Vaccination against the feline leukaemia virus: Outcome and response categories and long-term follow-up

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Abstract

Feline leukaemia virus (FeLV) is a pathogen inducing fatal disease in cats worldwide. By applying sensitive molecular assays, efficacious commonly used FeLV vaccines that protect cats from antigenaemia were found not to prevent proviral integration and minimal viral replication after challenge. Nonetheless, vaccines protected cats from FeLV-associated disease and prolonged life expectancy. The spectrum of host response categories was refined by investigating plasma viral RNA loads. All cats initially fought similar virus loads, although subsequently loads were associated with infection outcomes. Persistence of plasma viral RNA was moderately associated with reactivation of FeLV infection. In conclusion, sensitive molecular assays are important tools for reviewing pathogenesis of FeLV infection.

Keywords: Retrovirus; Vaccine; Pathogenesis

1. Introduction

1.1. The feline leukaemia virus (FeLV) infection in domestic cats

FeLV [1] belongs to the gammaretroviruses and is a well-known feline pathogen occurring worldwide in domestic cats [2]. FeLV isolates are grouped into three major subgroups, FeLV-A, -B and -C, according to their superinfection interference and neutralization patterns, which are determined by the virus envelope structures [3,4]. FeLV-A is the dominant subgroup and is found in all FeLV-infected cats; it is highly contagious, but low in pathogenicity [2,5]. FeLV-A is horizontally transmitted in nature and it is broadly accepted that vaccines directed against FeLV-A are protective against FeLV infection in general. Cats infected with FeLV-A usually exhibit a prolonged clinical asymptomatic phase; subsequently, they may develop diseases, such as malignant
lymphoma mainly of T-cell origin. FeLV-B and particularly FeLV-C are less prevalent [5]. They arise within the individual FeLV-A infected cat: FeLV-B via recombination with endogenous FeLV-related sequences and FeLV-C via mutations or insertions in the surface glycoprotein gene [2,6–8]. Some FeLV-B infected cats develop lymphoid malignancies, while FeLV-C infection is strongly associated with the development of aplastic anaemia. More recently, a fourth FeLV subgroup, FeLV-T, was described on the basis of sequence differences in the surface glycoprotein and on receptor interactions [9–11]. FeLV-T arises from FeLV-A via mutations and insertions in the surface glycoprotein gene. This subgroup includes T-cell-tropic cytopathic viruses that cause lymphoid depletion and immunodeficiency in infected cats.

1.2. Course of FeLV infection

The susceptibility of domestic cats to FeLV infection varies [2,12–18] and until recently, the outcome of infection in individual cats was categorized using mainly results of virus isolation and FeLV p27 antigen detection as a measure for viraemia. According to these parameters, many FeLV challenged cats develop a regressive infection characterized by transient or undetectable antigenaemia and an effective immune response [13,18–20]. Latent non-productive infection characterized by absence of viraemia and persistence of the virus in the bone marrow was identified in cats that ostensibly recovered from contained infection; the virus may be detected by culturing bone marrow cells in the presence of corticosteroids [21–23]. Most latent infections are resolved within 30 months after infection [24,25]. Some non-viraemic cats show localized FeLV infection in selected other tissues [26,27]. Finally, a proportion of cats develop a progressive infection with persistent antigenaemia, lack of FeLV-specific immunity [2,19,20] and they ultimately succumb to FeLV associated diseases [28].

1.3. FeLV vaccines

FeLV vaccines were developed many years ago and have been commonly used in veterinary practices. Comprehensive lists of vaccines, comparative efficiencies, etc., are compiled elsewhere [29–31]. While many of the FeLV vaccines are based on formaldehyde-inactivated FeLV proteins produced in cell culture [32–35], some others contain recombinant FeLV surface proteins [36]. Most of the vaccines are adjuvanted. More recently, a canarypox-vectored live virus vaccine expressing FeLV genes was introduced [37].

1.4. Molecular diagnostic methods and their impact

Most FeLV pathogenesis and vaccine studies were conducted before sensitive FeLV specific molecular diagnostic assays, such as real-time TaqMan polymerase chain reaction (PCR) for the quantification of FeLV proviral loads [38–41], were developed. Applying molecular methods, it was recently

<table>
<thead>
<tr>
<th>Putative response categories</th>
<th>Antigenemia</th>
<th>Proviral load (duration of detectability)</th>
<th>Plasma viral RNA load (duration of detectability)</th>
<th>Virus isolation from blood</th>
<th>Virus isolation from bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortive</td>
<td>Undetectable</td>
<td>Low to moderate (persistent)</td>
<td>Low to moderate (transient or persistent)</td>
<td>Negative or transiently positive</td>
<td>Negative or positive</td>
</tr>
<tr>
<td>Regressive with transient antigenemia</td>
<td>Undetectable</td>
<td>Low to moderate (persistent)</td>
<td>Low to moderate (transient or persistent)</td>
<td>Negative or transiently positive</td>
<td>Negative or positive</td>
</tr>
<tr>
<td>Regressive with undetectable antigenemia</td>
<td>Transient</td>
<td>Low to moderate (persistent)</td>
<td>Low to moderate (transient or persistent)</td>
<td>Negative or transiently positive</td>
<td>Negative or positive</td>
</tr>
<tr>
<td>Latent</td>
<td>Transient</td>
<td>Low to moderate (persistent)</td>
<td>Low to moderate (transient or persistent)</td>
<td>Negative or transiently positive</td>
<td>Negative or positive</td>
</tr>
<tr>
<td>Progressive</td>
<td>Persistent</td>
<td>Low to moderate (persistent)</td>
<td>Low to moderate (transient or persistent)</td>
<td>Positive or transiently positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

\[a\] Quantified in weeks 1–15 after challenge.
\[b\] Determined in weeks 3, 6, 9, 12 and 15 after challenge.
\[c\] Not determined.
\[d\] Determined in weeks 3, 6, 9, 12 and 15 after challenge.
\[e\] Determined in weeks 3, 6, 9, 12 and 15 after challenge.
\[f\] Determined in weeks 3, 6, 9, 12 and 15 after challenge.
\[g\] Not determined.
\[h\] Determined in weeks 3, 6, 9, 12 and 15 after challenge.
\[i\] Defined by absence of antigenemia but persistence of reactivatable virus in the bone marrow [21,22].
found that the detection of proviral DNA by real-time PCR is more sensitive as a marker for FeLV exposure than antigen detection or virus isolation [38]. Quantification of proviral loads led to the description of four putative categories of FeLV–host relationships after oronasal challenge: abortive, regressive, latent and progressive [39] (see also Table 1). However, plasma viral RNA loads had not been investigated for these four categories. Most recently, proviral loads and in one study plasma viral RNA loads were quantified during FeLV vaccination [39,41]. However, overall only limited information has been available on how the application of sensitive, quantitative molecular assays changes our views of FeLV infection and vaccination. Thus, it was the goal of the present study to provide information on some aspects of the host–virus interaction after FeLV vaccination and challenge that became only evident by using sensitive molecular assays.

2. Material and methods

2.1. Experimental studies

In the present study, partially published data from two FeLV vaccination studies [41,42] were reviewed and reanalyzed under a new perspective to call attention to some overlooked aspects, such as FeLV proviral and plasma RNA loads in cats of different host–virus relationship categories after intraperitoneal challenge. Moreover, the study presents new long-term follow-up data from the two FeLV vaccination studies. Study 1 was initiated to test the efficacy of a recombinant FeLV vaccine [42]; specific pathogen-free (SPF) cats (Ciba Geigy AG, Stein, Switzerland) were subsequently observed for up to 13 years. In study 2, molecular methods were applied to assess the efficacy of two vaccines [41]; SPF cats (Charles River Laboratories, Lyon, France) were subsequently observed for 3.3 years. All cats were kept under barrier conditions and housed in groups in large rooms under optimal ethological conditions as required by the Swiss law. Blood samples were collected prior to and at the time points of FeLV vaccination and challenge (week 0) and regularly thereafter (see also Fig. 1).

2.2. FeLV vaccines

For study 1, cats had been vaccinated in weeks −18 and −15 with a recombinant FeLV vaccine (for details see Table 2). In study 2, cats were vaccinated in weeks −7 and −4 with a canarypox-vectored live vaccine or a whole virus vaccine (Table 2). Cats of study 1 were revaccinated with the recombinant vaccine twice within a 3 week interval 2.9 years after the challenge.

2.3. FeLV challenge

For intraperitoneal FeLV challenge, $5 \times 10^5$ focus forming units of FeLV-A/Glasgow-1 [4] were used per cat based on the recommendation of the European Pharmacopoeia that persistent viraemia should be induced in at least 80% of the unvaccinated control cats [43]. At the time point of challenge, the cats of study 1 were 10 months and the cats of study 2 were 4.5 months of age.

2.4. Detection of FeLV infection

Plasma FeLV p27 antigen, antibodies to FeLV gp70 and total antibodies to FeLV were determined by ELISA [41,42,44]. Virus neutralizing antibodies were measured by focus-inhibition assay in study 1 on C81 cells [36,45] and in study 2 on QN10S cells [31]. The presence of infectious FeLV was determined in vitro by inoculation of heparinized plasma samples onto the respective cell lines. To detect latency, bone marrow was collected in weeks 24 (study 1), 17 and 43 (study 2) post challenge, respectively, and cultured for 4 weeks in the
Table 2
Study design of the two FeLV vaccine experiments included in the present study and observed vaccine efficacies

<table>
<thead>
<tr>
<th>Study</th>
<th>Vaccine group</th>
<th>Number of cats per group</th>
<th>Number of persistently infected cats (%)</th>
<th>Preventable fraction (%)(^a)</th>
<th>Significance of protection ((p_F))(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^c)</td>
<td>Recombinant FeLV vaccine(^d)</td>
<td>9</td>
<td>1 (11)</td>
<td>87%</td>
<td>0.0220</td>
</tr>
<tr>
<td></td>
<td>Unvaccinated controls</td>
<td>6</td>
<td>5 (83)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2(^e)</td>
<td>Whole virus vaccine(^f)</td>
<td>10</td>
<td>5 (50)</td>
<td>44%</td>
<td>0.1409</td>
</tr>
<tr>
<td></td>
<td>Canarypox-vectored vaccine(^g)</td>
<td>10</td>
<td>2 (20)</td>
<td>78%</td>
<td>0.0055</td>
</tr>
<tr>
<td></td>
<td>Unvaccinated controls</td>
<td>10</td>
<td>9 (90)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Preventable fraction of vaccines calculated as described [46].

\(^b\) Frequencies were tested for significant differences using the Fisher exact test (\(p_F\)).

\(^c\) Details of study design are reported elsewhere [42,48,49].

\(^d\) Leucogen (Virbac, Carros, France); recombinant vaccine containing p45, the non-glycosylated form of the envelope glycoprotein gp70 of FeLV-A.

\(^e\) Details of the study design are reported elsewhere [41].

\(^f\) Fel-O-Vax LV-K IV (Fort Dodge, Iowa, IA); whole virus vaccine.

\(^g\) Eurifel (Merial, Lyon, France); canarypox-vectorized live virus vaccine encoding FeLV A\(^{\text{env}}, \text{gag}\) and part of \(^{\text{pol}}\).

2.5. Statistics

Data were compiled and analyzed with Excel (Microsoft), Clinical Laboratory (Analyse-it, Leeds, United Kingdom) and Prism software (GraphPad, San Diego, CA). Frequencies were compared using the Fisher exact test for small numbers (\(p_F\)). Viral loads and antibody levels were tested for statistical differences among cats of several groups by the non-parametric Kruskal–Wallis test (\(p_{KW}\)) with Dunn’s multiple comparison post test (\(p_D\)), and between cats of two groups using the non-parametric Mann–Whitney U-test (\(p_{MWU}\)). Differences were considered significant if \(p < 0.05\). Vaccine efficacies were determined by calculating the preventable fraction [46].

3. Results

3.1. Indicators for non-sterilizing immunity in vaccinated cats protected from persistent antigenaemia after intraperitoneal challenge

Reviewing data from two previous FeLV vaccination studies [41,42], it became evident that cats vaccinated with a recombinant vaccine (study 1) were significantly protected from persistent antigenaemia compared to unvaccinated controls (Tables 1 and 2) and also showed a pronounced boost in ELISA-reactive anti-FeLV antibodies subsequent to challenge (Fig. 1A). In cats vaccinated with a canarypox-vectored vaccine (study 2) protection was found in the absence of pre-existing antibodies; however, antibodies were developed subsequent to challenge (Fig. 1B). In addition, an increase in virus neutralizing activity was observed after challenge in some cats vaccinated with the recombinant vaccine [42] and others developed virus neutralizing antibodies not until several weeks after challenge [41,42].

Using molecular assays to analyze the cats of study 2, it was found that all animals including vaccinated cats turned provirus and plasma viral RNA positive after FeLV challenge [41]. To further confirm this unexpected observation, we analyzed cell-associated RNA in peripheral blood cells. In week 5, samples collected from all 30 cats were positive for cell-associated viral RNA including those that were plasma viral RNA negative. In week 10, all samples from persistently infected cats independent of the vaccination status tested positive; however, those samples of cats that had overcome antigenaemia tested negative for cell-associated viral RNA.

3.2. Evaluation of the spectrum of host–virus interactions using molecular assays

Data from study 2 [41] were reanalyzed to assess the spectrum of FeLV–host interactions after vaccination and intraperitoneal challenge and to determine FeLV proviral and plasma viral RNA loads in cats with different infection outcomes.

3.2.1. Abortive infection

No abortive infection as defined by absence of antigen and provirus [39] was found after intraperitoneal challenge (Table 1).

3.2.2. Regressive infection with undetectable antigenaemia

Remarkably, nine vaccinated cats were provirus-positive in the absence of detectable antigen (Table 1). The cats had low to moderate plasma viral RNA loads (weeks 1–15: presence of \(10^{-6}\) mol/l hydrocortisone [41,42]. FeLV proviral and plasma viral RNA loads were quantified by real-time TaqMan PCR and reverse transcriptase (RT) PCR, respectively [40]. For quantification of cell-associated RNA, mRNA was extracted from 200 \(\mu\)L EDTA anticoagulated blood using the mRNA Isolation Kit I on a MagNA Pure LC Instrument with manual external lysis using Red Blood Cell Lysis Buffer as recommended by the manufacturer (Roche Diagnostics, Mannheim, Germany). mRNA extracted from samples collected in weeks 5 and 10 were analyzed by real-time RT-PCR. Molecular assays had not yet been available during the early phase of study 1.
Fig. 2. Proviral (A–D) and plasma viral RNA loads (E–H) in cats of different response categories independent of the vaccination status. Response categories are as follows (see also Table 3): progressive infection characterized by persistent antigenaemia (black squares) and regressive infection with either transient (black triangles pointing upwards) or absent antigenaemia (black triangles pointing downwards). A and E, week 1 after intraperitoneal FeLV challenge; B and F, week 2; C and G, week 8; and D and H, week 15. Significant differences were found in weeks 2, 8 and 15, but not in week 1. Comparison among the three groups was done using the Kruskal–Wallis test ($p_{KW}$ indicated in panels); for comparison of individual groups the Dunn’s multiple comparison post test was used (significant differences indicated by dotted lines and asterisks: **, $p_D < 0.01$; ***, $p_D < 0.001$).

median: $1.8 \times 10^3$ copies/ml plasma; range, $0–1.2 \times 10^6$ copies/ml plasma; Fig. 2). In six of the nine cats, plasma viral RNA was transiently detectable up to weeks 5–7, one cat intermittently showed positive results and two cats stayed plasma viral RNA positive until week 15 (cats #11 and #30). All analyzed blood and bone marrow samples were negative for virus isolation. However, the majority of the blood samples were provirus-positive (loads weeks 1–15: median: $1.0 \times 10^{-3}$ copies/cell; range, $0–5.6 \times 10^{-2}$ copies/cell; Fig. 2). The rare occurrence of a provirus-negative result (6 out of 135 samples) was always followed by a positive result in one of the following blood collections. The proviral and plasma viral RNA loads of cats with undetectable antigenaemia were lower than those of persistently infected cats from week 2 to 15 after challenge ($p_{KW} \leq 0.0020; p_D < 0.01$; and Fig. 2).

3.2.3. Regressive infection with transient antigenaemia

Five cats showed a regressive FeLV infection with transient antigenaemia (Table 1). No clearance of provirus was observed (loads weeks 1–15: median: $1.1 \times 10^{-2}$ copies/cell; range, $0–3.3$ copies/cell; Fig. 2). The cats in this category had low to moderate plasma viral RNA loads (weeks 1–15: median, $8.4 \times 10^4$ copies/ml plasma; range, $0–6.0 \times 10^7$ copies/ml plasma; Fig. 2). Four cats turned plasma viral RNA negative between weeks 5 and 21. One cat, #70, stayed plasma viral RNA positive and subsequently showed reactivation of the infection (Table 3). Cats with transient antigenaemia had lower proviral and plasma viral RNA loads than persistently infected cats starting from weeks 12 and 5, respectively ($p_{KW} \leq 0.0020; p_D < 0.05$; and Fig. 2).

3.2.4. Latent infection

Among the 14 cats with regressive infection, 2 (#22 and #70) were found to be latently infected (Tables 1 and 3). There was no difference in proviral and plasma viral RNA loads in latently infected cats when compared to cats without a latent infection.

3.2.5. Progressive infection

Finally, the majority of the unvaccinated cats and some vaccinated cats developed a progressive infection character-
Table 3
Long-term outcome of FeLV infection in selected cats

<table>
<thead>
<tr>
<th>Cat ID</th>
<th>Vaccine</th>
<th>Antigen</th>
<th>Provirus</th>
<th>Plasma viral RNA(^a)</th>
<th>Virus isolation (blood)(^b)</th>
<th>Latent infection(^c)</th>
<th>Long-term outcome of FeLV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>#22</td>
<td>Canarypox</td>
<td>Transiently positive</td>
<td>Persistently positive</td>
<td>Transiently positive</td>
<td>Transiently positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>#30</td>
<td>Canarypox</td>
<td>Undetectable</td>
<td>Persistently positive</td>
<td>Persistently positive</td>
<td>Persistently positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>#70</td>
<td>Whole virus vaccine</td>
<td>Transiently positive</td>
<td>Persistently positive</td>
<td>Persistently positive</td>
<td>Transiently positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

\(^a\) Determined by real-time TaqMan RT-PCR [40] between weeks 1 and 15 after challenge.
\(^b\) Performed by focus-inhibition assay on QN10S [31] cells in weeks 3, 6, 9, 12 and 15 after challenge.
\(^c\) Bone marrow collected at weeks 17 and 43 after challenge and cultured in the presence of corticosteroids [41].

ized by persistent antigenaemia and persistently high proviral loads (loads weeks 1–15: median: \(8.2 \times 10^{-1}\) copies/cell; range, \(0–4.9 \times 10^1\) copies/cell; Table 1). These cats had also persistently high plasma viral RNA loads (weeks 1–15: median, \(4.7 \times 10^7\) copies/ml plasma; range, \(2.9 \times 10^3\) to \(9.8 \times 10^8\) copies/ml plasma; Fig. 2) and were found to be virus isolation positive (Table 1).

Although cats with undetectable or transient antigenaemia subsequently had lower proviral and plasma viral RNA loads than cats that became persistently vireaemic, no significant difference was found in proviral and plasma viral RNA loads among cats of the three response categories in week 1 after challenge (Fig. 2A and E).

### 3.3. Long-term follow-up

Blood samples collected from cats of study 1 revealed a provirus-positive status during the last 6 years of the observation period, when PCR became available. Nevertheless, vaccinated animals lived significantly longer than unvaccinated control cats. The mean survival time of vaccinated cats was 11.3 years, while unvaccinated cats died in average after 5.7 years (Fig. 3).

In study 2, 12 out of 14 cats with regressive infection (Table 1) stayed FeLV antigen-negative and clinically healthy. No clearance of provirus was found for more than 3 years after infection, although transiently PCR-negative results were observed. One out of 12 surviving cats remained plasma viral RNA positive (#11).

Two of the cats (#30 and #70), which had been protected from persistent antigenaemia, subsequently became p27-positive (Table 3). Cat #30 was always negative in antigen ELISA and virus isolation from blood and bone marrow. Nevertheless, the cat turned FeLV antigen-positive in week 44 with increasing antigen levels thereafter and developed clinically manifest malignant lymphoma in week 53. The cat was co-housed with cats that had become persistently infected.

The second cat, #70, was transiently antigenaemic between weeks 3 and 7, virus isolation was positive in week 3 and latent infection was detectable by bone marrow culture collected in week 17 but not anymore in week 43 (Table 3). The cat was housed in a group separated from persistently infected cats from week 55 on. Reactivation of FeLV infection occurred in week 101 and the cat developed malignant lymphoma.

No association was found between reactivation and any of the following parameters: virus isolation from blood or bone marrow, presence of transient antigenaemia, proviral loads and absence of total or neutralizing antibodies. However, both cats with reactivation were not only persistently provirus but also plasma viral RNA positive. In contrast in cats without reactivation, seven out of eight animals with transient and all four cats with undetectable antigenaemia were found to reduce plasma viral RNA loads to undetectable levels. Thus, persistence of plasma viral RNA was to some extent associated with virus reactivation (\(p_F = 0.0659\)).

![Fig. 3. Long-term survival of FeLV vaccinated cats (n = 9) and unvaccinated control animals (n = 6) of study 1. Cats vaccinated with the recombinant FeLV vaccine survived significantly longer than unvaccinated control cats (\(p_{KW} = 0.0039\)).](image-url)
4. Discussion

The present study describes new insights into FeLV pathogenesis and immunity based on the application of new molecular assays. Using sensitive real-time TaqMan PCR assays, it was documented that cats believed to be immune to FeLV infection turned provirus-positive after virus challenge [38-41]. Moreover, we found that efficacious FeLV vaccines known to protect cats from antigenemia and FeLV-associated disease do not prevent minimal viral replication [41,47]. So far, the literature is contradicting to whether all or just a limited number of FeLV vaccinated cats turn FeLV provirus-positive subsequent to challenge infection [39,41].

Proviral and antigen loads in the peripheral blood have recently been used to redefine the response categories of domestic cats to FeLV challenge and four putative infection outcomes have been described [39]. Absence of FeLV antigen and provirus after challenge was categorized as abortive infection; this status was observed in five cats after oronasal FeLV challenge. Although we investigated more than 120 SPF cats after intraperitoneal challenge by real-time PCR assays, we were unable to document an abortive infection ([38,41] and unpublished observations). These experiments included cats vaccinated with different vaccines, such as an inactivated whole virus vaccine, a canarypox-vectored vaccine and a recombinant FeLV vaccine. The fact that all cats in our study became proviral and plasma viral RNA positive could be explained by particulars of the study setup, such as the sensitivity of the applied nucleic acid extraction methods and PCR assays, the challenge virus, dose and route. Nevertheless, our results obtained with molecular assays are in agreement with the serological data in that a pronounced boost effect of the specific antibody response was observed in vaccinated cats subsequent to challenge infection indicating non-sterilising immunity.

Nine vaccinated cats in study 2 were found to be provirus-positive in the absence of detectable antigenaemia, thus extending the recently described spectrum of host–virus relationships [39]. We suggest classifying these cats into the response category regressive infection. A very brief antigenaemia might have been missed in these cats, although blood samples were analyzed weekly. Alternatively, these cats might correspond to the abortive infection described above assuming differences in the sensitivity of the PCR systems applied. To investigate this discrepancy it would be desirable to directly compare the different PCR assays and evaluate the intraperitoneal versus the oronasal challenge.

Other factors that might have led to discrepant results in FeLV vaccine efficacy and pathogenesis studies include the genetic background of the SPF cats employed. We recently investigated endogenous FeLV-like sequences of SPF cats originating from different SPF catteries and of privately owned domestic cats and found significant differences in the proviral loads among the different groups (Tandon et al., submitted for publication). Thus, SPF cats might not necessarily mirror privately owned cats and the choice of SPF cats in future FeLV infection studies may need careful evaluation.

A third response category, the latent infection, has been described after oronasal FeLV challenge using molecular assays [39]; cats in this category were found to persistently exhibit moderate proviral loads. In the present study we found two cats with latent infection as characterized by the absence of viraemia and the persistence of reactivatable virus in the bone marrow [21-23]; however, their proviral loads were not different from the remaining cats with regressive infection but absence of latent infection. As long as the importance of the provirus-positive status is not clarified, we suggest maintaining the commonly used definition of latent infection according to results from virus isolation from bone marrow in the presence of corticosteroids.

Cats with different infection outcome were analyzed for plasma viral RNA loads. Remarkably, all cats including animals with undetectable, transient or persistent antigenaemia initially fought similarly high viral loads in the peripheral blood 1 week after challenge. This might correspond to the early lymphoreticular phases characterized by infection of circulating lymphocytes and monocytes described in both, cats with transient or persistent viraemia [16]. In cats that became persistently infected, FeLV infection was not contained at these early phases and extensive virus replication occurred in the germinal cell populations of lymphoid, hematopoietic and epithelial tissues [16]. The latter was associated with a second viraemia including infection of neutrophils and platelets. This might subsequently have led to the differences in plasma viral RNA and proviral loads observed in the present study between transiently and persistently antigenaemic cats starting in weeks 5 and 12 after challenge, respectively, and coincides with the development of virus neutralizing activity in recovered cats.

As early studies were conducted using immunofluorescence assays, cats with undetectable antigenaemia could not be assessed. By using molecular assays it seems that cats with undetectable antigenaemia also undergo the early phases of infection. However, proviral and plasma viral RNA loads in these cats with undetectable viraemia were already lower than those in persistently infected cats starting from week 2 on. Thus, it is possible that in cats with undetectable or transient antigenaemia different immune mechanisms lead to the decrease in FeLV provirus and plasma viral RNA. Moreover, we assume that not the total proviral and plasma viral RNA loads but rather the loads in specific leukocyte subsets are important for the infection outcome.

So far, it is unknown whether antigen-negative cats eventually are able to clear the provirus from the peripheral blood. In having followed cats with regressive infection for more than 3 years after initial virus challenge, very low loads but no permanent clearance of FeLV provirus was observed. Occasionally observed PCR-negative results were subsequently followed by positive results, although in some cases, multiple PCR assays had to be run from the same sample to obtain a positive result. This indicates that the infection had not been
cleared but rather that the proviral loads hovered around the detection limit of the PCR assay.

The biological relevance of the antigen-negative but provirus-positive cat population is unclear. In two of the cats, antigenaemia reoccurred and led to FeLV-associated disease. One cat had still been in contact with persistently infected cats; thus the source of infection could not be established. However, the second cat had been separated from any external FeLV source for almost a year before antigenaemia reappeared. The trigger initiating virus reactivation could not be determined; the cats were neither immune suppressed nor underwent any stressful procedures. Remarkably, the two cats showed persistence of plasma viral RNA in the absence of detectable antigen or virus in the blood and bone marrow. Thus, localized infection might have been present in these cats, in that minimal viral replication took place in a sequestered tissue and only small amounts of viral RNA but not replication competent viral particles were released in the peripheral blood. If FeLV antigen was indeed present in the peripheral blood, the amounts were below the detection limit of the ELISA. As the number of cats with reactivation is small, it cannot yet be concluded whether persistence of plasma viral RNA is a consistent feature in these cats.

In conclusion, we refined the range of possible FeLV infection outcomes by investigating the plasma viral RNA loads and broadening the spectrum of response categories. The molecular tools applied in this study are an important prerequisite to further investigate FeLV infection and pathogenesis. Noteworthy, efficacious FeLV vaccines are of great importance even if they do not prevent minimal viral replication and proviral integration. They protect cats from persistent antigenaemia and thus from FeLV-associated fatal disease. They significantly prolong the life expectancy of vaccinated cats. Nonetheless, the search for improved vaccines, which prevent FeLV proviral integration, should continue.

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