**765 (86.83)**	

*Titres ≥ 0.5 IU/ml.
[a]VtS: time from vaccination to sampling, expressed as days post-vaccination.
[b]FCI: International Canine Federation. FCI 1: Sheepdogs and Cattle dogs other than Swiss Cattle dogs); FCI 2: Pinschers, Schnauzers, Molossoid and Swiss mountains and Cattle dogs; FCI 3: Terriers; FCI 4: Dachshunds; FCI 5: Primitive type and Spitz dogs; FCI 6: Scent hounds and related breeds; FCI 7: Pointers and Setters; FCI 8: Retrievers, Water Dogs and Flushing Dogs; FCI 9: Companion and Toy dogs; FCI 10: Sighthounds.
[c]Small: ≤10 kg; medium: 11–25 kg; big: 26–44 kg; giant: ≥45 kg.
[d]The variable age was organized into 11 categories, but included in the model as a continuous variable.
[e]Observations collected from 8 breeds were selected from group Italy sub-data set and performances compared to those of cross-breeds.
Bold values correspond to the total observations (and percentages) for each variable considered in the study.

Table 2. Multivariate models with only significant variables ($P < 0.05$). Three models were applied to data set A, observations from dogs living in Italy and sampled before the intended travel. In Model 4, the interaction between the vaccine and the dog of geographical origin, intended as group Italy and group Eastern Europe (dogs entering Italy from an Eastern European country) observations, was assessed

			Independent variables							
Data sets	Multivariate models	Observations	Vaccine	Age	VtS	Breed (FCI)	Size	Breed[a]	Origin	Interactions
Data set A	Model 1	19 670	+[b]	+	+	+				Vaccine * VtS
	Model 2	18 485	+[b]	+	+		+			Vaccine * VtS
	Model 3[c]	10 278	+	+				+		
Data set B	Model 4	20 991	+[d]		+				+	Vaccine * VtS Vaccine * Origin

[a]Observations collected from 8 breeds were selected from group Italy sub-data set and performances compared to those obtained from cross-breeds. The selected breeds were English Setter, Pinscher, Chihuahua, Maltese, West Highland White Terrier, German Shepherd, Boxer, Beagle.
[b]Three vaccine categories (I, II and III) included.
[c]Results not shown.
[d]Two vaccine categories (I and II) included.

confirmed substantial differences in antibody response in different breed groups ($P < 0.001$). FCI 5 consisting of Primitive type and Spitz dogs was the category with the lowest vaccination success rate (89.38%), while FCI 3 and FCI 9 were the best categories with a success rate not lower than 95%. No FCI category showed a success rate higher than that of cross-breeds. Among the pure breeds selected for an in-depth analysis, boxers had the lowest performance to rabies vaccination, with a success rate of 88.82%, while the small-size breeds included in the analysis had the best performances, regardless of the FCI group they belonged to. Of particular note was the poor success rate of Beagles, with only 90.32% showing a protective titre.

Dog size inversely correlated with a protective titre, with a success rate ranging from 92.93% in giant to 95.48% in small-size dogs.

When considering age as a risk factor, those < 1 year showed the lowest probability of reaching the protective threshold for antibody titre (90.33%).

Multivariate analyses

Data set A

Results from Models 1 and 2 indicated that there was no association between time from vaccination to sampling and outcome, when animals were inoculated with vaccine I. On the contrary, vaccines II and III showed an increasing likelihood of higher titres as time progressed over the first two months. The relationship between vaccine used and outcome was explored in each category of VtS. Vaccine I showed much better performances, ranging from an odds ratio of two to more than four times higher than vaccine II in each category of sampling time. Vaccines II and III displayed similar results, except for samples collected between sixteen to thirty days p.v., where vaccine III showed an odds ratio more than twice higher (Table 3a).

According to the results of Model 1, animals belonging to FCI categories 2, 5 and 6 showed the lowest odds of yielding neutralizing titres ≥ 0.5 IU/ml when compared to cross-breeds ($P \leq 0.01$) (Table 3b, Model 1), with FCI category 5 showing the poorest response. Model 2 (including size instead of breed) showed equivalent results to Model 1 for the independent variables contained in both models. In addition, Model 2 indicated that, in comparison with smaller breeds, medium and big size breeds have a lower probability of reaching a positive outcome ($P < 0.01$), which means that the greater the size, the worse the response to vaccination (Table 3b, Model 2). Model 3 revealed that cross-breeds had a higher probability of reaching a protective titre compared to the English Setter, the German Shepherd, the Boxer and the Beagle ($P \leq 0.002$) (data not shown).

In relation to the age of the animals, results from the multivariate analyses confirmed the output from the univariate analysis, with younger dogs less likely to respond

Table 3. Results of multivariate logistic regression of data set A. Table 3a presents the results for variables vaccine and time from vaccination to sampling (VtS), considering an interaction between both variables, as obtained from Models 1 and 2. Table 3b presents the results obtained for the independent variables breed (Model 1) and size (Model 2)

3a

Interaction by vaccine type				Interaction by VtS			
Vaccine	VtS	Odds ratio (95% CI)	*P*-value	VtS	Vaccine	Odds ratio (95% CI)	*P*-value
I	<15	1 (–)	–	<15	I	3.53 (1.23–10.14)	0.019
	16–30	1.29 (0.55–3.03)	0.565		II	1 (–)	–
	31–45	1.79 (0.76–4.19)	0.181		III	0.86 (0.24–3.01)	0.810
	46–60	2.46 (0.96–6.31)	0.061	16–30	I	1.96 (1.31–2.95)	0.001
	61–75	1.26 (0.50–3.16)	0.619		II	1 (–)	–
	>75	0.78 (0.34–1.78)	0.551		III	2.39 (1.14–4.99)	0.021
II	<15	1 (–)	–	31–45	I	2.59 (1.86–3.59)	<0.001
	16–30	2.30 (1.10–4.84)	0.027		II	1 (–)	–
	31–45	2.43 (1.20–4.94)	0.014		III	1.40 (0.86–2.28)	0.176
	46–60	2.01 (0.94–4.31)	0.074	46–60	I	4.32 (2.38–7.82)	<0.001
	61–75	1.28 (0.59–2.80)	0.535		II	1 (–)	–
	>75	0.80 (0.41–1.58)	0.518		III	1.55 (0.70–3.46)	0.284
III	<15	1 (–)	–	61–75	I	3.48 (1.95–6.21)	<0.001
	16–30	6.43 (1.84–22.51)	0.004		II	1 (–)	–
	31–45	3.97 (1.26–12.48)	0.018		III	2.17 (0.81–5.85)	0.124
	46–60	3.63 (1.01–13.04)	0.048	>75	I	3.43 (2.91–4.04)	<0.001
	61–75	3.25 (0.81–13.09)	0.098		II	1 (–)	–
	>75	2.09 (0.70–6.25)	0.187		III	2.24 (1.68–3.00)	<0.001

3b

Model 1			Model 2		
Breed (FCI)	Odds ratio (95% CI)	*P*-value	Size	Odds ratio (95% CI)	*P*-value
Cross-breed	1 (–)		Small	1 (–)	
FCI1	0.65 (0.46–0.91)	0.013			
FCI2	0.60 (0.46–0.80)	<0.001			
FCI3	0.99 (0.72–1.37)	0.944	Medium	0.70 (0.58–0.86)	0.001
FCI4	0.79 (0.48–1.28)	0.337			
FCI5	0.39 (0.27–0.57)	<0.001			
FCI6	0.52 (0.35–0.78)	0.002	Big	0.76 (0.65–0.90)	0.001
FCI7	0.79 (0.64–0.98)	0.030			
FCI8	0.66 (0.50–0.87)	0.004			
FCI9	0.89 (0.67–1.17)	0.407	Giant	0.72 (0.50–1.05)	0.085
FCI10	0.74 (0.41–1.34)	0.328			

satisfactorily to vaccination. For each increase in age of one year, there was a 1.18 increase in the odds of obtaining a positive outcome ($P < 0.001$) (data not shown).

Data set B

Model 4 showed that the time interval between vaccination and sampling was associated with the outcome, with effects that were different according to vaccine type (Table 4). Specifically, when using vaccine II, dogs were more likely to obtain a titre ≥0.5 IU/ml between 15 and 45 days p.v. (Table 4a). Such a marked pattern in antibody response in relation to VtS was not noticed for vaccine I (Table 4a and 4c). Vaccines appeared to perform differently in groups Eastern Europe and Italy. Vaccine I showed a significantly lower likelihood of vaccine success in group Eastern Europe than in group Italy. The opposite effect was found for vaccine II, with a greater likelihood of vaccine success in group Eastern Europe, regardless of VtS (Table 4b and 4c).

Discussion

This study, based on a panel of over 20 000 observations collected in dogs over a period of 7 years, provides an updated analysis of variables associated with rabies vaccination response in dogs. Of interest, it also includes an analysis of the observations collected from dogs entering the Italian border both from EU and non-EU countries. Findings from our study are generally in agreement with those reported in the literature, identifying sex as the only variable not associated with serological response to rabies vaccination in dogs (Cliquet et al., 2003; Mansfield et al., 2004; Kennedy et al., 2007; Zanoni et al., 2010; Berndtsson et al., 2011).

Our investigation provides evidence that the most common vaccines used in dogs have different performances. More specifically, although the probability of a positive outcome (serum titre ≥0.5 IU/ml) generally increased progressively from the 15th day until the 2nd month p.v., vaccine I seems to induce a faster and longer-lasting antibody

Table 4. Results of multivariate logistic regression of data set B. Table 4a: results for variables vaccine types and time from vaccination to sampling (VtS); Table 4b: results for variables vaccine types and groups of geographical origin; Table 4c: results for variable vaccine types by VtS and by groups of geographical origin (Italy or Eastern Europe)

4a
Interaction between vaccine and VtS

Vaccine	VtS	Odds ratio (95% CI)	*P*-value
I	<15	1 (–)	–
	16–30	0.99 (0.45–2.19)	0.987
	31–45	1.40 (0.64–3.06)	0.406
	46–60	2.35 (0.97–5.68)	0.059
	61–75	1.08 (0.46–2.56)	0.859
	>75	0.66 (0.31–1.41)	0.282
II	<15	1 (–)	–
	16–30	2.27 (1.09–4.73)	0.028
	31–45	2.26 (1.12–4.54)	0.022
	46–60	1.72 (0.81–3.64)	0.159
	61–75	1.15 (0.53–2.48)	0.728
	>75	0.7 (0.35–1.35)	0.281

4b
Interaction between vaccine and group of geographical origin

Vaccine	Group	Odds ratio (95% CI)	*P*-value
I	Italy	1 (–)	–
	Eastern Europe	0.12 (0.08–0.17)	<0.001
II	Italy	1 (–)	–
	Eastern Europe	1.59 (1.23–2.06	<0.001

4c
Interaction between vaccine and VtS by group of geographical origin

Group	VtS	Vaccine	Odds ratio (95% CI)	*P*-value
Italy	<15	II	1 (–)	–
		I	3.77 (1.38–10.31)	0.010
	16–30	II	1 (–)	–
		I	1.65 (1.12–2.4)	0.012
	31–45	II	1 (–)	–
		I	2.33 (1.71–3.19)	<0.001
	46–60	II	1 (–)	–
		I	5.167 (2.88–9.24)	<0.001
	61–75	II	1 (–)	–
		I	3.56 (2.01–6.30)	<0.001
	>75	II	1 (–)	–
		I	3.58 (3.05–4.21)	<0.001
Eastern Europe	<15	II	1 (–)	–
		I	0.27 (0.09–0.82)	0.021
	16–30	II	1 (–)	–
		I	0.12 (0.07–0.22)	<0.001
	31–45	II	1 (–)	–
		I	0.17 (0.10–0.29)	<0.001
	46–60	II	1 (–)	–
		I	0.37 (0.18–0.76)	0.007
	61–75	II	1 (–)	–
I		0.26 (0.12–0.53)	<0.001	

response than vaccine II. Similarly, an experimental study identified different performances between two commercial vaccines in terms of persistence and magnitude of the neutralizing titre elicited (Minke et al., 2009), although a breed-specific immunological limitation may have affected the outcome of this study based on conventional laboratory Beagles, as previously reported (Van de Zande et al., 2009).

Our research has also identified a marked difference in the way vaccines perform in different geographical areas. Interestingly, vaccine I was administered more frequently and had the best performance in group Italy, while vaccine II was the most commonly used in group Eastern Europe, where it performed better than in group Italy. Such a discrepancy may be due to the conditions in which the two vaccines are produced, distributed and stored in different countries, although the authors acknowledge that differences in their use (i.e. route of administration or site of injection) cannot be totally excluded.

These findings suggest that the choice of the vaccine may influence the likelihood of an animal achieving a protective serological titre and that VtS should be considered when interpreting the serological test results.

In agreement with previously reported findings (Mansfield et al., 2004; Kennedy et al., 2007; Zanoni et al., 2010; Berndtsson et al., 2011), young dogs in our study showed a lower probability of achieving a positive outcome, with a steadily increasing success rate in adult dogs up to the age of 9 and declining thereafter. This finding is not unexpected, based on the dog immune system development and senescence (Day, 2007, 2010) and previously reported evidence that rabies vaccination induces a significantly lower response in old dogs than in adult ones (12.1 ± 1.3 and 3.15 ± 0.8 years, respectively) (HogenEsch et al., 2004). Our study found a positive association between age and outcome, even when controlling the confounding effect of size on the basis that life expectancy in dogs is usually inversely correlated with size (Kraft, 1998; Michell, 1999; Kraus et al., 2013).

Cross-breeds and FCI categories 3, 4, 9 and 10 showed the best response to vaccination, while FCI categories 2, 5, and 6 had the poorest response. Among the breeds studied independently from FCI group, the Boxer and the Beagle are worth a special mention, as they showed a remarkable failure rate (11.18% and 9.68%, respectively). While the inability of Beagles to effectively respond to vaccination has already been observed (Kennedy et al., 2007; Van de Zande et al., 2009), to our knowledge such an unexpectedly high rabies vaccine failure in Boxers has never been detected before. The fact that Beagles fall into the poor-responder category is especially noteworthy, as they are the breed most commonly used for vaccine trials, and it has been argued that vaccine performances and kinetics in this breed may not be truly representative (Van de Zande et al., 2009).

We found an overall 6.19% failure rate, in line with previous estimations in Europe (1.1–11.1%) (European Food Safety Authority, 2006). Higher failure proportions have been previously found in specific contexts, such as a 37% vaccine failure rate in dogs sampled at the Italian border (De Benedictis et al., 2008) and a more recently a 53% failure rate in rescued dogs from Romania and Hungary to Norway (Klevar et al., 2015). Similarly, we found a higher vaccine failure rate in dogs entering Italy from foreign countries (13.15%) than in those vaccinated in Italy (5.89%). Several factors should be considered as possible causes of an unfavourable post-vaccination outcome (Blancou et al., 1983), and most of them have been extensively discussed. Vaccine failure may be ascribable to most of the animals tested >75 days p.v. (86.5% and 43.19%, in groups Eastern Europe and Italy, respectively). Interestingly, the discrepancy in vaccine failure rates for this category >75 days p.v. is still significant between the two groups, meaning that even when testing >75 days after vaccination, the vaccination response is truly different in both groups. Interestingly, a significant difference between success rates obtained in Swedish owned dogs rather than in stray ones from Romania and Hungary and sampled within this same time lapse has been noticed (85.7% versus 45.5%, respectively, $P < 0.0001$), which may suggest poor compliance with the current EU regulation (Klevar et al., 2015).

One possible explanation for the poorer vaccination response in group Eastern Europe could be transport-related stress, as most observations from this group were made on dogs subject to commercial transport, which may not always be in line with international rules (Fevre et al., 2006; Tietjen et al., 2011; Klevar et al., 2015). In such cases, a failure in vaccine response may be attributed to counterfeit vaccine certificates and/or to the vaccination of puppies prior to the recommended 12 weeks, as frequently documented (European Parliament 2012: http://www.europarl.europa.eu/sides/getDoc.dotype=WQ&reference=E-2012-007168&language=EN) (Tietjen et al., 2011).

According to the results of our study and to the available literature, border controls still have a key role in assessing the full compliance of pet movements with EU legislation, and they should therefore be run timely and efficiently, not only to prevent the introduction of diseased pets into rabies-free areas, but to ensure that the animal welfare is maintained to an acceptable standard during international transport.

Limitations of the study

All observations were collected as part of routine antibody titration activities at the IZSVe, not specifically for the aim of this study. Being a retrospective analysis of a pre-existing database, we were not able to identify any potential data entry errors, or mistakes, made when filling in the accompanying submission forms Information on the factors of interest was available for a great number of dogs, resulting in a large database for our analysis, which contributed to the reliability of our results. Nevertheless, the data set lacked details of other very relevant factors that could be associated with the outcome of vaccination success or failure. For instance, information on whether the vaccine was administered alone (e.g. in monovalent form) or in combination with other vaccines was not recorded. Similarly, the database only contained the date of the last rabies vaccination, with no information on whether the animals had received any other rabies vaccine injections before the reported one. The administration type (intramuscular versus subcutaneous) was unknown. We cannot therefore exclude the possibility of other additional factors influencing the associations found in this study.

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Competing Interest

The authors declare no competing interest.

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