



Anti-rabies humoral immune response in cats after concurrent vs separate vaccination against rabies and feline leukaemia virus using canarypox-vectored vaccines

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Anna-Karina Weidinger¹ , Michèle Bergmann¹ ,
Matthias König², Yury Zablotski¹ and Katrin Hartmann¹

Abstract

Objectives Some expert groups recommend that cats should be vaccinated with non-adjuvanted feline leukaemia virus (FeLV) and rabies vector vaccines, which, in the European Union, are currently not licensed for concurrent use and have to be administered at least 14 days apart (different from the USA) and thus at separate visits, which is associated with more stress for cats and owners. The aim of this study was to assess the anti-rabies antibody response in cats after vaccination against rabies and FeLV at concurrent vs separate (4 weeks apart) visits using two canarypox-vectored vaccines (Purevax Rabies and Purevax FeLV; Boehringer Ingelheim) and to evaluate the occurrence of vaccine-associated adverse events (VAAEs).

Methods Healthy FeLV antigen-negative client-owned kittens (n = 106) were prospectively included in this randomised study. All kittens received primary vaccinations against rabies (week 0) and FeLV (weeks 4 and 8). After 1 year, the study group (n = 52) received booster vaccinations against rabies and FeLV concurrently at the same visit (weeks 50–52). The control group (n = 54) received booster vaccinations against rabies (weeks 50–52) and FeLV (weeks 54–56) separately. Anti-rabies virus antibodies (anti-RAV Ab) were determined by fluorescent antibody virus neutralisation assay at weeks 4, 50–52 and 54–56, and compared between both groups using a Mann–Whitney U-test.

Results Four weeks after the first rabies vaccination, 87/106 (82.1%) kittens had a titre ≥ 0.5 IU/ml and 19/106 (17.9%) had a titre < 0.5 IU/ml. Four weeks after the 1-year rabies booster, all cats had adequate anti-RAV Ab according to the World Organisation for Animal Health (≥ 0.5 IU/ml), and the titres of the study group (median = 14.30 IU/ml) and the control group (median = 21.39 IU/ml) did not differ significantly ($P = 0.141$). VAAEs were observed in 7/106 (6.6%) cats.

Conclusions and relevance Concurrent administration of Purevax FeLV and Purevax Rabies vector vaccines at the 1-year booster does not interfere with the development of anti-RAV Ab or cause more adverse effects and thus represents a better option than separate vaccination visits for cats and owners.

Keywords: canarypox-vectored vaccines; antibodies; concurrent vaccination; FeLV; immunisation

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Introduction

The necessity of vaccination is not only controversially discussed in human medicine, but it is also an important issue in feline veterinary practice due to the potential occurrence of vaccine-associated adverse events (VAAEs).¹ One of the most serious VAAEs in cats is feline injection-site sarcoma (FISS), which can develop at injection sites after vaccination.^{2,3} It has been reported that

¹LMU Small Animal Clinic, Centre for Clinical Veterinary Medicine, LMU Munich, Munich, Germany

²Institute of Virology, Faculty of Veterinary Medicine, Justus Liebig University, Giessen, Hessen, Germany

Corresponding author:

Anna-Karina Weidinger DVM, LMU Small Animal Clinic, Centre for Clinical Veterinary Medicine, LMU Munich, Veterinärstraße 13, Munich 80539, Germany

Email: a.weidinger@medizinische-kleintierklinik.de



in some jurisdictions, FISS occurs in 0.3–4/10,000 vaccinated cats.^{4,5} Some studies showed that there might be a potential link between the increase in FISS and more common use of vaccines against rabies^{3,6} and feline leukaemia virus (FeLV).⁷ A case-control study indicated that adjuvanted inactivated vaccines were more commonly associated with the development of FISS in certain locations than other vaccines ($P = 0.020$), although no vaccines were risk-free.⁸ The most widely discussed hypothesis is that chronic inflammatory reactions at the injection site could trigger malignant transformation,^{2,3,9,10} but the association between vaccine adjuvants and a higher risk of FISS development has not been proven.^{8,11} Some expert groups recommend that cats should be vaccinated with vaccines that cause less pronounced inflammation at the injection site to minimise the risk of FISS development. Therefore, they suggest that vaccines without adjuvants should be preferred over adjuvant-containing vaccines, as long as they are equally effective.^{2,12}

For vaccinations against rabies and FeLV, non-adjuvanted recombinant canarypox-vectored vaccines are available, which cause less inflammation at the injection site than adjuvanted vaccines.¹³ Some previous studies raised concerns regarding the concurrent administration of vectored vaccines and reduced efficacy, but these studies were performed with, for example, adenovirus-vectored vaccines in human medicine.^{14,15} In horses, on the other hand, it was shown that they did not develop detectable neutralising antibodies against canarypox virus after booster vaccinations using canarypox-vectored vaccines against West Nile encephalomyelitis, even after the repeated administration of high doses.^{16,17} In the European Union (EU), the available non-adjuvanted recombinant canarypox-vectored vaccines against rabies and FeLV (Purevax Rabies and Purevax FeLV; Boehringer Ingelheim) have to be administered during separate visits (at least 14 days apart) according to the manufacturer's instructions (different from the USA).¹⁸ Purevax Rabies has a validated 3-year duration of immunity after the primary vaccination series;¹⁹ revaccination is then recommended every 3 years.^{12,20} For FeLV, expert guidelines recommend 3-year booster vaccinations in low-risk cats.^{12,20,21} Therefore, cats with a potential risk of rabies exposure (outdoor access, contact to other cats, travel)^{20,22} could receive booster and revaccinations against rabies and FeLV at the same time. However, if vaccines cannot be given at the same visit, an additional visit to the veterinarian, which could possibly be associated with stress for cats and their owners, is necessary.^{23,24} So far, no independent field studies on the concurrent administration of non-adjuvanted recombinant canarypox-vectored vaccines have been performed.

The aim of the present study was to investigate the anti-rabies virus antibody (anti-RAV Ab) response of cats after vaccination against rabies (Purevax Rabies)

and FeLV (Purevax FeLV) at concurrent vs separate visits using canarypox-vectored vaccines by measuring anti-RAV Ab.

Materials and methods

Cats

This prospective study included 148 client-owned kittens between November 2018 and June 2021. Of these, 42 kittens were excluded for various reasons. Thus, 106 kittens entered the study. The study protocol was approved by the Government of Upper Bavaria (reference number ROB-55.2-2532.Vet_03-18-57). In addition, each owner signed an informed consent form before participation.

Kittens aged 8–12 weeks that had not been vaccinated against rabies and FeLV and had not received a systemic drug treatment (except deworming) within the past 4 weeks were included. Kittens had to be clinically healthy on physical examination and negative for feline immunodeficiency virus (FIV) and FeLV by ELISA (SNAP Combo Plus FeLV-antigen/FIV-antibody test; IDEXX Laboratories). Information on the kittens' signalment, origin (breeder, animal shelter, farm or private household), lifestyle (indoor or outdoor) and housing conditions (single- or multi-cat household) were collected.

All kittens received the same primary vaccination series against feline herpesvirus (FHV), feline calicivirus (FCV) and feline panleukopenia virus (FPV) (Purevax RCP; Boehringer Ingelheim), starting at 8–12 weeks of age, with subsequent revaccinations every 4 weeks until at least 20 weeks and a booster 1 year later;^{12,25–27} the response to vaccination against FHV, FCV and FPV was not a subject of the present study. Purevax RCP and Purevax FeLV were given concurrently at weeks 4, 8 and 50–52 (only in the 'concurrent booster group'); in study week 0 and weeks 50–52 (only in the 'group that received the booster vaccination at separate visits'), Purevax RCP vaccination was given on its own (Figure 1).

Before each vaccination, the cat's medical history was obtained and a physical examination was performed. Cats that did not return for follow-up visits (eg, due to the presence of diseases, pregnancy, car accidents, having run away) were excluded (Figure 1).

Most cats were purebred (77/106, 72.6%), belonging to the breeds British Shorthair (32/77, 41.6%), Bengal (24/77, 31.2%), British Longhair (5/77, 6.5%), Siberian (4/77, 5.2%), Norwegian Forest Cat (4/77, 5.2%), Maine Coon (3/77, 3.9%), Siamese (3/77, 3.9%) and Russian Blue (2/77, 2.6%). Of the 106 cats, 29 (27.4%) cats were non-pedigree. Overall, 57 (53.8%) cats were male and 49 (46.2%) were female. The 106 cats originated from registered breeders ($n = 62$, 58.5%), private households ($n = 28$, 26.4%), farms ($n = 10$, 9.4%) or animal shelters ($n = 6$, 5.7%). At the time of the kitten vaccination series, all 106 (100%) kittens were intact and lived indoors only. Most cats (93/106, 87.7%) had been neutered when they were

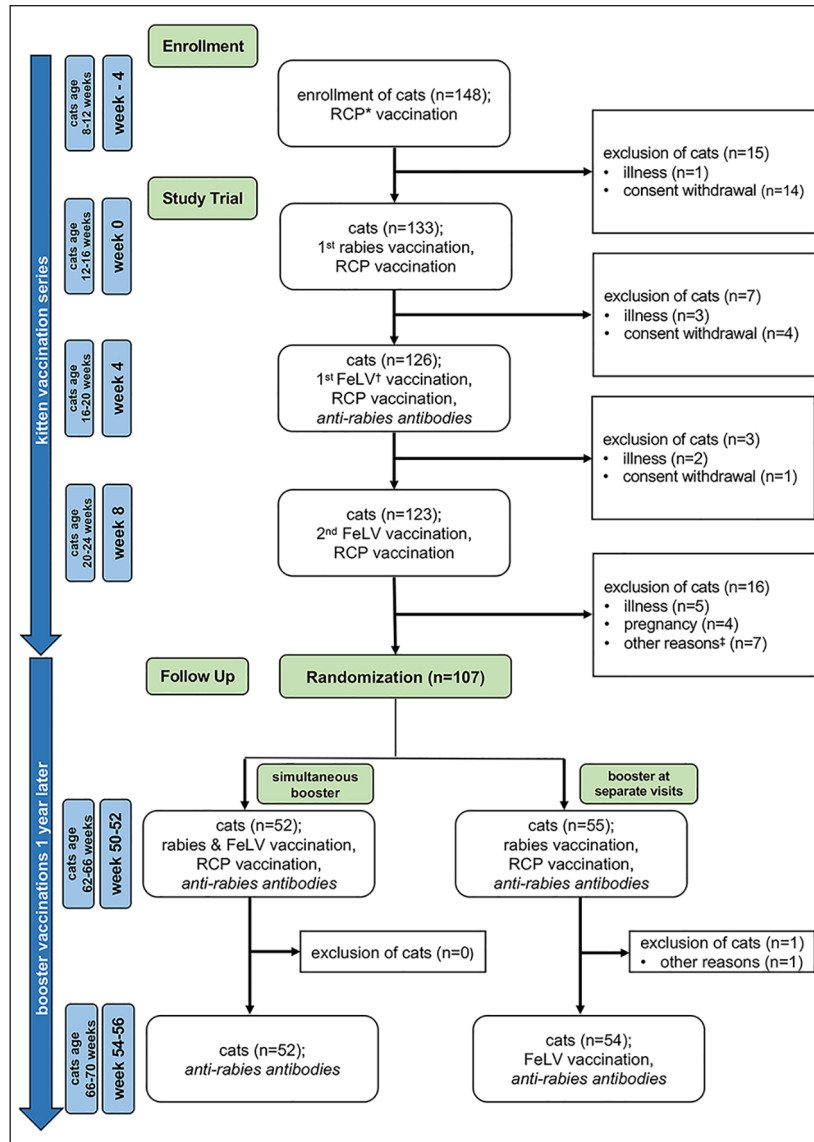


Figure 1 Flow chart illustrating the study course, enrolment, inclusion and allocation process for cats that received 1-year booster vaccinations against rabies and feline leukaemia virus with canarypox-vectored vaccines. *Italic text refers to the measurements of anti-rabies virus antibodies in blood by fluorescent antibody virus neutralisation.* *RCP = vaccination against feline herpesvirus, feline calicivirus and feline panleukopenia virus; †FeLV = feline leukaemia virus; ‡other reasons = other reasons for exclusion: ran away, death by car accident, examination not possible

presented for the 1-year booster vaccinations, and at that time, 55 (51.9%) cats had outdoor access. Overall, 81/106 (76.4%) cats lived together with other animals.

Vaccinations against rabies and feline leukaemia virus

Vaccination against rabies was started at the age of 12–16 weeks (study week 0) (Figure 1). Starting 4 weeks after the first rabies vaccination, kittens were vaccinated against FeLV twice at a 4-week interval, at the age of 16–20 and 20–24 weeks (study weeks 4 and 8) (Figure 1).

For the 1-year booster vaccinations against rabies and FeLV, cats were randomly divided into two groups

(QuickCalcs; GraphPad Software): the study group ($n = 52$) received booster vaccinations against rabies and FeLV concurrently at the same visit in study weeks 50–52, and the control group ($n = 55$) received separate booster vaccinations against rabies in study weeks 50–52 and FeLV 4 weeks later in study weeks 54–56 ($n = 54$) (Figure 1).

The administered vaccines were taken out of the refrigerator approximately 15 mins before injection and brought to room temperature.² After shaving the injection site, vaccines were administered subcutaneously using a 2 ml syringe with a 23 G needle. The rabies vaccine was administered in the right abdominal wall and the FeLV

vaccine (together with FHV, FCV and FPV) in the left lateral abdominal wall. The abdominal wall, which is an alternative site for subcutaneous vaccine injection,^{2,12} was chosen due to the small size and difficult handling of the kittens. In addition, the abdominal wall is still a location that meets the requirements for radical surgical resection of FISS including margins of at least 3 cm, but preferably 5 cm.²⁸ The vaccination against rabies was performed with an adjuvant-free vaccine containing rabies recombinant canarypox virus strain vCP65 with a viral titre of $\geq 10^{6.8}$ fluorescent assay infectious dose 50% (Purevax Rabies). Vaccination against FeLV was performed with an adjuvant-free vaccine containing FeLV recombinant canarypox virus strain vCP97 with a viral titre $\geq 10^{7.2}$ cell culture infective dose 50% (Purevax FeLV). Cats were observed for approximately 15 mins after vaccination for anaphylactic reactions. Owners were instructed to report possible VAAEs (eg, swelling and/or pain at the injection site, fever, lethargy, hypo-/anorexia, vomiting).

Detection of anti-rabies virus antibodies

For detection of anti-RAV Ab, serum samples were collected at different time points (weeks 4, 50–52 and 54–56) (Figure 1) and frozen at -80°C until all samples had been collected. Samples were shipped on dry ice from the LMU Small Animal Clinic, Centre for Clinical Veterinary Medicine, LMU Munich, Germany, to the Institute of Virology, Faculty of Veterinary Medicine, Justus Liebig University, Giessen, Germany. Before testing, sera were heat inactivated at 56°C for 30 mins. Anti-RAV Ab were measured by fluorescent antibody virus neutralisation using the prescribed protocol of the World Organisation for Animal Health (WOAH).²⁹

All samples from each individual cat were included in the same test run. Anti-RAV Ab were determined using immunofluorescence and calculated as \log_{10} neutralising dose 50 according to the Spearman–Kärber method.³⁰ Titres were converted to international units per millilitre (IU/ml) by comparison to the Office International des Epizooties (WOAH) standard serum of dog origin. Tests were validated using control charts (titre of the test virus, titres of control sera [WOAH dog standard, internal standard serum, naive dog serum (WOAH), internal negative serum]). Anti-RAV Ab titres ≥ 0.5 IU/ml were considered an adequate antibody response after vaccination against rabies as defined by the World Health Organization (WHO)³⁰ and WOAH.²⁹

Statistical analysis

A one-sided non-inferiority test for log-normal distribution or geometric mean ratio was used to determine the sample sizes of the cats. The non-inferiority margin was set at 0.5 and a significance level of $\alpha = 0.05$ was chosen. The power was set to $1 - \beta = 0.80$. As a result, 51 cats per group were required. As some dropouts were expected,

a higher number of cats ($n = 148$) was initially included. Throughout the study period, 42 kittens were excluded for various reasons (Figure 1).

The normality of the data was assessed using the Shapiro–Wilk normality test. Levene's test was used to assess the homogeneity of variances. Due to mostly non-normally distributed data, medians and interquartile ranges were calculated, and the Mann–Whitney U-test was used to compare anti-RAV Ab titres between the concurrently vaccinated group and the separately vaccinated group. Results with $P < 0.05$ were considered statistically significant. Data were analysed using R statistical software version 3.6.3 (R Foundation for Statistical Computing). Fisher's exact test was used to determine the associations between VAAEs and anti-RAV Ab ≥ 0.5 IU/ml after rabies vaccination. Welch's t-test was used to determine significant differences in the mean anti-RAV Ab titres in cats with and without VAAEs.

Results

Antibody response after rabies vaccination

Four weeks after the first rabies vaccination at the age of 12–16 weeks, all 106 kittens (100%) had anti-RAV Ab (median titre 1.81 IU/ml; range 0.05–37.05 IU/ml); of the kittens, 87 (82.1%) had a titre ≥ 0.5 IU/ml, and 19 (17.9%) had a titre < 0.5 IU/ml.

On the day of the 1-year booster vaccination, anti-RAV Ab were still detectable in all 106 cats (median titre 0.6 IU/ml; range 0.03–16.25); of the cats, 55 (51.9%) had a titre ≥ 0.5 IU/ml, and 51 (48.1%) had a titre < 0.5 IU/ml.

Four weeks after the 1-year booster rabies vaccination, anti-RAV Ab ≥ 0.5 IU/ml were detected in all 52 cats (100%) that had received concurrent booster vaccinations against FeLV (median titre 14.3 IU/ml; range 1.37–146.29) and in all 54 cats (100%) that had received separate booster vaccinations (median titre 21.39 IU/ml; range 1.04–253.39). At this time point, there was also no significant difference in the anti-RAV Ab titres between concurrently vaccinated cats and separately vaccinated cats ($P = 0.141$) (Figure 2).

Table 1 summarises the median anti-RAV Ab titres of the cats during the entire study period. Cats with anti-RAV Ab titres ≥ 0.5 IU/ml at the three different study periods are presented in Table 2.

Occurrence of VAAEs

Throughout the study period, VAAEs were observed by the owners in 7/106 (6.6%) cats. After the first rabies vaccination, 2/7 kittens showed pain at the rabies vaccine injection site for 1–5 days and one kitten showed a slightly reduced general condition (lethargy). During the kitten vaccination series against FeLV, 3/7 kittens showed a slightly reduced general condition for 1–2 days. After the 1-year rabies booster vaccination, 1 cat (1/7; separate vaccinations group) showed pain at the rabies vaccine

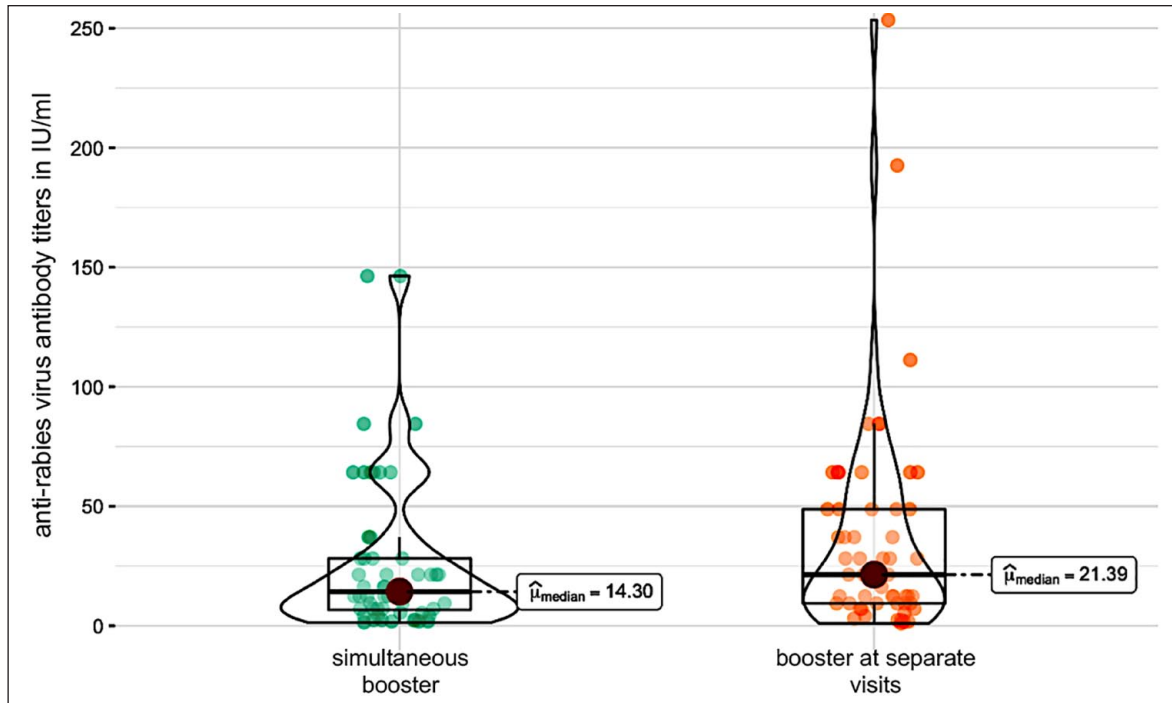


Figure 2 Anti-rabies virus antibody titres at 4 weeks after the 1-year booster vaccination against rabies and feline leukaemia virus (FeLV). One group (green; $n = 52$) received booster vaccinations against rabies and FeLV concurrently at the same visit, and one group (orange; $n = 54$) received separate booster vaccinations against rabies and FeLV at a 4-week interval. No significant difference in anti-rabies virus antibody titres between the concurrently vaccinated group and the separately vaccinated group was determined by Mann–Whitney U-test ($P = 0.141$)

Table 1 Anti-rabies virus antibody titres of the cats during the entire study course

Group	Anti-rabies virus antibody titres		
	4 weeks after first rabies vaccination (IU/ml)	50–52 weeks after first rabies vaccination, directly before 1-year booster (IU/ml)	4 weeks after 1-year booster vaccination (IU/ml)
Concurrent booster* ($n = 52$)	1.81 (0.05–28.15)	0.48 (0.05–3.13)	14.30 (1.37–146.29)
Booster at separate visits† ($n = 54$)	1.81 (0.12–37.05)	0.60 (0.03–16.25)	21.39 (1.04–253.39)

Data are presented as median (range)

*Concurrent booster vaccinations against rabies and feline leukaemia virus

†Separate booster vaccinations against rabies and feline leukaemia virus 4 weeks apart

injection site for 1 day. None of the cats from the concurrent booster group showed VAAEs and no cats from the separate vaccinations group had VAAEs after the single FeLV booster vaccination. Overall, no anaphylactic reactions were observed.

Cats with VAAEs were not more likely to have high anti-RAV Ab (titre ≥ 0.5 IU/ml) after rabies vaccination ($P = 1.000$). There were no differences in the mean anti-RAV Ab titres of cats with VAAEs in comparison with those without VAAEs ($P = 0.780$).

Discussion

Recombinant vectored vaccines have several advantages.^{17,31,32} In comparison with modified live viruses (MLVs), their production does not require the handling of potentially dangerous viruses, such as rabies virus, and there is no risk of virulence reversion.^{17,33} In contrast to inactivated vaccines with adjuvants, recombinant vectored vaccines cause less inflammation at the injection site¹³ and are therefore recommended for vaccination against rabies and FeLV by some cat expert

Table 2 Numbers of cats with different anti-rabies virus antibody titres 4 weeks and 50–52 weeks after the first rabies vaccination, and 4 weeks after the 1-year booster vaccination against rabies

Titre (IU/ml)	Study week			
	Week 4	Weeks 50–52	Weeks 54–56	
	Cats (n = 106)	Cats (n = 106)	Concurrent booster* (n = 52)	Booster at separate visits† (n = 54)
0.5–<1.0	14	21	0	0
1.0–<2.0	23	22	4	3
2.0–<4.0	13	10	4	3
4.0–<8.0	22	1	10	5
8.0–<12.0	3	0	3	6
12.0–<24.0	10	1	15	11
24.0–<48.0	2	0	6	9
≥48.0	0	0	10	17
Summary				
<0.5	19	51	0	0
≥0.5	87	55	52	54

*Concurrent booster vaccinations against rabies and feline leukaemia virus

†Separate booster vaccinations against rabies and feline leukaemia virus 4 weeks apart

groups worldwide.^{2,25} The two available recombinant vectored vaccines against rabies and FeLV are currently not licensed for concurrent use in the EU,¹⁸ which is disadvantageous for practical reasons as two visits to the veterinarian are required to administer them separately.

The present study was the first independent field study that investigated the concurrent use of two recombinant canarypox-vectored vaccines. The results indicate that concurrent application did not negatively influence the cats' humoral immune response, at least not concerning the development of anti-RAV Ab. The efficacy of the used canarypox-vectored vaccines is based on the expression of foreign immunogens: the glycoprotein G of rabies virus (Purevax Rabies; vCP65), and the envelope glycoproteins (p15E and gp70) and capsid proteins of FeLV A (Purevax FeLV; vCP97).^{31,34–36} One concern about the concurrent use of two recombinant canarypox-vectored vaccines was that an increased presence of canarypox virus might lead to a competitive inhibition of host cells, resulting in an imbalanced response to the rabies and FeLV vaccines. Pre-existing anti-vector antibodies could bind to the vector vaccine and therefore inhibit cell entry, antigen presentation and, thus, the induction of immune responses to the encoded antigens in the vaccine.^{14,15} However, the present study demonstrated that 4 weeks after the 1-year booster rabies vaccination, anti-RAV Ab ≥0.5 IU/ml were detected in all cats (regardless of whether the vaccine was administered concurrently or at two separate visits), which implies that there was no interference with potentially pre-existing anti-vector antibodies and

vaccination. All cats in the present study had anti-RAV Ab ≥0.5 IU/ml after the booster vaccination, indicating an adequate antibody response according to the WOAHA legal requirements²⁹ and EU pet travel regulations³⁷ and, thus, no disadvantage of concurrent vaccination was demonstrated.

The cats in the present study were not tested for FeLV-neutralising antibodies, since cell-mediated immunity (cytotoxic T lymphocytes) plays a major role in immunity to FeLV^{35,38} and specifically because Purevax FeLV usually does not induce detectable antibodies.³⁹

Besides efficacy, a further aspect of concurrent vaccination with canarypox-vectored vaccines was safety. In the present study, VAAEs after concurrent administration of the two canarypox-vectored vaccines were not observed in any of the cats. VAAEs occurred in only 7/106 cats (6.6%). Clinical signs were either local (pain at the injection site) or mild systemic (slightly reduced general condition), and all clinical signs resolved without treatment. Local reactions at the injection site, including pain and swelling, represented the second most common VAAE, occurring in approximately 25% of cats in a retrospective study (in which various different vaccines were used),⁴⁰ whereas, in the present study, only 2.8% of cats showed pain at the injection site. None of the vaccines administered in the present study contained adjuvants, which could explain the lower number of local reactions.¹³

Systemic VAAEs (slightly reduced general condition in 4/7 cats) occurred only after vaccination with canarypox-vectored vaccines in combination with FHV, FCV

and FPV during the kitten vaccination series. Therefore, these VAAEs could have also been caused by vaccination with the core components FHV, FCV and FPV. It is likely that the MLVs (FPV, FHV) were responsible as they are able to replicate in the cat.⁴¹ In fact, a reduced general condition after FPV MLV vaccination has been shown to be associated with a strong humoral immune response.⁴² FCV, as an inactivated component of Purevax RCP, does not replicate within the cat, and so is less likely to be responsible for the systemic signs.²¹ A statistical analysis failed to demonstrate a correlation between VAAEs and a high anti-RAV Ab titre (≥ 0.5 IU/ml) after rabies vaccination in the present study, but this could also be due to the overall low number of cats with VAAEs.

A higher risk for VAAEs, especially anaphylactic reactions, has been described after the application of a higher number of vaccines per visit.⁴⁰ Anaphylactic reactions were not observed in the cats in the present study, which supports the safety of concurrent vaccination against rabies, FeLV and combinations with vaccines against FHV, FCV and FPV.

The concurrent administration of the rabies and FeLV vaccinations is advantageous since cats do not have to be presented to the veterinarian twice for the booster vaccination. This, as a consequence, reduces stress for cats and owners.^{23,24,43–45} Previous studies^{23,43,45} investigated the physiological parameters in cats (blood pressure, rectal temperature, heart rate and respiratory rate) during veterinarian visits and found that several parameters were significantly increased compared with those of the same cats at home.²³ This indicates that the visit to the veterinarian itself causes stress in cats. Several owner compliance studies also showed that stress experienced by cats during a veterinary visit was a very important factor in the owner's decision on whether to vaccinate their cats.^{24,44}

In the present study, the percentage of cats with an anti-RAV Ab titre ≥ 0.5 IU/ml decreased within the first year (from 82.1% 4 weeks after the first rabies vaccination to 51.9% 50–52 weeks after the first rabies vaccination). Thus, the longer the time between vaccination and sample collection, the smaller the number of cats that had anti-RAV Ab ≥ 0.5 IU/ml. This has also been shown within a framework of pet travel schemes⁴⁶ and in various studies in dogs.^{47–50} It has to be mentioned that an anti-RAV Ab titre < 0.5 IU/ml does not indicate a lack of protection in vaccinated animals; in an experimental study, vaccinated cats with anti-RAV Ab < 0.5 IU/ml (and even some cats without detectable anti-RAV Ab; $n = 2$) were protected against rabies challenge,¹⁹ indicating the involvement of other immune mechanisms, such as cell-mediated immunity.^{19,35,38,51,52} However, the results of the present study endorse the recommendations that a booster vaccination with the Purevax Rabies vaccine is necessary after 1 year (or even earlier) to increase the probability that cats will reach a titre ≥ 0.5 IU/ml (especially if travel documents

are required).²⁹ Veterinarians should proactively make owners aware of revaccinations and titre testing in time.

The major limitation of the present study was that anti-RAV Ab titre reactions (≥ 0.5 IU/ml) were used as a measure of immunity, which does not directly correlate with protection, and it is known that cell-mediated immunity plays a major role in protection, but measurements of cell-mediated immunity are not well developed.^{19,35,38,51–53} True protection can be demonstrated only by virulent rabies challenge, which was not performed since the present study was conducted under field conditions with privately owned cats. Another limitation of the study was that FeLV responses were not assessed, since cell-mediated immunity (cytotoxic T lymphocytes) plays a major role in immunity to FeLV^{35,38} and Purevax FeLV usually does not induce detectable antibodies.³⁹

Conclusions

Anti-RAV Ab titres ≥ 0.5 IU/ml were detected in all concurrently vaccinated cats after the administration of Purevax FeLV and Purevax Rabies vector vaccines. These results indicate that concurrent vaccination is possible, representing a better option by reducing the number of vaccination appointments for cats and consequently the stress for cats and owners. In the present study, it was also shown that a booster vaccination with Purevax Rabies vaccine is necessary after 1 year to increase the likelihood that cats will reach a titre ≥ 0.5 IU/ml, which is required for travel to endemic areas.²⁹

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Conflict of interest This research was funded by Boehringer Ingelheim, Ingelheim Rhine, Germany. Boehringer played no role in the collection and interpretation of data or in the decision to submit the manuscript for publication. There is no commercial conflict of interest of the authors as the information generated here is solely for scientific dissemination.

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Ethical approval The work described in this manuscript involved the use of non-experimental (owned or unowned) animals and procedures that differed from established internationally recognised high standards ('best practice') of veterinary clinical care for the individual patient. The study therefore had prior ethical approval from an established committee as stated in the manuscript.

Informed consent Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

ORCID iD Anna-Karina Weidinger  <https://orcid.org/0009-0004-0206-0872>

Michèle Bergmann  <https://orcid.org/0000-0002-8856-3849>

Yury Zablotzki  <https://orcid.org/0000-0001-6928-4089>

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