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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. 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

Reisen mit Hunden und Katzen: Tollwutserologie im Zusammenhang mit internationalen Reisebestimmungen

In dieser Arbeit wurden die Daten von 13'469 Blutproben von 10'999 Hunden und 2'470 Katzen, die im Zusammenhang mit Reisebestimmungen für Haustiere auf Tollwutneutralisierende Antikörper untersucht wurden, auf einzelne und kombinierte Faktoren mit Einfluss auf Titerhöhe und Versagerquote untersucht. Die Zeitspanne zwischen Blutentnahme und Impfung konnte als eine wichtige Quelle für ungenügende Titer bei Hunden bestätigt werden mit einer Quote von 23 % 4 Monate nach Erstimpfung (eine Impfstoff-Dosis). Ein signifikanter Rückgang von Versagern (Titer < 0.5 IU) ergab sich bei doppelter Grundimmunisierung (2 Impfungen im Abstand von 7–10 Tagen), wobei dieser Effekt bei Hunden bereits nach 6 Monaten wieder verschwand. Nach einem Jahres-Booster lag die Versagerquote hingegen bei Hunden generell unter 5 % und bei Katzen praktisch bei Null. Statistisch signifikante Unterschiede zeigten sich zwischen den Versagerquoten der Impfstoffe «Rabisin» (1.5 %), «Defensor» (6.7 %), «Nobivac Rabies» (11.0 %) und «Rabdomun» (18.2 %) bei Hunden und zwischen den Titern bei Katzen. Ebenfalls signifikante Unterschiede wurden zwischen verschiedenen Hunderassen gefunden. Aufgrund dieser Daten scheint ein neues Tollwut-Impfschema mit doppelter Grundimmunisierung in kurzem Intervall von 7–10 Tagen und einem Jahresbooster für Hunde und Katzen als sehr empfehlenswert.

Schlüsselwörter: Tollwut, Pet Travel Schemes, Serologie, Impfstoff, Impfschema, Rasse

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 (Zanoni 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 $p/m \pm 1.96 * SE$ (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 (Sihvonen et al., 1995). This effect

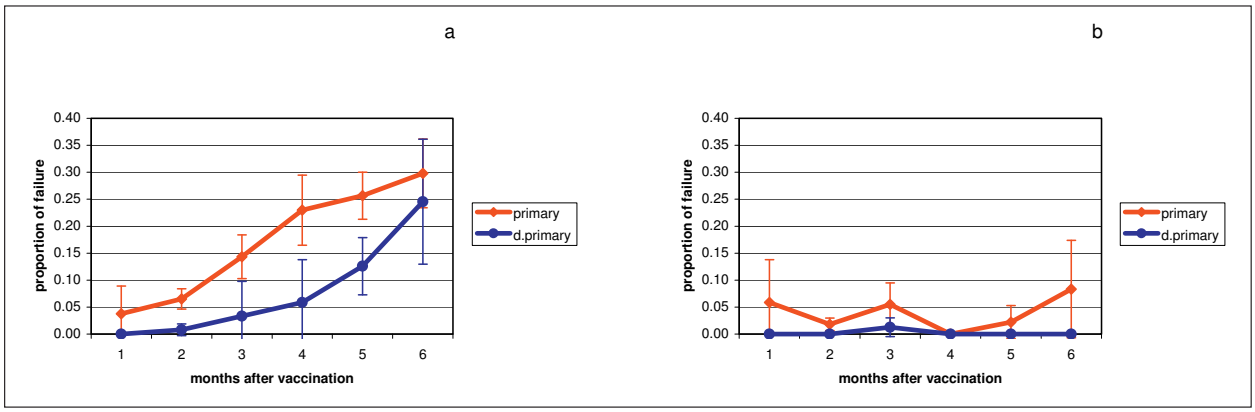

Figure 1: Proportion of failure after single or double primary rabies vaccination in dogs (a) and cats (b). The proportion of failure in rabies serology (< 0.5 international units [IU]) is significantly reduced in dogs after double primary vaccination. Bars represent 95 % confidence intervals (CI).

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 a 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

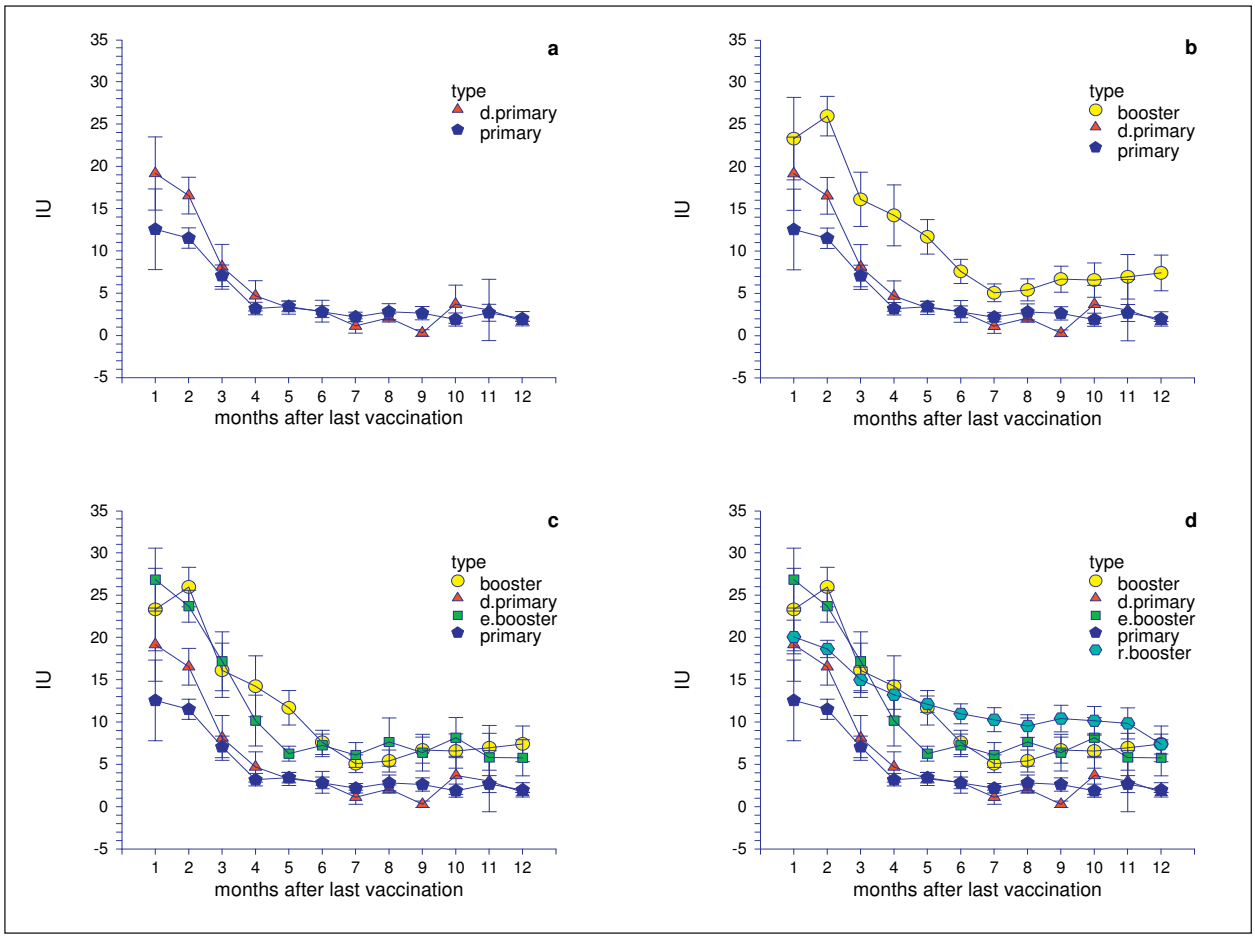

Figure 2: Kinetics of rabies antibody titres in dogs after a given «type» of last vaccination.
(a) Titre decrease after double primary («d.primary») in comparison to single primary vaccination («primary»), (b) Titre decrease after a booster vaccination («booster») applied at the earliest 12 months after single primary vaccination, (c) Intermediate titre course after an early booster vaccination («e.booster») between 30 days and one year after single primary vaccination, (d) Rabies antibody titres after repeated booster vaccination («r.booster», altogether > 2 rabies vaccinations). The respective time of the application of a given last vaccination is defined as time point zero of the x-axis. Bars represent 95 % CI. IU = international units.

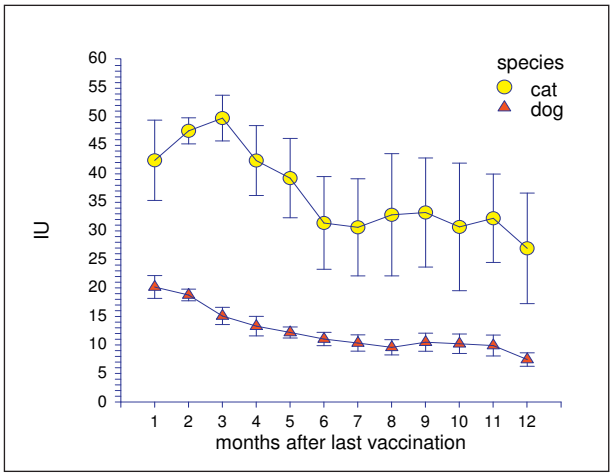

Figure 3: Comparative rabies antibody titre kinetics in dogs (n = 4740) and cats (n = 548) after repeated booster vaccinations (altogether > 2 rabies vaccinations). Bars represent 95 % CI. IU = international units.

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) or 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;

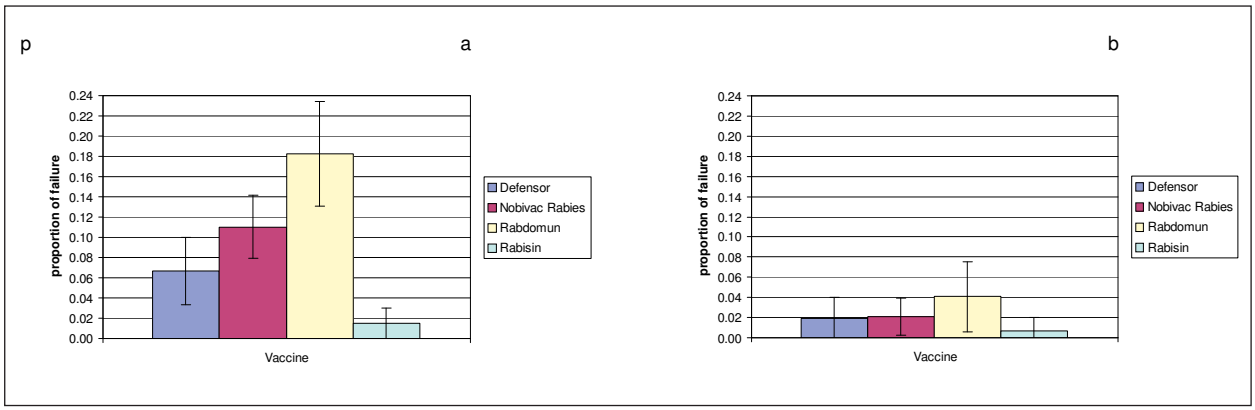

Figure 4: Proportions of failure (neutralizing titre < 0.5 IU) in dogs (a) and cats (b) after a single primary vaccination over a range of 4 months after vaccination with different vaccine brands. A significantly lower proportion of failure after the use of «Rabisin» is evident for dogs but not for cats with generally lower failure rates.

Mansfield et al., 2004; Kennedy et al., 2007). According to our data, about 1/3 of the dogs appear to fall below the threshold (serorevert) within a year after initial vaccination. This is reminiscent of a similar situation described for preexposure rabies vaccinations in humans, where so-called serological low responders were identified, who needed at least one booster for long-term persistence of rabies neutralizing antibodies (Strady et al., 1998). As also noted by others (Tepsumethanon et al., 1991; Kennedy et al., 2007), the titres after primo-vaccination shown here are in line with primary immune response kinetics. It must be noted that the classification of titres into pass and failure categories both in humans and animals is based on an arbitrary decision (Fishbein et al., 1987) being applied in PETS in a strictly decisive sense. In spite of the clear correlation with humoral neutralizing antibodies (Dietzschold et al., 1990; Wunderli et al., 1991), protection in vivo is just as much related to cellular immunity (Dietzschold et al., 1987; Bunschoten et al., 1989; Kawano et al., 1990; Dietzschold et al., 1990; Herzog et al., 1992; Xiang et al., 1995; Mansfield et al., 2004). As long as efficacy and potency of rabies vaccines are confirmed on the basis of animal challenge experiments (Bunn, 1991; Cliquet et al., 2003; Anonymous, 2005), protection in the absence of neutralizing antibodies might reasonably be assumed. This notwithstanding, in view of several possible shortcomings in practical vaccine application, regularly reported vaccine failures in vaccinated animals (Schwendenwein and Gerstl, 1993; Clark and Wilson, 1996; Okoh, 2000; Weber et al., 2003; De Benedictis et al., 2009; Murray et al., 2009) might also be attributed to overconfidence in the efficacy of vaccines or in the duration of protection. Furthermore, protection of the population due to a sufficient level of herd immunity is quite different from protection of a single individual, which is aimed at in PETS.

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., 2005). 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

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.

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Voyages avec des chiens et des chats: sérologies rabiques en rapport avec les dispositions internationales

Dans ce travail, les données de 13'469 échantillons sanguins, provenant de 10'999 chiens et de 2'470 chats, prélevés au vu des dispositions liées à des voyages, ont été examinés quant aux anticorps rabiques pour rechercher des facteurs isolés ou combinés influençant le titre et les échecs vaccinaux. Le délai entre la vaccination et la prise de sang a pu être confirmé comme une source importante de titre insuffisant chez les chiens avec une prévalence de 23 % 4 mois après la primo vaccination (1 dose). On a constaté une réduction significative des échecs vaccinaux (titre inférieur à 0.5 IU) après une double immunisation de base (2 vaccins avec un intervalle de 7 à 10 jours), cet effet disparaissait toutefois chez les chiens déjà après 6 mois. Après un rappel annuel, le taux d'échec était, par contre, chez les chiens inférieurs à 5 % et chez les chats, pratiquement nuls. On a constaté des différences significatives dans les taux d'échecs entre les vaccins «Rabisin» (1.5 %), «Defensor» (6.7 %) «Nobivac Rabies» (11.0 %) et «Rabdomun» (18.2 %) chez les chiens ainsi qu'entre les titres chez les chats. Des différences significatives ont également été trouvées entre différentes races de chiens. Sur la base de ces données, un nouveau schéma vaccinal comportant une double immunisation de base dans un intervalle court de 7 à 10 jours et un rappel annuel pour les chiens et les chats semblent très recommandables.

Viaggiare con cani e gatti: sierologia della rabbia in relazione alle disposizioni internazionali di viaggio

In questo studio sono stati analizzati i dati di 13'469 campioni di sangue provenienti da 10'999 cani e 2'470 gatti sui fattori singoli o combinati che influenzano i risultati della titolazione e della percentuale di insuccessi, in relazione alle disposizioni di viaggio per animali da compagnia riguardo gli anticorpi neutralizzanti contro la rabbia.
Nei cani, il periodo trascorso tra la raccolta del sangue e la vaccinazione potrebbe essere una fonte importante per confermare l'insufficienza di titolo, con un tasso del 23 % dopo 4 mesi dalla prima vaccinazione (una dose di vaccino). Un significativo rallentamento degli insuccessi (titolo < 0.5 IU) risultava da una doppia immunizzazione primaria (2 vaccinazioni a distanza di 7–10 giorni), anche se questo effetto nei cani spariva dopo circa 6 mesi. Dopo un richiamo annuale il tasso di insuccessi rimaneva generalmente inferiore al 5 % e nei gatti praticamente a zero. Differenze statisticamente significative sono risultate tra i tassi di insuccessi nei vaccini «Rabisin» (1.5 %), «Defensor» (6.7 %), «Nobivac Rabies» (11.0 %) e «Rabdomun» (18.2 %) in cani e tra i titoli nei gatti. Inoltre si sono riscontrate differenze consistenti tra le diverse razze di cani. Sulla base di questi dati sembra necessario raccomandare un nuovo schema di vaccinazioni contro la rabbia con doppia vaccinazione primaria praticata ad un breve intervallo di 7–10 giorni e un richiamo annuale sia per i cani che per i gatti.

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