Walking the dog and moving the cat: Rabies serology in the context of international pet travel schemes

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Summary

Data of 13’469 blood samples from 10’999 dogs and 2’470 cats tested for rabies neutralizing antibodies within the framework of pet travel schemes were analysed for single and combined factors influencing antibody titres and failures. The time span between vaccination and drawing the blood sample was confirmed as a major source of failure in dogs with a proportion of 23 % at 4 months after primary vaccination (single dose). Failures in dogs and cats (titre < 0.5 IU) were significantly reduced after double primary vaccination (2 doses within 7 – 10 days), although failures reached comparable levels in dogs as early as 6 months after vaccination. In contrast, failure after vaccination was generally below 5 % in dogs and absent in cats after a booster applied at earliest 12 months after single primary vaccination. Statistically significant differences between the failures of the vaccine brands «Rabisin» (1.5 %), «Defensor» (6.7 %), «Nobivac Rabies» (11.0 %) and «Rabdomun» (18.2 %) were found in dogs but also between the titres induced in cats. Statistically significant differences were found between different dog breeds with some small breeds showing a significantly higher responsiveness. Taken together, a new regimen for rabies vaccination consisting of double primary vaccination with a short interval of 7 – 10 days and a one-year booster appears to be highly recommended for dogs and cats.

Keywords: rabies, pet travel schemes, serology, vaccine, regimen, breed

Introduction

Rabies serology by serum neutralization assays (Smith et al., 1973; Cliquet et al., 1998) has become widely used in dogs and cats after the first introduction of a pet travel scheme (PETS) in Sweden and Norway instead of classical quarantine regulations (Klingeborn and Krogsrud, 1993). This has been an important progress toward a more humane procedure for the safe import of pets from dog rabies endemic areas into rabies free countries. In...
PETS, quarantine was replaced by vaccination, serological control of neutralizing antibodies correlating with protection and a waiting period before import. The waiting period accounts for the incubation of a possible rabies exposure before vaccination since a single dose would not ensure protection as postexposure treatment (Cho and Lawson, 1989; Blancou et al., 1991; Clark and Wilson, 1996; Mitmoonpitak et al., 2002; WHO, 2005; WHO, 2007). The UK PETS was established in 2000 (Fooks et al., 2002), followed by the European PETS implemented in Switzerland in 2003 (Bundesamt für Veterinärwesen, 2003b) and in Europe in 2004, respectively, and other national schemes based on the same principles (Takahashi-Omoe et al., 2008). Within the framework of the Swedish/Norwegian model, where testing of rabies neutralizing antibodies must not be performed earlier than 4 months after the last rabies vaccination, the short-lived character of neutralizing antibodies in a certain proportion of the animals became apparent right away (Klingeborn and Krogsrud, 1993) and was confirmed by several laboratories involved (Cliquet et al., 2003; Mansfield et al., 2004). The obvious operational solution to this was a double primary vaccination in a short interval of 7–10 days, which was also adopted as an official recommendation by the Swiss authorities (Bundesamt für Veterinärwesen, 2003a). Over the years of practical experience since its introduction, PETS have proven to be a safe alternative to quarantine if not circumvented by illegal import (Zanonni and Breitenmoser, 2003; Cliquet et al., 2005; French multidisciplinary investigation team, 2008; Eurosurveillance, 2008; Van Gucht and Le Roux, 2008; Dacheux and Bourhy, 2008). The purpose of this work was to identify and confirm risk factors for failure in serological testing on the basis of a large number of animals tested in Switzerland, to study the effect of repeated vaccinations and to identify possible implications for the recommendations for rabies vaccination regimens in pets.

Animals, Material and Methods

Animals and data collection

The data of 13'469 blood samples taken from 10'999 dogs and 2'470 cats between July 23, 1997 and June 26, 2009 (12 years) with complete information on the mandatory variables were included in this study. Data on the animals were collected on the basis of the request form for animal rabies serology and stored in a Windows Access® database. Apart from personal data on the veterinarian and the owner, the following epidemiologically relevant data were recorded: unique identification of the animal (microchip number or tattoo), species, date of collection of blood sample, date of the last vaccination (mandatory variables for reporting), date of birth, sex, breed, total number of rabies vaccinations, date of first vaccination, name (brand) of vaccine.

Blood collection

Whole blood samples without anticoagulants or sera of dogs and cats were obtained by veterinarians within the framework of the diagnostic services provided by the Swiss Rabies Center for the determination of rabies neutralizing antibody titres. These animals had been vaccinated against rabies for varying periods of time before intended international travelling with their owners. Sera were recovered as supernatant from coagulated whole blood by centrifugation at 1’400 g for 10 min at room temperature. All samples were stored at – 20 °C before testing (usually within a week).

Rabies Neutralization Test (Rapid Fluorescent Focus Inhibition Test, RFFIT)

A microtitre adaptation of RFFIT in 96 well microtitre tissue culture trays was performed essentially as described (Smith et al., 1973; Zalan et al., 1979). A pool of human sera calibrated with the 2nd international standard preparation for rabies immunoglobulin (Lyng, 1994) was used for the determination of the neutralizing potency of test sera and CVS-11 (Challenge Virus Strain, Cliquet et al., 1998) was used as challenge virus. The virus neutralizing antibody (VNA) titres were calculated according to Spearman-Kaerber (Spearman, 1908; Kaerber, 1931) by extrapolating the dilution of the sample reducing the number of fluorescent microscopic fields to 50% and international units (IU) were determined via standard control (9.0 IU/ml).

Data evaluation

The test result was stored quantitatively as a titre in international units (IU) and qualitatively as insufficient (< 0.5 IU) or sufficient (≥ 0.5 IU) and used as the dependent (response) variable in statistical analyses. Samples of animals with repeated submissions were included only once. Depending on the type of analysis, only subsets of the whole dataset with complete data could be analyzed. All statistical procedures were performed with the NCSS 2007 software (Number Cruncher Statistical Systems, Kaysville, Utah, USA; Anonymous, 2007). Confidence intervals for proportions (p) and means (m) were calculated as p/m ± 1.96 * sE (standard error).

Results

Time of serological testing after vaccination

Soon after the initiation of pet travel schemes (PETS) in lieu of quarantine in 1994 in Sweden and Norway (Klingeborn and Krogsrud, 1993) it became obvious that the period of time elapsed since vaccination is a critical factor in test failures (Silvonen et al., 1995). This effect
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was strongest after a single primary rabies vaccination in dogs. Therefore, we recommended several years ago to generally apply a regimen of two rabies vaccinations within a short interval of 7–10 days (double primary vaccination). While the proportion of failure (titre < 0.5 IU) in dogs after four months (minimal delay for antibody determination after vaccination in the Swedish/Norwegian PETS) was 23% after single primary vaccination, it was significantly reduced to 6% after double primary vaccination (Fig. 1a). The proportion of failure after a single primary vaccination stabilized at above 30% in the period of 7 to 12 months after vaccination. The same effect of double primary vaccination was much less pronounced and not significant at single time points in cats (Fig. 1b). The proportion after a single primary vaccination stabilized in cats at around 10% in the period of 7 to 12 months after vaccination. Conspicuously, the proportion of failure in dogs with double primary vaccination progressively approximated that of singly vaccinated individuals with time, reaching comparable levels at 6 months (Fig. 1a). In sharp contrast to that, after a booster applied at the earliest 12 months after primary vaccination, the proportion of failure up to 12 months after vaccination was generally below 5% in dogs and absent in cats.

In order to clarify the effect of the double primary vaccination and its non-sustained effect, the kinetics of the seroconversion in dogs (n = 10’318) was also studied quantitatively. As expected, double primary vaccination (interval between doses < 30 d, n = 628) resulted, at least initially, in significantly higher titres than single primary vaccination (n = 2’341), but this effect disappeared as early as after 5 months (Fig. 2a). A booster vaccination applied at the earliest 12 months after single primary vaccination (n = 1’147) led to significantly different postvaccinal antibody kinetics. Titres peaked higher and remained significantly higher throughout the 12 months observation period (Fig. 2b). The application of an earlier booster vaccination (between 30 and 365 days after single primary vaccination, n = 1’462) triggered intermediate antibody kinetics (Fig. 2c) whereas repeated boosters (altogether more than 2 rabies vaccinations, n = 4’740) topped all other regimens in terms of long-term titre stability (Fig. 2d). According to multiple analysis of variance, both time after vaccination and type of vaccination were highly significant effects on postvaccinal antibody titres (p < 0.001). Throughout all types of vaccinations, cats reacted at significantly higher levels than dogs (Fig. 3).

Vaccine, breed, age and gender

Besides time interval and type of vaccination, the different vaccine brands were also found to exert a significant influence on the test results, particularly on failures over a range of 4 months after a single primary vaccination in dogs (p Chi Square < 0.001, Fig. 4a). The proportion of failure of «Rabisin» (RABISIN® ad us. vet., Biokema SA, n = 266) was 1.5%, of «Defensor» (Defensor 3® ad us. vet., Pfizer AG, n = 210) 6.7%, of «Nobivac Rabies» (Nobivac® RABIES ad us. vet., Veterinaria AG, n = 390) 11.0% and that of «Rabdomun» (Rabdomun ad us. vet., Veterinaria AG, n = 214) 18.2%. In cats, no significant differences were evident between the proportions of failure of the vaccine brands used (Fig. 4b) in spite of significant differences of the mean titres (not shown). The frequency distribution of blood sampling time span in months after vaccination for the different vaccine brands was comparable in both cats and dogs (not shown).

The breed was a significant factor in both quantitative (neutralizing antibody titres up to one year after a single primary vaccination) and qualitative analysis (proportions of failure) in dogs (n = 1’248). Using multiple logistic regression, the breeds «Yorkshire Terrier», «Maltese» and «Jack Russell Terrier» exhibited a significantly lower risk of failure in comparison to the «Bernese Mountain Dog» as reference. In cats (n = 686) neither titres nor
proportions of failures seen up to one year after a single primary vaccination differed significantly between the breeds analysed. Neither age in dogs (n = 1344; min = 1 year; max = 18 years; mean = 2.3 years; median = 1 year) nor cats (n = 727; min = 1 year; max = 20 years; mean = 5.1 years; median = 4 years) nor gender in dogs (50.8 % females) or cats (50.9 % females) had a significant influence on neutralizing titre or proportion of failures in univariate analysis (p > 0.05).

**Discussion**

Since the introduction of pet travel schemes (PETS) instead of quarantine in 1994, the time of taking blood samples after vaccination has repeatedly been recognized as the most critical factor for failures with a proportion of around 25 % at 4 months after primovaccination (Sihvonen et al., 1995; Anonymous, 1996; Cliquet et al., 2003;
so-called serological low responders were identified, who for preexposure rabies vaccinations in humans, where threshold (serorevert) within a year after initial vaccination. This is reminiscent of a similar situation described according to Mansfield et al., 2004; Kennedy et al., 2007). According to «Rabisin» is evident for dogs but not for cats with generally lower failure rates.

The positive effect of double primary vaccination on the risk of serological failure (Sihvonen et al., 1995; Anonymous, 1996; Cliquet et al., 2003; Mansfield et al., 2004) was clearly confirmed for dogs. Double primary vaccination also helps to minimize the risk for cats, although cats generally reacted much more strongly than dogs. Interestingly, the titre kinetics observed in dogs were less impressive than expected with titres and failure rates comparable to single primary vaccination reached as early as after 5–6 months. By contrast, clearly different titre kinetics with sustained immunity and low failure rates were observed after a one-year booster. Interestingly, earlier boosters between 30 days and one year remained associated with both significantly different kinetics and a higher failure rate. By modelling over the range of intervals between the first and second rabies vaccination in dogs, the earliest boosting time for a sustained effect on immunity was observed to be around 10 months (not shown). This is compatible with findings in humans vaccinated against rabies, who showed long-term immunity after a one-year booster after triple primary preexposure vaccination for at least 10 years (Strady et al., 1998; Strady et al., 2001) but also with more general principles of maturation of humoral immunity and emergence of the long-lived memory repertoire (Bernasconi et al., 2002; Traggiai et al., 2003).

The effect of the vaccine brand used on serological failure was quite noticeable as described also by others (Mansfield et al., 2004; Kennedy et al., 2007). It is difficult to speculate on the reasons for this, all of the vaccine brands used being inactivated and alum-adjuvanted vaccines produced in cell culture. A possible explanation might be the application of a seed viral strain for vaccine production like the Flury-LEP strain used in «Rabdomun» as in purified chick embryo cell rabies vaccine (PCECV) for human use (Barth et al., 1990), which is not very closely related to the challenge virus strain used to measure the neutralizing antibodies (Moore et al., 2009). Nevertheless, apart from the potential implication on protection, this might be an important factor for travellers and their veterinary advisors in terms of costs and time minimization for compliance with the

![Figure 4: Proportions of failure (neutralizing titre < 0.5 IU) in dogs (a) and cats (b) after a single primary vaccination over a range of 4 months after vaccination with different vaccine brands. A significantly lower proportion of failure after the use of «Rabisin» is evident for dogs but not for cats with generally lower failure rates.](image-url)
regulations. Furthermore, also a significant effect of breed could be confirmed in this work. Taken together, in order to minimize the failure rate for PETS and presumably also to improve protection, a new regimen for rabies vaccination consisting of double primary vaccination with a short interval of 7–10 days and a one-year booster appears to be highly recommended for dogs and cats.

**Acknowledgements**

The scrutinizing proofreading and valuable expert suggestions for improvement of the manuscript by Dr. G. Bertoni and two reviewers were highly appreciated. The indulgence of the respective author’s families for lost private time is gratefully recognized.

**References**


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Received: 17 December 2009
Accepted: 5 February 2010