Veterinary Microbiology 213 (2018) 1–4

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Factors influencing the outcome of primary immunization against rabies in young dogs

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ARTICLE INFO

Keywords:
Rabies
Vaccination
Canines
FAVN

ABSTRACT

There is currently limited information on the factors influencing the outcome of rabies vaccination in dogs based on the primary immunization schedule. The objective of this study was to investigate whether selected variables (signalment, number of vaccinations, vaccine brand and multivalence, and time interval between the most recent vaccination and blood sampling) were associated with the achievement of an acceptable titer threshold (based on international standards) and with absolute antibody titers in young dogs vaccinated with commercially available vaccines. Serologic data from 662 dogs tested prior to their first annual booster for rabies were retrospectively reviewed. Neutralizing antibody titers were determined using a fluorescent antibody neutralization test. An acceptable titer threshold (≥ 0.5 IU/ml) was achieved in 86.5% of the dogs. Dogs that had been vaccinated twice had significantly ($P < 0.001$) higher antibody titers compared with dogs vaccinated once. The odds of achieving seropositivity and the median absolute antibody titer tended to decrease with increasing time between vaccination and blood sampling. Dogs vaccinated with monovalent vaccines were more likely to achieve an acceptable titer than dogs vaccinated with polyvalent vaccines. Dogs that were vaccinated after 3–6 months of age were more likely to develop higher antibody titers. These results indicate that the administration of two vaccines rather than one vaccine in the primary immunization schedule for rabies, result in a superior vaccination response and may be a more beneficial policy for ensuring pre-exposure prophylaxis and for travel certification of young dogs.

1. Introduction

Rabies is a fatal viral zoonosis, causing more than 60,000 human deaths annually, worldwide (WHO, 2013). After 25 years of rabies-free status, an outbreak emerged recently in Greece. Rabies was diagnosed in 48 animals between October 2012 and May 2014, including 40 red foxes, 5 dogs, 2 cattle and one cat (Tasioudi et al., 2014; Korou et al., 2016). The outbreak rekindled the national debate on the most effective rabies vaccination strategy, especially with regard to the primary immunization schedule for young dogs. Current guidelines recommend that puppies should be vaccinated for rabies at 12 weeks of age and revaccinated with a booster one year later (Day et al., 2016).

Although several studies have examined factors that can affect the outcome of rabies vaccinations in dogs (Cliquet et al., 2003; Mansfield et al., 2004; Kennedy et al., 2007; Berndtsson et al., 2011; Rota Nodari et al., 2017), only a few studies have focused on factors influencing the outcome of rabies vaccination based on the primary immunization schedule of young dogs (Minke et al., 2009; Zanoni et al., 2010; Shiraishi et al., 2014; Wallace et al., 2017). In a study by Zanoni et al. (2010), puppies given two vaccines for rabies, 7–10 days apart, had a significantly lower failure rate compared with puppies vaccinated only once. These data suggest that in the primary immunization schedule, two vaccines rather than a single vaccine may better ensure the achievement of a protective titer against rabies.

The objective of this study was to investigate whether selected variables (signalment, number of vaccinations, vaccine brand and multivalence, and time between the most recent vaccination and blood sampling) were associated with the ability of dogs to achieve an

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https://doi.org/10.1016/j.vetmic.2017.11.006
Received 14 August 2017; Received in revised form 4 November 2017; Accepted 6 November 2017

acceptable rabies titer threshold, based on international standards, and with the absolute antibody titer in young dogs (< 1 year of age) vaccinated with commercially available vaccines.

2. Materials and methods

2.1. Serum samples

Archived data from 3,861 canine serum samples submitted as part of the pet travel scheme, between December 2008 and January 2015 for serologic testing for rabies neutralizing antibodies were retrospectively reviewed. Samples were included in the study if the dogs were: 1) < 12 months of age at the time of their most recent rabies vaccination (regardless of whether one or two vaccines were given) and 2) serologically tested prior to receiving their first annual booster after of 12 months of age. Data compiled from the records included the unique identifier of the animal (microchip number or tattoo); signalment (age, breed, body weight and sex); the date of the most recent rabies vaccination; brand name and type (monovalent or polyvalent) of vaccine and the date of blood collection for serologic testing.

2.2. Sample handling and serologic testing

Serum samples from dogs, accompanied by a submission form, were sent by routine surface mail by private veterinary practitioners to the Greek National Reference Laboratory for Rabies (GNRLR), which is under the auspices of the Ministry of Rural Development and Food. Upon receipt, samples were stored at −20 °C, until testing. Titers (IU/ml) were determined using a fluorescent antibody virus neutralization (FAVN) test, according to a previously described protocol (Cliquet et al., 1998). The GNRLR is one of the approved rabies serology laboratories in Europe and the only one in Greece for verifying the effectiveness of rabies vaccination by measurement of titers. Based on international standards, titers ≥0.5 IU/ml or < 0.5 IU/ml were considered acceptable or a vaccine failure, respectively (OIE, 2013).

2.3. Statistical analysis

Data were analyzed using Stata13 (StataCorp, College Station, TX, USA). The following variables were evaluated for potential association with an acceptable antibody titer and with the absolute value of the antibody titers: 1) the number of prior rabies vaccinations (1 or 2) given during the primary immunization schedule, 2) the age of the most recent vaccination (< 3 months; 3–6 months; 6–9 months; or 9–12 months), 3) sex (male, female), 4) breed (purebred versus mixed breeds), 5) dog size (small [body weight < 10 kg]; medium [body weight 10-25 kg]; large [body weight > 25 kg]), 6) the time interval between the most recent vaccination and blood sampling, 7) the type of vaccine (monovalent versus polyvalent), and 8) the brand of vaccine.

A logistic regression model was used to assess the effect of the variables on the odds of obtaining a serologic result ≥0.5 IU/ml. Initially, univariate analysis was used to identify candidate variables associated with acceptable antibody titers at a significance level of $P \leq 0.25$. Subsequently, all variables with $P \leq 0.25$ were simultaneously analyzed in a full model, which was then reduced by backwards elimination. All variables with $P < 0.05$ were retained in the final model. The Hosmer-Lemeshow goodness-of-fit test was used to assess the fitness of the model to the data.

A linear regression model was used to assess the effect of the variables on the absolute values of antibody titers. Initially, univariate analysis was used to identify candidate variables associated with the antibody titers at a significance level of $P \leq 0.25$. Subsequently, all variables with $P \leq 0.25$ were simultaneously analyzed in a full model, which was then reduced by backwards elimination. All variables with $P < 0.05$ were retained in the final model. Goodness-of-fit for the final model was assessed using the Pearson chi-square statistic.

3. Results

Of the 3,861 canine samples received for serologic testing for rabies, 662 met the inclusion criteria and were entered into the study. The samples were from 332 (50.2%) male dogs and 330 (49.8%) female dogs, and from 317 (47.9%) purebred and 344 (52.1%) mixed-breed dogs; breed was not reported for one dog. Of the samples for which body weight was recorded, 215 (32.5%) were from small dogs, 75 (11.3%) from medium-sized dogs, and 149 (22.5%) from large dogs; body weight was not recorded for 223 (33.7%) dogs.

Of the 662 dogs, 573 (86.5%) had acceptable titer threshold (≥0.5 IU/ml) and 89 (13.5%) failed to achieve an acceptable titer (vaccine failure). The median absolute titer for the 662 dogs was 6.01 IU/ml (range 0.04–13.77 IU/ml). One vaccine had been administered to 546 (82.5%) dogs and two vaccines had been administered to 116 (17.5%) dogs. The median titer in dogs vaccinated once was 4.56 IU/ml (range 0.04–13.77 IU/ml); the median titer in dogs vaccinated twice was 13.77 IU/ml (range 0.17–13.77 IU/ml). The time interval between vaccinations in the 116 dogs receiving two vaccines was not reported (the information was not required in the submission form). The most recent rabies vaccination (the single vaccine, or the second of two vaccines) had been given at the age of 0–3 months in 35 (5.3%) dogs, at 3–6 months in 275 (42.5%) dogs, 6–9 months in 168 (25.4%) dogs and 9–12 months in 142 (21.5%) dogs. Of the 142 dogs vaccinated at 9–12 months of age, 127 had been vaccinated only once. The age of the most recent vaccination was not recorded for 42 (6.3%) dogs, but it could reasonably be assumed to be less than 12 months. The median time interval between the most recent vaccination and blood sampling was 5.29 weeks (range 0.14–64 weeks). Of the 662 dogs, 587 (88.7%) received a monovalent vaccine and 74 (11.3%) received a polyvalent vaccine (for one dog this information was not available). Of the 74 dogs receiving polyvalent vaccines, 71 received a single vaccine and 3 received two vaccines. Three commercially available monovalent vaccines were used in 573 (86.5%) dogs and two polyvalent vaccines were used in 69 (10.4%) dogs. In 20 dogs, other brands (monovalent or polyvalent) or unspecified products were used. Vaccination failure rates for dogs receiving one or two vaccines, age at vaccination, and vaccine type were summarized (Table 1).

Based on the final logistic regression model, dogs that were vaccinated twice were more likely to have achieved an acceptable titer compared with dogs vaccinated only once (Table 2). The odds for seropositivity tended to decrease with increasing time between the most recent vaccination and blood sampling. Dogs vaccinated with a monovalent vaccine were more likely to have achieved an acceptable titer compared with those vaccinated with polyvalent vaccines. Dogs that were vaccinated after 3 months of age were more likely to be seropositive.

Based on the final linear regression model, dogs that were vaccinated twice tended to have higher antibody titers than dogs vaccinated once (Table 3). Mixed-breed dogs also had higher antibody titers than purebred dogs. Antibody titers decreased with increasing time between the most recent vaccination and blood sampling. Dogs that were

Table 1
Rabies vaccination failure rates (antibody titer < 0.5 IU/ml) in 662 dogs, based on the number of rabies vaccinations, the vaccine type and the vaccination age.

Variable	Definition	Failure rate (%)
Prior vaccinations	1	16.1
	2	0.9
Vaccine type	Monovalent	9
	Polyvalent	46
Vaccination age	< 3 months	31
	3–6 months	19
	6–9 months	7
	9–12 months	8