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Antibody response to feline panleukopenia virus vaccination in cats with asymptomatic retrovirus infections: a pilot study

Michèle Bergmann[1], Stephanie Schwertler[1], Stephanie Speck[2], Uwe Truyen[2] and Katrin Hartmann[1]

Abstract

Objectives Currently, there are only a few studies on how immunocompromised cats, such as cats infected with feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV), respond to vaccination. Therefore, this study measured feline panleukopenia virus (FPV) antibodies in retrovirus-infected cats within a period of 28 days after FPV vaccination, and compared the immune response to that of non-infected cats.

Methods Eight asymptomatic retrovirus-infected cats (four FeLV, four FIV), and non-infected age-matched control cats (n = 67) were vaccinated with a commercial FPV modified live virus (MLV). Pre- and post-vaccination antibody titres were measured by haemagglutination inhibition (HI) on days 0, 7 and 28. An HI titre ⩾1:40 was defined as protective. An adequate response to vaccination was defined as a four-fold titre increase or higher. Comparison of the immune response of retrovirus-infected and non-infected cats was performed.

Results Pre-vaccination FPV antibody titres ⩾1:40 were present in 100% (n = 8/8; 95% confidence interval [CI] 62.8–100) of retrovirus-infected and in 77.6% (n = 52/67; 95% CI 66.2–86.0) of non-infected cats. An adequate response to vaccination (titre increase ⩾four-fold) was seen in 1/8 retrovirus-infected cats (12.5%; 95% CI 0.1–49.2) compared with 22/67 non-infected cats (32.8%; 95% CI 22.8–44.8). In cats with high pre-vaccination titres (⩾1:160), a four-fold titre increase or higher was observed in 1/8 retrovirus infected cats (12.5%; 95% CI 0.1–49.2) compared with 4/42 non-infected cats (9.5%; 95% CI 3.2–22.6). None of the eight retrovirus-infected cats developed illness or vaccination side effects after vaccination with MLV against FPV within the 28 days. There were no significant differences between groups: for pre-vaccination titres; for at least four-fold titre increases following vaccination in either all cats or the cats with high pre-vaccination titres; and concerning adverse effects.

Conclusions and relevance All retrovirus-infected asymptomatic cats had pre-vaccination FPV antibodies indicating protection against panleukopenia. Response of retrovirus-infected cats to vaccination was similar to the response of non-infected cats.

Keywords: FIV; FeLV; immunosuppression; FPV; active immunisation; protection

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Introduction

Currently, there is debate whether vaccination with modified live virus (MLV) is effective and safe in retrovirus-infected cats. Safety is a concern as MLV vaccines might regain pathogenicity. Thus, current guidelines advise veterinarians to avoid vaccination with MLV in retrovirus-infected cats, although there is no definitive evidence for an increased risk.[1,2] In addition, it has been

[1]Clinic of Small Animal Medicine, Centre for Clinical Veterinary Medicine, LMU, Munich, Germany
[2]Institute of Animal Hygiene and Veterinary Public Health, University of Leipzig, Leipzig, Germany

Corresponding author:
Michèle Bergmann Dr med vet, Clinic of Small Animal Medicine, Centre for Clinical Veterinary Medicine, LMU, Veterinaerstrasse 13, 80539 Munich, Germany
Email: n.bergmann@medizinische-kleintierklinik.de

discussed whether vaccine-induced immune stimulation might lead to progression of retrovirus infection by altering the unstable balance between the immune system and virus.[3] Thus, especially in cats with feline immunodeficiency virus (FIV) infection, unspecific immune stimulation due to vaccination could lead to increased virus replication caused by activation of latently infected lymphocytes and macrophages, and therefore result in progression of FIV infection.[4]

Another concern of vaccination in retrovirus-infected cats is the efficacy of the vaccines. The immune response to vaccination might not be comparable to the response in non-infected cats and it is unclear whether vaccines work at all or whether duration of immunity is shortened in retrovirus-infected cats. In experimental studies, FIV-infected cats (except in the terminal phase of infection) were able to mount appropriate levels of protective antibodies after vaccination.[5,6] In contrast, in one study, vaccination against feline leukaemia virus (FeLV) failed to protect cats naturally infected with FIV.[7] Similarly, FeLV-infected cats were not able to mount an appropriate immune response to vaccines, such as rabies vaccination.[8]

So far, no information is available on how FIV-infected or FeLV-infected cats in the field respond to vaccination with MLV, such as a vaccine against feline panleukopenia. Presence of serum antibodies against feline panleukopenia virus (FPV) correlates with protective immunity against FPV infection, and thus measurement of antibodies can be used to evaluate the specific immune status of individual cats.[9] The aim of this pilot study was to determine differences in the efficacy and safety of vaccination of asymptomatic retrovirus-infected cats compared with age-matched non-infected cats.

Materials and methods

Study population

The protocol of this study was approved by the Government of Upper Bavaria (reference number 55.2-1-54-2532.3-62-11). All cats included in the prospective study were presented to the Clinic of Small Animal Medicine, Centre for Clinical Veterinary Medicine, LMU Munich, or to different animal shelters for vaccination. All samples, from retrovirus-infected, as well as from non-infected, cats were collected between April 2012 and September 2014. Included were healthy cats that were presented for vaccination. All cats were tested for FIV antibodies and FeLV antigen and then were enrolled into either retrovirus-infected cats or controls.

Only cats between 2 and 6 years of age were included. Only cats found to be healthy during physical examination were able to enter the study. Mild gingivitis up to grade 1 ('slight inflammation, no ulceration, no proliferation, no spontaneous bleeding induced by gentle pressure' in the alveolar/buccal mucositis score)[10] did not lead to exclusion. Except prophylactic deworming and application of ectoparasiticides, none of the cats had received any medications. FIV and FeLV status was determined in each cat using a commercial rapid ELISA (SNAP Combo Plus FeLV/FIV Antibody Test; IDEXX). As FIV vaccination is not available in Germany, there was no concern that cats could be antibody-positive due to vaccination. Cats were only included if FPV vaccination was given more than 12 months ago. Cats were excluded if they had received immunosuppressive drugs or passive immunisation in the 4 weeks prior to vaccination.

Data on signalment (age, breed, sex, neutering status, body weight), origin (breeder, private household, animal shelter, foreign country), housing conditions (multi-, single-cat household), lifestyle (indoor, outdoor), cohabitation with dogs, vaccination status (previous vaccination; complete vaccination series; time since last vaccination) were collected.

In total, eight retrovirus-infected cats were included: four were FIV antibody positive and four were FeLV antigen positive. Of the retrovirus-infected cats, two were female (25.0%) and six were male (75.0%) (Table 1). Age ranged between 2 and 6 years (median 4 years). All retrovirus-infected cats were domestic shorthairs (DSHs). Six cats lived in multi-cat households (75.0%) and two in single-cat households (25.0%). Outdoor access was allowed in two cats (25.0%). Seven cats originated from shelters (87.5%), and one was a client-owned cat. All of the cats had a mild gingivitis but no other clinical signs.

A healthy, non-retrovirus-infected age-matched control group was included, which was presented at the same time period as the retrovirus-infected group. Samples from healthy, non-infected cats had been collected as part of a previous study evaluating the antibody response against FPV in healthy cats after vaccination.[11] Of the 67 non-infected cats, 40 cats were female (59.7%) and 27 cats were male (40.3%). Age ranged between 2 and 6 years (median; 4 years). Twenty-two cats were purebred (32.8%) and 45 cats were DSHs (67.2%). The majority (52 cats) lived in multi-cat households (77.6%) and 15 cats lived in single-cat households (22.4%). Outdoor access was allowed in 12 cats (17.9%). Twenty-six cats (38.8%) originated from private households, 29 came from shelters (43.3%) and 12 came from breeders (17.9%).

Study protocol

Besides obtaining a detailed history, health status of the cats was evaluated by physical examination on days 0, 7 and 28. On day 0, each cat received a single dose of a MLV FPV strain PLI IV with a viral titre of $10^{3.5}$ cell culture infective dose 50%, also containing feline calicivirus (FCV) and feline herpesvirus (FHV-1) antigen (Purevax RCP, Merial); FCV and FHV-1 vaccination were not the subject of this study. Owners were advised to record

Table 1 Signalment, history and pre-vaccination antibody titre of the eight feline immunodeficiency virus (FIV)- and feline leukaemia virus (FeLV)-infected cats and their response to vaccination

Cat	FIV/FeLV infection status	Signalment	Origin	Household, access	Physical examination	Any vaccination in the past	Time since last vaccination	Complete vaccination series	FPV antibody titre			Adequate response to vaccination (⩾four-fold titre increase)	Vaccination adverse effects
									Day 0	Day 7	Day 28		
1	FIV	5 y, DSH, male, castrated	Shelter	Multi-cat, indoor	Mild gingivitis	Unknown	Unknown but >1 y	Unknown	640	1280	1280	No	None
2	FIV	4 y, DSH, male, castrated	Shelter	Multi-cat, indoor	Mild gingivitis	Unknown	Unknown but >1 y	Unknown	640	640	640	No	None
3	FIV	6 y, DSH, female, spayed	Shelter	Multi-cat, indoor	Mild gingivitis	Yes	Within the past 1.5–3 y	No	320	640	1280	Yes	None
4	FIV	5 y, DSH, female, spayed	Shelter	Multi-cat, indoor	Mild gingivitis	Unknown	Unknown, but >1 y	Unknown	640	640	640	No	None
5	FeLV	2 y, DSH, male, castrated	Shelter	Multi-cat, indoor	Mild gingivitis	Yes	Within the past 1.5–3 y	No	320	320	160	No	None
6	FeLV	2 y, DSH, male, castrated	Private	Multi-cat, indoor	Mild gingivitis	Yes	Within the past 1.5–3 y	No	640	1280	1280	No	None
7	FeLV	3 y, DSH, male, castrated	Shelter	Single-cat, outdoor	Mild gingivitis	Yes	Within the past 1.5–3 y	No	320	320	320	No	None
8	FeLV	4 y, DSH, male, intact	Shelter	Single-cat, outdoor	Mild gingivitis	Yes	Within the past 1.5–3 y	No	1280	1280	1280	No	None

FPV = feline panleukopenia virus; DSH = domestic shorthair; y = years

possible vaccine-associated adverse events (VAAEs) until day 28. Serum samples were taken on days 0, 7 and 28 for evaluation of pre- and post-vaccination antibody titres.

Four FeLV-infected cats (4/4) and one FIV-infected cat (1/4) had received a vaccination in the past (>1 year ago), but none of the retrovirus-infected cats had received a complete vaccination series according to current guidelines (Table 1). Of the 67 non-infected cats, 43 (64.2%) had received a vaccination in the past, and 12 of these cats (27.9%; n = 12/43) had received a complete vaccination series. A complete vaccination series against FPV was defined as a primary FPV vaccination series with an MLV starting at an age of 6–8 weeks, with subsequent booster vaccinations in 3–4 week intervals until the cat was at least 16 weeks of age, followed by a booster vaccination given 11–13 months later. In cats older than 12 weeks, vaccination was considered complete if they had received two vaccinations with a 3–4 week interval followed by a booster after 11–13 months. After the primary vaccination series, cats had to have received subsequent revaccinations in at least 3 year intervals.

Detection of antibodies by haemagglutination inhibition

Serum samples for the determination of FPV antibodies were frozen at −20°C until end of the trial and tested by haemagglutination inhibition (HI) as previously described.[11] After heat inactivation (56°C, 30 mins), sera were diluted 1:5 in barbital-acetate buffer (BAB; pH 6.2). Five hundred microlitres of these dilutions were mixed with 15 µl of a 50% porcine erythrocyte suspension (PES) and incubated for 1 h at 4°C. Thereafter, sera were retrieved by centrifugation, and the erythrocyte pellets were discarded. Following a serial two-fold dilution in BAB (starting serum dilution was 1:10), sera were mixed with an equal volume of FPV, strain 292 (8 haemagglutinating units/ml). After an incubation period of 1.5 h at 37°C, 50 µl of a 0.5% PES was added. Samples were subsequently incubated overnight at 4°C and evaluated optically. Sera from the non-infected control group and sera from retrovirus-infected cats were examined as one batch; positive and negative control sera were included.

Antibody titres ⩾1:40 were considered as 'protective against FPV'.[9,12] Cats with a four-fold titre increase or higher were defined as 'adequately responding to vaccination'.[12] Cats without detectable parvovirus antibodies on day 0 that did not develop an antibody titre increase during the whole study course were defined as 'non-responders'.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 6.0. Confidence intervals (CIs) were determined by an exact binomial test.[13] The exact binomial test was one-tailed and was used to prove the alternative hypothesis that the number of vaccine responders was within the 95% CI. A significance level of <0.05 was chosen. Fisher's exact test was used to compare: (1) presence of pre-vaccination antibodies between retrovirus-infected and non-infected cats; (2) adequate antibody responses of cats with all possible pre-vaccination titres; (3) adequate antibody response of cats with pre-vaccination antibodies ⩾1:160; and (4) occurrence of vaccination side effects.

Results

Response to vaccination

Of the retrovirus-infected cats, 100% (n = 8/8; 95% CI 62.8–100) had antibody titres ⩾1:40 on day 0 (Table 1). In 77.6% (n = 52/67; 95% CI 66.2–86.0) of the non-infected cats, antibody titres ⩾1:40 were present on day 0.

Table 2 summarises the response to vaccination of all cats. An adequate response to vaccination (titre increase ⩾four-fold) was observed in 1/8 retrovirus-infected cats (12.5%; 95% CI 0.1–49.2) vs 22/67 non-infected cats (32.8%; 95% CI 22.8–44.8). Table 3 summarises the

Table 2 Comparison of cats with and without retrovirus infection using Fisher's exact test

		Total	Retrovirus-infected cats (n = 8)	Non-infected cats (n = 67)	*P* value
Pre-vaccination antibody titre (n = 75)	⩾1:40	60	8 (100.0)	52 (77.6)	0.345
	<1:40	15	0 (0)	15 (22.4)	
⩾Four-fold titre increase (n = 75)	Yes	23	1 (12.5)	22 (32.8)	0.422
	No	52	7 (87.5)	45 (67.2)	
⩾Four-fold titre increase in cats with a pre-vaccination antibody titre ⩾1:160 (n = 50)	Yes	5	1 (12.5)	4/42 (9.5)	0.531
	No	45	7 (87.5)	38/42 (90.5)	
Non-responders (n = 75)	Yes	1	0 (0)	1 (1.5)	1.000
	No	74	8 (100.0)	66 (98.5)	
Vaccine-associated adverse events*	Yes	9	0 (0)	9 (13.4)	0.585
	No	66	8 (100.0)	58 (86.6)	

Data are numbers of cats (n) and percentage of cats (%)
*Based on owner reports and veterinary examination on days 7 and 28

Table 3 Feline panleukopenia pre-vaccination antibody titre on day 0 and number of cats with an at least ⩾four-fold titre increase during the course of the study

	Number of cats with a ⩾four-fold antibody titre increase with the respective pre-vaccination antibody titre on day 0	
Pre-vaccination FPV antibody titre on day 0	Retrovirus-infected cats	Non-infected cats
<1:10	0/0 (0)	7/8 (87.5)
1:10	0/0 (0)	2/2 (100)
1:20	0/0 (0)	3/5 (60.0)
1:40	0/0 (0)	3/4 (75.0)
1:80	0/0 (0)	3/6 (50.0)
1:160	0/0 (0)	1/7 (14.3)
1:320	1/3 (33.3)	1/8 (12.5)
1:640	0/4 (0)	1/8 (12.5)
1:1280	0/1 (0)	1/10 (10.0)
1:2560	0/0 (0)	0/8 (0)
1:5120	0/0 (0)	0/0 (0)
1:10240	0/0 (0)	0/1 (0)
Total number of cats with ⩾four-fold antibody titre increase	1/8 (12.5)	22/67 (32.8)

Data are n (%)
FPV = feline panleukopenia virus

number of cats with an adequate response to vaccination (⩾four-fold antibody titre increase) in subgroups with different pre-vaccination antibody titres on day 0; 50–100% of the non-infected cats with pre-vaccination titres from >1:10 to 1:80 had an adequate titre increase (⩾four-fold) vs 0–14% of the non-infected cats with a pre-vaccination titre of ⩾1:160. Of the retrovirus-infected cats, 1/8 (12.5%; 95% CI 0.1–49.2) with a pre-vaccination titre of ⩾1:160 showed an adequate response (⩾four-fold antibody titre increase) vs 4/42 non-infected cats (9.5%; 95% CI 3.2–22.6). One of the non-infected cats was identified as non-responder.

No VAAEs were reported after vaccination in the retrovirus-infected cats. In 13.4% (9/67) of non-infected cats, VAAEs were detected by the owners, limited to slightly reduced general condition (less activity, more frequent resting periods) after vaccination for a few days. Physical examination of these cats (on days 7 and 28) was unremarkable.

Comparison of retrovirus-infected and non-infected cats

There was no significant difference in the presence of pre-vaccination antibody titres ⩾1:40 on day 0 (P = 0.345; odds ratio [OR] 5.019, 95% CI 0.27–92.01) between asymptomatic retrovirus-infected and non-infected cats. There was no significant difference in the response to vaccination between all retrovirus-infected and all non-infected cats with respect to an adequate antibody titre increase (⩾four-fold) (P = 0.422; OR 3.422, 95% CI 0.40–29.58); there was also no significant difference in the response to vaccination between retrovirus-infected (n = 8) and non-infected cats (n = 53) with high pre-vaccination antibodies (⩾1:160) with respect to an adequate antibody titre increase (⩾four-fold) (P = 0.531; OR 1.679, 95% CI 0.16–17.27). There was also neither a significant difference in the occurrence of VAAEs (P = 0.585; OR 0.362, 95% CI 0.02–6.81), nor in the number of non-responders (P = 1.000; OR 2.608, 95% CI 0.10–69.31) between retrovirus-infected and non-infected cats.

Discussion

It has been suggested that cats with immunosuppression might be more likely to lack protective antibodies against FPV than healthy cats and that they might not react adequately to vaccination.[14] Retrovirus infection can cause severe immunosuppression in cats. In one study, an impaired response to develop neutralising antibodies after FCV vaccination was detected in FIV-infected cats.[15] Another study showed that after rabies vaccination, FeLV-infected cats were only protected for 6 months.[8]

The present study showed that all recruited retrovirus-infected cats had antibody titres ⩾1:40 on day 0 of the study, suggesting that they had responded to vaccination or clinically inapparent field infection in the past, but the stage of retrovirus infection in the cats was unknown. The majority of control cats also had FPV antibodies due to vaccination or field virus exposure. Given the fact that all retrovirus-infected cats had pre-vaccination antibodies, they were likely protected against panleukopenia.[9]

The main reason for an impaired immune function in FIV infection is a decrease in the number and proportion of $CD4^+$ cells that play an important role in promoting and maintaining humoral and cell-mediated immunity. However, in the asymptomatic stage of infection, decrease of $CD4^+$ cells is mild, and a rapid and severe decrease might only occur in the terminal stage of infection. In the present study, cats with FIV infection were asymptomatic (except for mild gingivitis), and thus in a stage in which the immune function was likely to be comparable to that of non-infected cats. Cats with progressive FeLV infection can be more severely immunosuppressed than cats with FIV infection, as FeLV can destroy all haematopoietic cells.[16] It has been shown that protection after vaccination is not as complete and long-lasting in FeLV-infected cats as in non-infected cats.[8,14] In contrast, all FeLV-infected cats in the present study had pre-vaccination antibodies ⩾1:40, although previous vaccinations had been performed at least 1 year ago. Thus, the protection rate of progressively FeLV-infected cats was comparable to that of healthy non-FeLV-infected cats, at least for those in the early asymptomatic stages of infection.

The present study is the first to evaluate the response to FPV vaccination of asymptomatic retrovirus-infected cats in the field. Studies in naturally retrovirus-infected cats are important owing to potential differences in the response to vaccination depending on the immune status of the individual retrovirus-infected cat. Adequate response to vaccination (⩾four-fold titre increase) was observed in only one of the retrovirus-infected cats. Thus, only a minority of retrovirus-infected cats (12.5%), as well as control cats (32.8%), responded to vaccination with an adequate (⩾four-fold) rise in HI antibody titre. The most likely reason for the poor response to vaccination is the presence of high pre-vaccination antibodies in all retrovirus-infected and most of the control cats (n = 42/67; 62.7%).

The lack of response to vaccination in cats with pre-existing antibodies (⩾1:160) has already been demonstrated in non-retrovirus-infected cats.[11] However, it has been discussed that in retrovirus-infected cats poor response to vaccination could be due to the underlying immunosuppression. This, however, seems unlikely as all retrovirus-infected cats in the present study showed only mild gingivitis on physical examination and no other signs of disease or immunosuppression. In one retrovirus-infected cat that had pre-existing antibodies, a titre decrease after vaccination was observed. A possible explanation might be the binding of the pre-existing antibodies to the vaccine virus, a phenomenon that has been described after FPV vaccination of healthy cats,[11] although the observed titre increase could also be explained by experimental error. Therefore, booster vaccination is unnecessary and not recommended in cats with pre-existing antibody titres. The results of the present study suggest that similar vaccination recommendations should be applied to retrovirus-infected cats, and titre testing instead of routine vaccination should be emphasised even more in retrovirus-infected cats than in non-infected cats.

In the present study, all asymptomatic retrovirus-infected cats had protective antibody titres before and after vaccination, indicating that the immune system of the cats was not markedly compromised. As cats with pre-vaccination antibodies (⩾1:160) are less likely to react to vaccination,[11] comparison of response to vaccination was also performed in retrovirus-infected vs non-infected cats with high pre-vaccination antibodies (⩾1:160). There was no significant difference in vaccine efficacy between asymptomatic retrovirus-infected and non-infected cats with high titres. However, only a small number of retrovirus-infected cats were investigated and further studies are needed.

It has been proposed that retrovirus-infected cats are at an increased risk of developing illness after vaccination with MLV, although this has never been proven.[17] None of the eight retrovirus-infected cats in the present study developed clinical signs consistent with FPV. In addition, none of the eight retrovirus-infected cats showed VAAEs. This indicates that vaccination is safe in asymptomatic retrovirus-infected cats, at least in the short term.

This study included age-matched adult control cats (between 2 and 6 years). Young cats were not included in the study in order to rule out age influence on immune response. Interference with maternally derived antibodies can lead to vaccination failure in kittens.[18] In addition, there is some evidence for a generally weaker antibody response following booster vaccination compared with primary vaccination series,[19] and thus kittens receiving their primary vaccination series were not considered. Therefore, only cats >2 years were included. To further rule out the influence of age, older cats (>6 years) were excluded. A lower number of circulating lymphocytes is known to cause a reduced immune response in elderly people.[20,21] Studies in cats[22,23] demonstrated an age-related remodelling of the immune system, with a gradual decline in relative percentage of lymphocytes in cats older than 6 years of age vs younger cats.[23] An absolute reduction in B cells was also found in older cats (10–14 years) compared with younger cats (aged 2–5 years).[24] Therefore, only cats between 2 and 6 years were included in the present study.

For detection of antibodies, HI and not virus neutralisation (VN) was performed in the present study. Although there is a correlation between these tests, only VN can detect antibodies that neutralise infectious particle and prevent infection.[25] However, historically, HI is considered the gold standard in FPV antibody detection.[9,11,18] It is based on the principle that antibodies bind to the virus particle and, by doing so, block the determinants that bind erythrocytes. The antibodies prevent the binding of erythrocytes and therefore block haemagglutination. The neutralising epitopes on the virus surface are positioned right next to the erythrocyte binding site. As the antibody molecule is large when compared with the virion size (antibody 10 nm, virion 20 nm) probably any antibody molecule binding in that area can impair or even inhibit receptor binding and haemagglutination.[26] This can explain why HI results and VN results are very similar, although not identical.

The results of the present study only apply to asymptomatic retrovirus-infected cats. The main limitation of the study was the relatively small number of cats with retrovirus infections, which makes statistical assessment of a difference in response to vaccination difficult. Unfortunately, more retrovirus-infected cats could not be recruited. On the one hand, this was likely due to the low prevalence of retrovirus infections in Germany.[27,28] On the other hand, owners were very reluctant to vaccinate a retrovirus-infected cat, even if they were asymptomatic, (1) due to fear of progression of retrovirus infection or VAAEs, or (2)

due to the assumption that by keeping their cats indoors (which is mandatory for retrovirus-infected cats), vaccination would no longer be necessary. Further studies should be performed involving larger numbers of immunosuppressed cats. Additionally, measuring $CD4^+$ and $CD8^+$ cells should be performed to evaluate the immune status of the cats, as well as measurement of the FIV and FeLV load to determine whether vaccination of the cats might result in progression of retrovirus infection.

Conclusions

All retrovirus-infected asymptomatic cats in the current study had pre-vaccination antibodies against FPV, indicating protection against panleukopenia. Response of retrovirus-infected cats to vaccination was similar to the response of non-infected cats. Thus, at least in an asymptomatic stage, the immune function of retrovirus-infected cats seems comparable with that of non-infected cats and MLV vaccination can be regarded as safe in retrovirus-infected cats in the short term. However, further studies should be performed, involving larger numbers of immunosuppressed cats with different immunosuppression status and with a longer follow-up period.

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ORCID iD Michèle Bergmann https://orcid.org/0000-0002-8856-3849

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