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### ► To cite this version:

M.A. Gomes-Keller, E. Gönczi, B. Grenacher, R. Tandon, R. Hoffman-Lehman, et al.. Fecal shedding of infectious feline leukemia virus and its nucleic acids: A transmission potential. *Veterinary Microbiology*, 2009, 134 (3-4), pp.208. 10.1016/j.vetmic.2008.08.011 . hal-00532463

**HAL Id: hal-00532463**

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Submitted on 4 Nov 2010

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## Accepted Manuscript

Title: Fecal shedding of infectious feline leukemia virus and its nucleic acids: A transmission potential

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PII: S0378-1135(08)00329-5  
DOI: doi:10.1016/j.vetmic.2008.08.011  
Reference: VETMIC 4115

To appear in: *VETMIC*

Received date: 4-2-2008  
Revised date: 10-8-2008  
Accepted date: 14-8-2008

Please cite this article as: Gomes-Keller, M.A., Gönczi, E., Grenacher, B., Tandon, R., Hoffman-Lehman, R., Lutz, H., Fecal shedding of infectious feline leukemia virus and its nucleic acids: A transmission potential, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2008.08.011

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1 **Title**

2

3 **Fecal shedding of infectious feline leukemia virus and its nucleic acids: a**  
4 **transmission potential**

5

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15

16 Running title: Fecal shedding of infectious FeLV and its nucleic acids

17

18 Number of words in the Abstract section: 239

19 Number of figures: 4

20 Number of tables: 3

21

22 **Abstract**

23

24 Although it is assumed that fecal shedding of feline leukemia virus (FeLV)  
25 constitutes a transmission potential, no study has been performed showing that feces  
26 of infected cats can be a source of infection. In this study, we investigated fecal viral  
27 shedding of FeLV and its role in viral pathogenesis with the goal to improve infection  
28 control. FeLV RNA and DNA levels were determined in rectal swabs of  
29 experimentally infected cats by real-time PCR, and the results were correlated with  
30 proviral and viral loads in whole blood and plasma, respectively, and plasma p27  
31 levels. All antigenemic cats shed FeLV RNA and DNA in feces. To determine  
32 whether the viral RNA detected was infectious, virus isolation from feces was also  
33 performed. Infectious virus was isolated from feces of antigenemic cats, and these  
34 results perfectly correlated with the isolation of virus from plasma. Naïve cats  
35 exposed to these feces seroconverted, showing that infection through feces took  
36 place, but remained negative for the presence of FeLV provirus and p27 in blood, an  
37 outcome so far not described. Some of the organs collected after euthanasia were  
38 provirus positive at low copy numbers. From these results it is concluded that fecal  
39 shedding of FeLV plays a role in transmission, but it is probably of secondary  
40 importance in viral pathogenesis. Nevertheless, sharing of litter pans by susceptible  
41 and viremic cats could increase the environmental infectious pressure and  
42 appropriate measures should be taken to avoid unnecessary viral exposure.

43

44 **Keywords**

45

46 FeLV, feces, transmission, nucleic acids, virus isolation

47

## 48 Introduction

49

50 Feline leukemia virus (FeLV) is an enveloped, positive-sense, single-stranded  
51 RNA virus, member of the family Retroviridae, genus Gammaretrovirus. FeLV is one  
52 of the most common agents causing cancerous diseases, blood disorders and  
53 immunodeficiencies in cats worldwide.

54 It is assumed that FeLV initially infects the mucosae from the respiratory and  
55 gastrointestinal tracts of the head, and replicates subsequently in the tonsils and  
56 local lymph nodes (Rojko et al., 1979). The germinal centers from the lymph nodes  
57 and tonsils play an important role in this initial replication, functioning as an early  
58 reservoir for the virus (Racz et al., 1990). From these lymphoid tissues, infected  
59 lymphocytes, monocytes and probably mucosal dendritic cells (Langerhans cells)  
60 spread the virus to the bone marrow, thymus, spleen, and intestine, which favors the  
61 initiation of a systemic infection (Rohn and Overbaugh, 1999; Rojko et al., 1979).  
62 Infectious FeLV is found in great numbers in saliva (Hoover et al., 1972), and  
63 transmission occurs mainly via the oronasal route, by mutual grooming and sharing  
64 of eating dishes and drinking bowls (Hoover and Mullins, 1991). Persistently infected,  
65 antigenemic cats shed large quantities of infectious virus in saliva and nasal  
66 secretions, and pose a great risk to susceptible cats (Hardy et al., 1973; Jarrett et al.,  
67 1973).

68 Although saliva is the most important vehicle for the transmission of FeLV,  
69 infectious virus has also been found in milk, urine, and rectal specimens (Hardy et  
70 al., 1976; Hinshaw and Blank, 1977). However, the importance of these clinical  
71 specimens in the horizontal transmission of the virus has been far less studied when  
72 compared to saliva.

73           Gastrointestinal dysfunction can be observed in cats persistently infected with  
74 FeLV, but the etiology of this FeLV-associated enteritis has not been clearly defined  
75 (Lutz et al., 1995; Reinacher, 1987, 1989). It was shown that p27 is expressed in  
76 intestinal epithelial cells not only of cats showing clinical signs of enteritis, but also in  
77 FeLV-positive cats without gastrointestinal disorders, suggesting that there is viral  
78 replication in those cells (Kipar et al., 2000). To our knowledge, although there are  
79 grounds for suspecting that FeLV can be shed in feces, no study has verified fecal  
80 viral shedding and its importance for the transmission of FeLV. In addition, no report  
81 has examined the correlation between FeLV nucleic acids detection and isolation of  
82 infectious FeLV in feces and blood.

83           Recently we demonstrated that the shedding of FeLV RNA in saliva is a  
84 consistent feature in viremic cats, and that latently infected cats (low proviral load, no  
85 antigenemia) sporadically shed viral RNA in saliva, but no virus could be detected by  
86 an infectivity assay (Gomes-Keller et al., 2006). To improve diagnosis and infection  
87 control, and to gain a better understanding on the importance of latently infected  
88 cats, it is essential that possible viral transmission routes are better investigated. In  
89 this study we aimed at investigating the possible role of feces in the transmission of  
90 the virus in cats harboring different proviral loads. We have applied noninvasive  
91 methods for the detection of FeLV. Rectal swabs from 24 experimentally infected  
92 cats were tested for the presence of FeLV RNA and DNA. In addition, the presence  
93 of infectious virus in the feces of these cats was also investigated and a transmission  
94 experiment was carried out to determine whether FeLV can be transmitted solely by  
95 contact of naïve cats with feces from persistently infected cats, harboring infectious  
96 virus.

97

98 **Material and methods**

99

100 ***Animals and study design***

101 Two separate studies were performed with specific-pathogen-free (SPF) cats.  
102 In the first study, 24 animals were acquired from Charles River Laboratories  
103 (L'Arbresle, France), and were infected intraperitoneally with FeLV-A/Glasgow strain  
104 (kindly provided by Dr. O. Jarrett) at 17 to 19 weeks of age. We examined shedding  
105 of FeLV nucleic acids and infectious virus in the feces of these animals at week 98  
106 post-infection, and correlated our findings with the presence of FeLV RNA, DNA, p27  
107 and infectious virus in blood.

108 The second study consisted of a transmission experiment. Eight cats were  
109 acquired from Liberty Research, Inc. (Waverly, NY), and entered the study at 12  
110 weeks of age. For the transmission experiment, feces of 8 persistently infected cats  
111 were carefully collected and placed in the litter pan (size of 0.4 X 2.0 m) localized in  
112 the room that housed these 8 SPF cats. Feces were spread randomly on the surface  
113 of the litter pan. This procedure was performed twice daily, once in the morning and  
114 once in the afternoon, for a period of 26 weeks. Blood samples and rectal swabs  
115 were collected weekly until week 12, and at weeks 18 and 26. Blood samples were  
116 evaluated for the presence of FeLV-specific RNA and DNA sequences, anti-FeLV  
117 antibodies, and p27. Rectal swabs were used for the detection of FeLV-specific DNA  
118 and RNA.

119 In addition, 30 SPF cats acquired from Liberty Research, Inc., not infected by  
120 FeLV, were used as negative controls. These animals were acquired at the same  
121 time and at the same age as the animals from the first experiment, and were handled  
122 in the exact same manner. They were tested prior to the beginning of the experiment  
123 and shown to be negative for the presence of FeLV DNA and RNA sequences in

124 plasma and rectal swabs and p27 in plasma. Blood samples and rectal swabs from  
125 these negative controls were used to test the specificity of our extraction and  
126 detection methods.

127 All cited animals were kept under barrier conditions in large rooms furnished  
128 with running boards, climbing trees, ladders, hammocks and elevated sleeping  
129 places, under optimal ethological conditions at the Vetsuisse Faculty, Zurich,  
130 Switzerland. Animal handling protocols were reviewed and approved by the Swiss  
131 Federal Veterinary Office and followed strictly its guidelines.

132

### 133 ***Sample collection and processing***

134 In study 1, to test whether cats shed FeLV-nucleic acids and infectious virus in  
135 their feces, blood samples and rectal swabs were collected from the 24 animals  
136 previously cited on the same day and under identical conditions. Blood samples were  
137 obtained by jugular venipuncture using evacuated tubes (Becton Dickinson,  
138 Plymouth, UK) containing K<sub>3</sub>EDTA. For virus isolation, an additional blood sample  
139 was collected using evacuated tubes (Becton Dickinson, Plymouth, UK) containing  
140 lithium heparin. Rectal swabs were collected with the aid of commercially available  
141 cotton wool swabs, similar to Q-tips (Primella, Migros Genossenschafts-Bund,  
142 Switzerland). Swabs were inserted into the anus of the cats and rubbed gently. After  
143 collection, swabs were immediately placed into sterile 1.5-ml microcentrifuge tubes,  
144 the external tip was removed, and the microcentrifuge tubes were closed. Swabs  
145 were then processed as previously described (Gomes-Keller et al., 2006). Briefly,  
146 200 µl of Hank's balanced salt solution (without calcium chloride, magnesium  
147 chloride, or magnesium sulfate, GIBCO, Paisley, Scotland, UK) were pipetted into the  
148 tubes and briefly mixed by pulse-vortexing. Samples were incubated at 42°C for  
149 10 min. The tubes were subsequently centrifuged at 8'000 X g for 1 minute to remove

150 drops from the inside of the lid. Swabs were inverted and samples were eluted by  
151 centrifugation at 8'000 X g for 1 min. Swabs were then discarded, and 140 µl of the  
152 whole eluate were used for extraction of total nucleic acids.

153 For study 2 (transmission experiment), 1 ml of blood was collected from 8 SPF  
154 cats using the closed collection system K<sub>3</sub>EDTA S-Monovette (Sarstedt, Nümbrecht,  
155 Germany). Blood was collected weekly, starting from week 0 (no exposition to feces)  
156 until week 12, and at weeks 18 and 26.

157 For both experiments, plasma was obtained by centrifuging blood at 2300 X g,  
158 for 10 min. Blood and plasma samples were immediately frozen at -80°C until they  
159 were processed.

160

#### 161 ***Nucleic acid extractions***

162 One million blood leukocytes were used for DNA extraction using MagNA Pure  
163 LC DNA Isolation Kit I and MagNA Pure LC Instrument (Roche Diagnostics,  
164 Mannheim, Germany), according to the manufacturer's recommendations. Leukocyte  
165 counts were determined by an electronic cell counter (Cell-Dyn 3500, Abbott, Baar,  
166 Switzerland), and the appropriate volume containing 10<sup>6</sup> cells were used for  
167 extraction. Total nucleic acids were extracted from 140 µl of fecal eluate or 140 µl of  
168 plasma using MagNA Pure LC Total Nucleic Acid Isolation Kit and MagNA Pure LC  
169 Instrument (Roche Diagnostics, Mannheim, Germany) as recommended by the  
170 manufacturer. Each sample was eluted in a final volume of 60 µl of elution buffer.  
171 Extracted samples were stored at -80°C until analysis.

172

#### 173 ***Detection and quantification of FeLV-DNA and RNA***

174 FeLV DNA was detected by real-time PCR, and RNA by real-time RT-PCR  
175 (ABI 7700, Applied Biosystems, Foster City, USA). Five microliters of the eluate

176 obtained by nucleic acid extraction were used in each reaction. Primers, probe and  
177 assay conditions were as described (Tandon et al., 2005). The detection limit of the  
178 assays were determined using serial 10-fold dilutions of purified DNA and RNA from  
179 FEA/FeLV-A, -B, and -C spiked into a given sample (sample extracted either from  
180 blood, plasma or rectal specimens). The results obtained were compared to a  
181 standard curve. In the case of FeLV DNA detection in blood and rectal swabs, the  
182 lower limit of sensitivity was one proviral molecule per PCR reaction. For FeLV RNA  
183 detection, the RT-PCR procedure had a detection limit of 4.93 log<sub>10</sub> copies/ml for  
184 plasma RNA and 3.16 log<sub>10</sub> copies/RT-PCR reaction for fecal RNA, which cannot be  
185 compared directly as amounts of material in rectal swabs are not precisely  
186 quantifiable.

187

#### 188 ***Stability of total nucleic acids in rectal swabs***

189 Four rectal swabs each from five FeLV-infected cats were collected as  
190 mentioned above. Dry swabs were kept at room temperature, and processed and  
191 assayed as cited on days 0, 7, 21 and 42 post-collection for the evaluation of the  
192 stability of FeLV nucleic acids in rectal swabs.

193

#### 194 ***Spiking control experiment***

195 To test for the presence of nonspecific inhibitors in fecal specimens, a spiking  
196 control experiment was performed. The experiment consisted of combining aliquots  
197 of pooled fecal swab eluates from SPF, non-infected cats, and supernatant of  
198 infected QN10S cells (virus stock), to reach an end volume of 200 µl. The following  
199 volumes of virus stock were used: 0 (negative control), 15, 40, 60, 80, 100, 140, 160,  
200 and 200 µl. The same procedure was performed using Hank's balanced salt solution  
201 (without calcium chloride, magnesium chloride, or magnesium sulfate, GIBCO,

202 Paisley, Scotland, UK), in place of fecal swab eluate. Combined samples were used  
203 for total nucleic acid isolation using the MagNA Pure LC total nucleic acid isolation kit  
204 and MagNA Pure LC instrument (Roche Diagnostics), as recommended by the  
205 manufacturer and tested for the presence of RNA by real-time RT-PCR, as described  
206 (Tandon et al., 2005).

207

### 208 ***ELISA for the detection of FeLV p27 in plasma and cell supernatant***

209 FeLV p27 antigen was detected by a sandwich ELISA, as previously described  
210 (Lutz et al., 1983). Results are represented as percentages of absorbance in  
211 comparison to a positive control, FL-74 feline lymphoblastoid cell culture supernatant  
212 (Cockerell et al., 1976), which was considered to be 100%. In this study, p27 values  
213 above 5% were considered to be positive. Cats positive for p27 in plasma were  
214 considered to be antigenemic.

215

### 216 ***Measurement of anti-FeLV IgG in plasma***

217 We detected anti-FeLV IgG in plasma, using ELISA wells coated with  
218 200 ng/well of gradient-purified FL-74 FeLV under conditions previously described  
219 (Lutz et al., 1980). Samples were analyzed in duplicate and the absorbances were  
220 read using a microplate reader. For study 1, the results were calculated as the ratio  
221 of the mean optical density (OD) in comparison to the mean OD of a serum pool of  
222 the same 24 cats before inoculation with FeLV. In the case of study 2, the  
223 transmission study, ratios were calculated using the individual ODs obtained pre- and  
224 post-exposition to feces containing infectious FeLV. Ratios below 1.00 were  
225 considered negative for the presence of FeLV-specific antibodies. In addition, for the  
226 transmission experiment, Western blotting analysis (WB) was performed as  
227 described previously (Lutz et al., 1988), using 1 µg of gradient-purified FL-74 per

228 nitrocellulose strip. We analyzed samples from weeks 0 (before experiment begin)  
229 and 18.

230

### 231 ***In vitro virus isolation from rectal swabs and plasma***

232 Virus was isolated by cell culture assay from rectal swabs and plasma  
233 specimens from cats from study 1 as described by Jarrett and Ganiere (Jarrett and  
234 Ganiere, 1996), with few modifications (Gomes-Keller et al., 2006). Rectal swabs  
235 were obtained from 21 experimentally infected cats as described above (same 24  
236 cats cited above; 3 animals were not sampled), but swabs were immediately placed  
237 into a 1.5 ml-microcentrifuge tube containing 500  $\mu$ l of cell culture medium. Swabs  
238 were processed essentially as described (Gomes-Keller et al., 2006). The  
239 supernatant was filtrated into a sterile 1.5 ml-microcentrifuge tube using a syringe-  
240 driven filter unit (Millex-HV filter unit, 0.45  $\mu$ m, Millipore Corporation, Bedford, MA).  
241 Three hundred and fifty microliters of the filtrate were used for the infection of QN10S  
242 cells. Alternatively, 200  $\mu$ l of lithium heparin plasma were used for inoculation of  
243 QN10S cells. Two hours after inoculation, the supernatant was replaced by fresh  
244 medium. Development of cytopathic effects (CPE) was monitored daily. Supernatants  
245 were tested on days 0, 10 and 18 post-inoculation by the detection of p27 in  
246 supernatant using ELISA, and by RT-PCR using total nucleic acids extracted from  
247 200  $\mu$ l of cell supernatant as described above. Medium was replaced by 50% once a  
248 week.

249 To test whether infectious virus was found in feces present in the litter pan of  
250 FeLV persistently infected cats, we randomly collected 1 g of 20 different fecal  
251 samples, being 10 collected in the morning and 10 collected in the afternoon. Fecal  
252 samples were resuspended in cell culture medium, and incubated for 10 min at RT.  
253 Samples were then centrifuged at 8000 X g for 1 min, and supernatant was filtrated

254 as described above. Three hundred and fifty microliters of the filtrate were used for  
255 the infection of QN10S cells.

256

257 ***Detection of FeLV-DNA and virus isolation from tissues of cats in the***  
258 ***transmission experiment***

259 Cats used in the transmission study (study 2) were euthanized in week 26,  
260 and tissues samples from 27 organs were collected within 30 min post-mortem.  
261 Samples were immediately snap-frozen in liquid nitrogen. Approximately 10 to 20 mg  
262 of tissue were used for the extraction of DNA (DNeasy Blood and Tissue Kit,  
263 QIAGEN, Hombrechtikon, Switzerland), following manufacturer's instructions. FeLV  
264 DNA was detected by real-time PCR as described above, using 800 ng of total DNA  
265 per reaction. For every eight cat samples, one water sample was co-extracted and  
266 tested by real-time PCR as negative control for extraction. In addition, four water  
267 samples were included in each 96-well real-time PCR plate to detect any possible  
268 contamination due to pipetting and processing.

269 In addition, we collected under sterile conditions samples from mesenteric  
270 lymph node, urinary bladder, lung, thymus, spleen, and duodenum for virus isolation.  
271 Tissue samples were processed and co-cultured with QN10S cells, in an attempt to  
272 isolate small quantities of FeLV. Assay conditions were as described (Jarrett and  
273 Ganiere, 1996), but tissue samples were cocultured with QN10S cells for 10 days,  
274 instead of being removed after an initial incubation of approximately 2 h.  
275 Supernatants of the cocultures (200 µl) collected at day 10 post-inoculation were  
276 used for total nucleic acid isolation (MagNA Pure LC Total Nucleic Acid Isolation Kit,  
277 Roche Diagnostics) and samples were tested for the presence of FeLV RNA as cited  
278 above.

279

280 **Statistical analysis**

281 The software StatView (Version 5. SAS Institute Inc., Cary, NC) was used to  
282 determine whether a correlation between different parameters characterizing FeLV  
283 infection and FeLV-RNA and DNA in feces existed (Spearman correlation analysis),  
284 to access the stability of total nucleic acids in rectal swabs (Kruskal-Wallis analysis),  
285 to analyze the results from virus isolation and to compare data in the spiking  
286 experiment (Mann-Whitney U test). All statistical analyses were two-tailed and P-  
287 values  $< 0.05$  were considered as significant. Distributions were tested for normality  
288 by the Kolmogorov-Smirnov test using GraphPad Prism (version 3.00 for Windows,  
289 GraphPad Software, San Diego California USA).

290

291 **Results**

292

293 ***Animal contact with infected feces in the transmission study***

294 Before beginning the transmission study (study 2), we checked for the  
295 presence of infectious virus in the feces of 8 FeLV persistently infected cats. We  
296 randomly collected 20 fecal samples (10 in the morning and 10 in the afternoon) and  
297 individually tested them for the presence of FeLV RNA at the day of collection.  
298 Regardless the time of collection, all samples tested positive for the presence of  
299 FeLV RNA. Samples from morning had in average a cycle threshold (Ct) value  
300 ( $\pm$  SD) of 22.73 ( $\pm$  1.87), while samples collected in the afternoon had a Ct value of  
301 23.99 ( $\pm$  0.78). There was no statistically significant difference between samples  
302 collected in the morning and in the afternoon. Fecal samples were also used for viral  
303 isolation to test for the presence of infectious virus, considering that FeLV loses  
304 relatively rapidly its infectiousness in the environment (Francis et al., 1979). We were  
305 able to detect infectious virus in all samples collected, except one from the morning

306 pool, as shown by the presence of FeLV RNA in the supernatant of QN10S cells,  
307 detected 10 days after inoculation. There was no statistically significant difference  
308 between samples collected in the morning and in the afternoon, regarding the Ct  
309 values found by the real-time RT-PCR analysis of FeLV RNA in the supernatants of  
310 positive samples. Of note, the amount of virus isolated from feces exposed to the  
311 environment was relatively small in comparison to freshly collected feces (see results  
312 below for virus isolation). Based on these results, we conclude that infectious virus is  
313 commonly found in the feces of persistently infected cats and the virus remains  
314 infectious, a prerequisite for its use in the transmission experiment, even though at a  
315 lower load.

316 For the transmission study (study 2), feces from the same 8 FeLV persistently  
317 infected cats cited above were spread over the litter pan in a room housing 8 naïve,  
318 SPF cats. The procedure was performed daily in the morning and in the afternoon,  
319 for a period of 26 weeks. We observed that, after the spread of the feces, naïve cats  
320 entered in contact with the infected material as they immediately covered the  
321 deposited feces with litter. In this manner, we could assure that naïve cats were  
322 exposed twice daily to infectious virus present in the feces of persistently infected  
323 cats.

324

### 325 ***Stability of total nucleic acids in rectal swabs***

326 The stability of total nucleic acids over time was monitored in rectal swabs  
327 collected from experimentally FeLV infected cats known to be shedding virus in the  
328 feces by cell culture assay. Rectal swabs were stored at room temperature, and  
329 individually tested for the presence and the amount of FeLV RNA and DNA on days  
330 0, 7, 21 and 42 post-collection (Figure 1). We did not observe any statistically  
331 significant difference among different time points with reference to FeLV RNA

332 (Kruskal-Wallis,  $P = 0.9719$ ) or DNA Ct values (Kruskal-Wallis,  $P = 0.7089$ ). These  
333 results indicate that FeLV RNA and DNA present in feces are stable without  
334 refrigeration for up to 42 days post-collection. Considering the Ct values obtained,  
335 our results highly suggest that viral RNA is the predominant viral nucleic acid species  
336 in these clinical samples.

337

### 338 ***FeLV-DNA and RNA detection in blood and rectal swabs by real-time PCR***

339 We subsequently investigated the presence of RNA and DNA in blood  
340 specimens and rectal swabs of experimentally FeLV infected cats from study 1. We  
341 were able to confirm infection of all 24 cats by detecting provirus in whole blood by  
342 real-time PCR (Table 1). FeLV RNA was detected in plasma of 22 (91.5%) of the 24  
343 animals by RT-PCR. The levels of FeLV RNA and DNA in rectal swabs were  
344 quantitated by RT-PCR and PCR, respectively. Twenty-two (91.5%) of the 24 cats  
345 tested positive for both fecal RNA and DNA. Cats exhibiting an elevated proviral load  
346 were likely to have a higher plasma viral burden (Spearman's correlation coefficient  
347  $r_s = 0.850$ ,  $P < 0.0001$ ), higher viral RNA burden in rectal swabs (Spearman's  
348 correlation coefficient  $r_s = 0.626$ ,  $P = 0.0027$ ), and higher viral DNA levels in rectal  
349 swabs (Spearman's correlation coefficient  $r_s = 0.612$ ,  $P = 0.0033$ ) than cats  
350 harboring a low proviral load (Table 2). In addition, cats showing high plasma viral  
351 load had higher levels of RNA (Spearman's correlation coefficient  $r_s = 0.551$ ,  $P =$   
352  $0.0082$ ) and DNA (Spearman's correlation coefficient  $r_s = 0.664$ ,  $P = 0.0014$ ) in rectal  
353 swabs (Table 2). Rectal swabs obtained from 30 control SPF cats tested negative for  
354 the presence of both FeLV RNA and DNA (data not shown). To ensure that negative  
355 RT-PCR results were not due to the presence of inhibitors in the feces, which could  
356 affect the RNA extraction and detection procedures, we spiked pooled rectal swab  
357 eluates with a cell-free virus stock, using different volumes. The results were

358 compared to HBSS spiked with the same virus stock and identical volumes. As  
359 shown in Figure 2, there was no statistically significant difference between Ct values  
360 obtained from eluates of rectal swabs or HBSS spiked with virus stock at any volume  
361 tested. This implies that negative results obtained for rectal swabs were not due to  
362 the presence of elements, which might inhibit the efficient RNA extraction and  
363 amplification, but rather due to the absence of FeLV RNA or too low amounts, not  
364 detectable by our assay.

365 Cats from the study 2 (transmission study) did not become provirus positive in  
366 any week tested (data not shown). Considering that our assay is able to detect one  
367 copy per PCR reaction, we believe that these cats did not harbor FeLV in cells from  
368 whole blood or the amount of FeLV present was below our detection limit. Similarly,  
369 we were also not able to detect RNA in plasma of these cats at any occasion tested.

370

### 371 ***Detection of p27 in plasma***

372 Plasma samples were also assayed for the presence of p27 using a sandwich  
373 ELISA. In study 1, the antigen p27 was detected in plasma of 22 (91.5%) of the 24  
374 cats tested, indicating an antigenemic state. There was a positive correlation  
375 between levels of p27 in plasma and RNA (Spearman's correlation coefficient  $r_s =$   
376  $0.662$ ,  $P = 0.0015$ ), and DNA (Spearman's correlation coefficient  $r_s = 0.534$ ,  $P =$   
377  $0.0105$ , Table 2) from rectal swabs. On the other hand, there was no correlation  
378 between proviral load or FeLV RNA in plasma, and levels of plasma p27. Latently  
379 infected cats (cats 24 and 68), with no detectable levels of p27 and plasma viral  
380 RNA, tested negative for the presence of both RNA and DNA in rectal swabs.

381 We were not able to detect p27 at any occasion in cats from the transmission  
382 study (study 2, data not shown). Either these cats did not become antigenemic or the  
383 levels of p27 in the circulation were below the detection limit from our assay.

384

385 ***Detection of FeLV-specific IgG in plasma***

386 The levels of FeLV-specific IgG were determined by ELISA in plasma. In study  
387 1, the levels of FeLV-specific IgG in plasma of the 24 experimentally FeLV-infected  
388 cats showed a strong negative correlation with proviral load (Spearman's correlation  
389 coefficient  $r_s = -0.865$ ,  $P < 0.0001$ ) and with plasma RNA load (Spearman's  
390 correlation coefficient  $r_s = -0.865$ ,  $P < 0.0001$ ). In addition, we also observed a strong  
391 negative correlation between the antibody levels in plasma and the RNA load  
392 (Spearman's correlation coefficient  $r_s = -0.592$ ,  $P = 0.0045$ ) and DNA load  
393 (Spearman's correlation coefficient  $r_s = -0.719$ ,  $P = 0.0006$ ) in rectal swabs. The  
394 levels of viral-specific IgG showed no correlation with the levels of p27 in plasma  
395 (Spearman's correlation coefficient  $r_s = -0.332$ ,  $P = 0.1114$ ).

396 To test for seroconversion by ELISA in the transmission study (study 2),  
397 samples collected at week 0 served as individual negative controls for samples  
398 collected at week 18 and 26. The mean OD obtained by testing samples from these  
399 two latter weeks was divided by the mean OD of the samples collected at week 0,  
400 providing a ratio. Ratios below 1.00 were considered as negative for the presence of  
401 specific antibodies against FeLV. Results were confirmed by WB analysis (only week  
402 18). Results are presented in Table 3 and Figure 3. We observed seroconversion in 5  
403 out of 8 cats (62.5%), as shown by the ELISA and WB results. Interestingly, not all  
404 positive ratios obtained by ELISA proved to be due to specific reactions to FeLV  
405 proteins, as revealed in the WB analysis. We were not able to detect antibodies to  
406 FeLV proteins other than p12 and p15. However, the seroconversion observed took  
407 place clearly after exposition of susceptible cats to infected feces.

408

409 ***Isolation of infectious FeLV from plasma and fecal specimens from***  
410 ***experimentally infected cats***

411 QN10S cells were inoculated with either a filtrate obtained from rectal swabs  
412 or heparin-plasma from 21 FeLV experimentally infected cats of study 1 (same  
413 animals cited above; 3 animals were not sampled). We monitored development of  
414 CPE, and the results were confirmed by the detection of FeLV RNA extracted from  
415 QN10S cell supernatant by real-time RT-PCR, and by the detection of p27 present in  
416 the cell supernatant using ELISA on days 10 and 18 post-inoculation. FeLV was  
417 isolated from rectal swabs and plasma of 17 (81.0%) out of 21 experimentally  
418 infected cats (Figure 4). Samples testing positive for virus isolation by ELISA and RT-  
419 PCR consistently showed CPE (data not shown). Cats 24 and 68, which were  
420 negative for the presence of p27 in plasma and did not shed fecal FeLV nucleic  
421 acids, tested negative for the presence of infectious virus. In addition, we were not  
422 able to isolate virus from cats 18 and 53, which tested positive for the presence of  
423 both p27 in plasma and FeLV RNA and DNA in rectal swabs. Cats with a negative  
424 result in virus isolation had a significantly lower proviral load (Mann-Whitney U Test,  
425  $P = 0.0023$ ), plasma RNA load (Mann-Whitney U Test,  $P = 0.0023$ ), RNA (Mann-  
426 Whitney U Test,  $P = 0.0023$ ) and DNA loads in rectal swabs (Mann-Whitney U Test,  
427  $P = 0.0023$ ), and levels of p27 in plasma (Mann-Whitney U Test,  $P = 0.0031$ ). In  
428 addition, cats testing negative for the presence of infectious virus in rectal swabs and  
429 in plasma showed significantly higher concentrations of IgG in plasma than cats  
430 testing positive for virus isolation (Mann-Whitney U Test,  $P = 0.0031$ ). In 28.5% of  
431 the cats, detection of virus in positive cultures inoculated with rectal swabs eluate  
432 required a longer incubation time than that required for detection of virus in plasma  
433 cultures (Figure 4).

434

435 ***Presence of FeLV proviral sequences in tissue samples from cats positive only***  
436 ***in serological assays***

437 At week 26 of feces exposure, cats from the transmission study (study 2) were  
438 euthanized, and different tissue samples were collected and analyzed for the  
439 presence of FeLV DNA sequences. In cats J1 and J2, which did not seroconvert, we  
440 were not able to detect FeLV DNA in any tissue examined. However, although we  
441 were also not able to detect seroconversion in cat F1, we were able to amplify viral  
442 DNA sequences in rectum, parotid gland and aorta. In cats that seroconverted (G1,  
443 G2, G3, I1 and I2), we found FeLV DNA in different tissues, such as aorta,  
444 diaphragm and myocardial muscles, thymus, popliteal lymph node, rectum, urinary  
445 bladder, thyroid, mandibular gland and lungs. The copy numbers were in average  
446 very low (2.5 copies/reaction). We were not able to detect viral DNA in tissue  
447 samples from the following organs: bone marrow, spleen, liver, kidneys, brain  
448 (cortex), spinal cord, ischiadic nerve, mesenteric, submandibular and sternal lymph  
449 nodes, tonsils, duodenum, jejunum, ileum, colon, and thigh muscle. Water samples  
450 co-extracted together with cat tissue samples and water negative controls added to  
451 real-time PCR plate tested negative for FeLV-specific DNA.

452 We also attempted to isolate infectious FeLV from mesenteric lymph node,  
453 urinary bladder, lungs, thymus, spleen and duodenum. However, we were not  
454 successful in isolating virus from any of these tissues.

455

456 **Discussion**

457

458 To gain a better knowledge on the pathogenesis, routes of transmission and  
459 control measures for FeLV, viral shedding patterns in different body fluids and  
460 secretions must be comprehensively elucidated. Although it has been cited

461 extensively that feces might play a role in the transmission of FeLV, there are no  
462 published studies on the fecal shedding of infectious virus and the importance of this  
463 route for transmission of FeLV. In addition, no report is available, which  
464 demonstrates the correlation between proviral and viral loads in blood, and FeLV  
465 fecal shedding, in the form of either nucleic acids or infectious virus. With the advent  
466 of real-time PCR and RT-PCR, an excellent diagnostic tool with high analytical  
467 sensitivity and specificity, and fast turnaround time became available for the  
468 detection of FeLV in different specimens.

469 In this study we demonstrated that FeLV nucleic acids are consistently found  
470 in rectal swabs of antigenemic cats. Our results showed that the proviral load  
471 strongly correlates with the viral load in rectal swabs and in plasma, as well as with  
472 the proviral load in rectal swabs. Latently infected cats, however, do not appear to  
473 shed either FeLV RNA or DNA in feces. These cats were positive only for the  
474 presence of provirus in whole blood, and tested negative for the presence of viral  
475 RNA and p27 in plasma.

476 We also observed that the FeLV-specific IgG levels strongly and negatively  
477 correlate with the proviral load in whole blood, viral load in rectal swabs and in  
478 plasma, and DNA load in rectal samples. Interestingly, no correlation between the  
479 levels of p27 in plasma and antibody titers could be observed. These results are in  
480 agreement with a previous study performed in this laboratory (Gomes-Keller et al.,  
481 2006), suggesting that IgG plays an important role in viral containment. Animals  
482 testing negative for virus isolation in feces tended to have higher plasma IgG levels  
483 than those shedding infectious virus. We can only speculate that increased levels of  
484 anti-FeLV specific antibodies may lead to an increase in the rates of viral removal  
485 from the circulation, which would justify the strong negative correlations observed. Of  
486 note, we did not evaluate in this study whether the FeLV-specific antibodies found

487 were present in the form of circulating immune complexes and whether they  
488 possessed neutralizing activity.

489         There are several studies reporting the detection of RNA and DNA in fecal  
490 specimens in viral infections causing gastrointestinal clinical signs for diagnostic  
491 purposes (Decaro et al., 2004; Pang et al., 2004; Phan et al., 2005). Cats persistently  
492 infected with FeLV can present viral-associated enteritis, characterized by  
493 histopathologic features in the small intestine similar to those observed in cats with  
494 feline panleukopenia (Reinacher, 1987). Of note, none of the 24 cats examined  
495 showed signs of gastrointestinal alterations, such as diarrhea, constipation or  
496 vomiting, suggesting that despite the involvement of the gastrointestinal tract, the  
497 FeLV-specific nucleic acids are consistently shed in feces of antigenemic and viremic  
498 cats without apparent clinical signs. It is known that tissues frequently infected in  
499 persistently infected cats include the gastric, intestinal and colonic epithelia (Rojko  
500 and Kociba, 1991), and villous stromal cells (Kästner, 1992). The FeLV RNA  
501 detected in rectal samples by our system could originate both from viral particles and  
502 from FeLV-infected cells originating from those tissues. In view of the fact that  
503 antigenemic cats shed also FeLV DNA, which had to be expected to originate from  
504 cellular material, at least part of the FeLV RNA detected most certainly originated  
505 from cells. However, the mean difference between Ct values from samples extracted  
506 from rectal swabs obtained by PCR (DNA measurement) and RT-PCR (RNA  
507 detection) was of 4.54 (SD = 2.30), and, therefore, FeLV DNA accounts for only 4.3%  
508 of the total signal obtained. Consequently, although total nucleic acids were extracted  
509 from fecal swabs, the signal obtained in the real-time RT-PCR originates mainly from  
510 viral RNA and not DNA. Of note, by performing a spiking experiment, we could  
511 exclude the presence of inhibitors affecting RNA extraction and amplification as a  
512 cause for a lack of a signal in the detection of the specific FeLV nucleic acid by real-

513 time RT-PCR. The PCR assay for detecting the presence of viral nucleic acids in  
514 rectal swabs of FeLV experimentally infected cats was shown to be of high diagnostic  
515 sensitivity and specificity, taking into consideration that we correctly diagnosed all  
516 antigenemic cats and we did not obtain a single false-positive result when testing  
517 fecal samples from 30 control SPF cats processed in an identical manner. These  
518 results are very similar to results obtained previously for the detection of FeLV  
519 nucleic acids in saliva (Gomes-Keller et al., 2006). We also observed that FeLV RNA  
520 and DNA are very stable at RT, considering that we were able to detect FeLV nucleic  
521 acids in rectal swabs stored for at least 42 days, even though very high  
522 concentrations of RNases appear to be present in fecal specimens. However, if we  
523 compare the collection of buccal swabs with the one of fecal swabs, it is  
524 comprehensible that saliva samples are easier to collect than rectal swabs.  
525 Nevertheless, the use of fecal swabs, rather than crude feces, due to the presence of  
526 large amounts of debris in the latter ones, remains an additional alternative as clinical  
527 sample that can be used for the diagnosis of FeLV. When this clinical specimen is  
528 used for diagnosis, it is important to keep in mind that cats with access to outdoors  
529 should necessarily be sampled by using rectal swabs, considering that feces from  
530 these animals are rarely available for collection. In addition, in a multiple cat  
531 household, collection of feces from a litter pan leads to cross-contamination with  
532 samples originating from different cats. Therefore, collection of individual rectal  
533 swabs avoids the risk of a false diagnosis.

534 Infectious virus was isolated from 17 (81%) fecal and plasma samples out of  
535 21 tested. All 21 cats tested positive for the presence of provirus in whole blood,  
536 confirming infection with FeLV. Nineteen cats out of 21 (90.5%) tested positive for the  
537 presence of both FeLV RNA and DNA in rectal samples, as well as for the presence  
538 of viral RNA and p27 in plasma. Therefore, the presence of FeLV RNA in feces does

539 not absolutely reveal the production of infectious virions by intestinal and colonic  
540 epithelia. This is reflected by the fact that we were not able to isolate infectious virus  
541 from two cats, which tested positive for the presence of FeLV RNA in feces.  
542 However, the fact that virus was detected in rectal samples and concomitantly in  
543 plasma of 81% of the cats tested demonstrates that fecal shedding is a frequent  
544 feature of the infection. The reason why we were not able to isolate infectious virus in  
545 rectal swabs and plasma from two cats, which tested positive for the presence of  
546 FeLV RNA in both specimens, could be the presence of virus neutralization activity in  
547 these samples. Nevertheless, this possibility remains to be determined. This study  
548 confirms that antigenemia (presence of p27 in plasma) is a good marker for infection  
549 but a less reliable marker for viremia. We were not able to detect either FeLV RNA in  
550 plasma or isolate infectious virus from plasma from two cats testing positive for the  
551 presence of p27, indicating that antigenemia is not a synonym for viremia.

552 This is the first study to show that feces from persistently infected cats harbor  
553 an infectious potential to susceptible cats. However, it appears that viral load present  
554 in feces exposed to environmental conditions is relatively low, leading to a novel  
555 outcome, in which cats only seroconvert, indicating infection, but virus replicates at  
556 very low levels, below the detection limit of very sensitive molecular assays. Based  
557 on this outcome, we can conclude from our results that the bone marrow was not  
558 successfully infected by FeLV, considering that cats tested always negative for the  
559 presence of viral DNA in whole blood. In addition, we could not detect FeLV in bone  
560 marrow samples from any of the 8 cats tested. The likelihood that all cats have been  
561 exposed to the virus is high, because we could observe that all cats equally entered  
562 in contact with feces harboring infectious virus. The level of antibodies remained low.  
563 This could be associated to either a limited initial viral replication (undetectable to our  
564 assay), or to a weak immune response directed to the viral particles present in the

565 feces. We were not able to test in this study whether the antibodies found possessed  
566 neutralizing activity. Interestingly, we were only able to detect seroconversion to p12  
567 and p15 by WB analysis. In a previous experiment, we observed that antibodies to  
568 these two proteins appear very early after FeLV experimental infection (week 4 post-  
569 challenge) and last, in some individuals, up to week 98 (M. A. Gomes-Keller and H.  
570 Lutz, unpublished data). However, in this previous experiment, it appears that  
571 antibodies specific to p12 and p15 were not protective.

572         Although positive findings for seroconversion in a small population are difficult  
573 to generalize, our results suggest that infection with FeLV can occur by exposing  
574 susceptible cats to feces containing infectious virus, leading to solely development of  
575 a weak humoral response. However, virus was not completely eliminated,  
576 considering that we were able to find FeLV DNA sequences in a number of tissue  
577 samples from the euthanized cats. In an attempt to isolate infectious virus from  
578 mesenteric lymph node, urinary bladder, lungs, duodenum, spleen and thymus, we  
579 were not able to obtain a positive result. Tissues for virus isolation were collected at  
580 the same time as tissues for DNA isolation, and there were no means to know which  
581 samples were positive for the presence of DNA before sample collection. With the  
582 exception of thymus, we were not able to find DNA in any other tissue used for virus  
583 isolation. In addition, the number of DNA copies found in a single positive thymus  
584 sample was very low (1.21 copies/reaction). Thus, it appears that the presence of  
585 very low copy numbers of DNA in a certain tissue or its complete absence could be  
586 the reason for a negative result *in vitro* for virus isolation. In addition, our PCR assay  
587 amplifies both integrated and unintegrated FeLV DNA sequences. For viral  
588 replication, DNA must integrate before transcription can take place. The FeLV DNA  
589 sequences found in different tissues could originate from unintegrated DNA, justifying  
590 the negative result obtained. Low copy numbers could also be explained by

591 contamination of some of the samples. However, contaminations are very unlikely as  
592 all negative controls, which were routinely included during RNA and DNA extraction,  
593 as well as during the real-time PCR testing, tested negative.

594

## 595 **Conclusion**

596

597 The present study raises some important points. First, the fact that FeLV RNA  
598 and DNA, and infectious virus are consistently shed in feces of persistently viremic  
599 cats, and that susceptible cats exposed to these feces seroconvert, demonstrates a  
600 role of feces in the transmission of the virus. Although this route of transmission  
601 appears to have a secondary importance, when compared to saliva, special care  
602 should be taken when handling litter pans. However, to utterly evaluate the potential  
603 for contact transmission via feces, the minimum dose required leading to infection of  
604 susceptible cats, the duration and the titers of fecal shedding by persistently infected  
605 cats remain to be determined. Second, the finding that FeLV nucleic acids and  
606 infectious virus are shed in feces from persistently viremic cats, but not from latently  
607 infected cats, i.e. cats testing positive only for the presence of provirus in whole  
608 blood, but negative for p27 in plasma, provides a better understanding of the FeLV  
609 epidemiology. This finding confirms that latently infected cats may not pose a direct  
610 risk to susceptible cats at the time of proviral determination. Nevertheless, under  
611 stressful conditions or coinfections, the infection may be reactivated, and latently  
612 infected cats could become viremic. Finally, we were able to detect a novel outcome  
613 of FeLV, which resulted to the exposition of susceptible cats to very low loads of the  
614 virus. Whether this outcome has clinical importance or contributes to the  
615 pathogenesis of FeLV remains to be defined. However, considering that these cats  
616 tested negative for the presence of DNA and RNA in blood, and that the only signs

617 for an infection were a weak immune response and the presence of FeLV DNA in  
618 some organs, we believe that such infection state does not pose a risk to susceptible  
619 cats. Nevertheless, it would be interesting to induce such outcome in a larger  
620 population to further investigate these findings.

621

## 622 **Acknowledgements**

623

624 We thank Dr. O. Jarrett, who kindly provided the FeLV virus stock, and Dr.  
625 Marina Meli, Velia Fornera, Elisabeth Rogg, and Theres Meili for excellent assistance  
626 in this project. This work was supported by the Forschungskredit grant 55230201  
627 from the University of Zurich. The laboratory work was performed at the Center for  
628 Clinical Studies from the Vetsuisse Faculty, University of Zurich, under the  
629 supervision of Dr. M. Meli. This study was conducted by M. A. Gomes-Keller in partial  
630 fulfillment of the requirements for a Ph.D. degree at the Vetsuisse Faculty, University  
631 of Zurich.

632

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707 *Methods*.
- 708
- 709

710 **Figure captions**

711

712 FIG. 1. Stability of FeLV RNA and DNA in fecal swabs stored at room temperature for  
713 up to 42 days. Results are expressed as the mean of cycle threshold (Ct) values from  
714 5 experimentally infected cats and the respective standard deviations; RNA (□), DNA  
715 (■).

716

717 FIG. 2. Virus spiking control experiment. Supernatant of infected QN10S cell cultures  
718 (virus stock) that contained FeLV RNA were added into either pooled fecal swab  
719 eluates or HBSS before extraction and amplification of viral RNA. NC, negative  
720 control; PC, positive control. Results are expressed as cycle threshold (Ct) values  
721 from samples spiked with different volumes ( $\mu$ l) of virus stock (X axis); HBSS (□),  
722 pooled fecal swab eluates (■). As there were only two measurements, no standard  
723 deviation or error could be calculated.

724

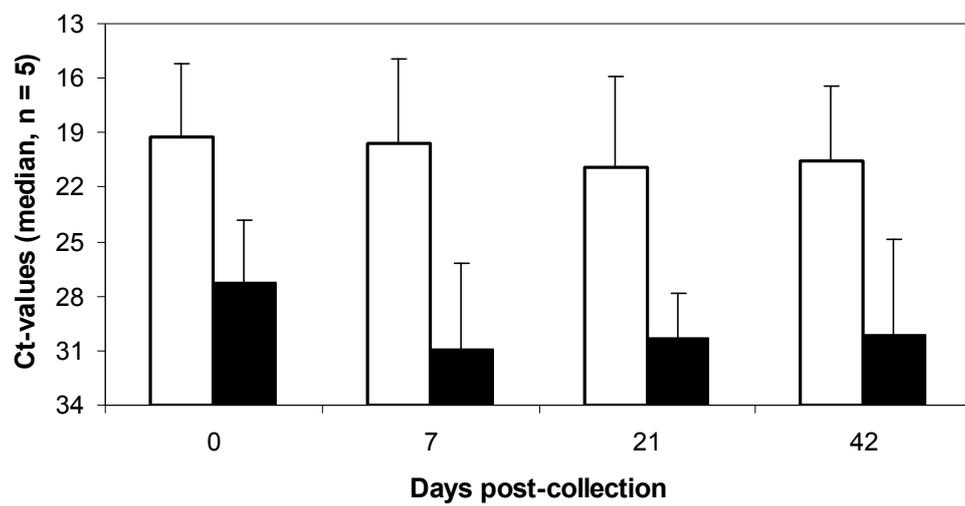
725 FIG. 3. Western blotting using gradient-purified FL-74 and sera from susceptible cats  
726 exposed to feces containing infectious FeLV. Strips 1 and 9, negative controls; strips  
727 8 and 20, positive controls; strips 2 (cat G1), 4 (cat G2), 6 (cat G3), 10 (cat F1), 12  
728 (cat I1), 14 (cat I2), 16 (cat J1) and 18 (cat J2), sera before exposure to infected  
729 feces (week 0); strips 3 (cat G1), 5 (cat G2), 7 (cat G3), 11 (cat F1), 13 (cat I1), 15  
730 (cat I2), 17 (cat J1) and 19 (cat J2), sera after exposure to infected feces (week 18).

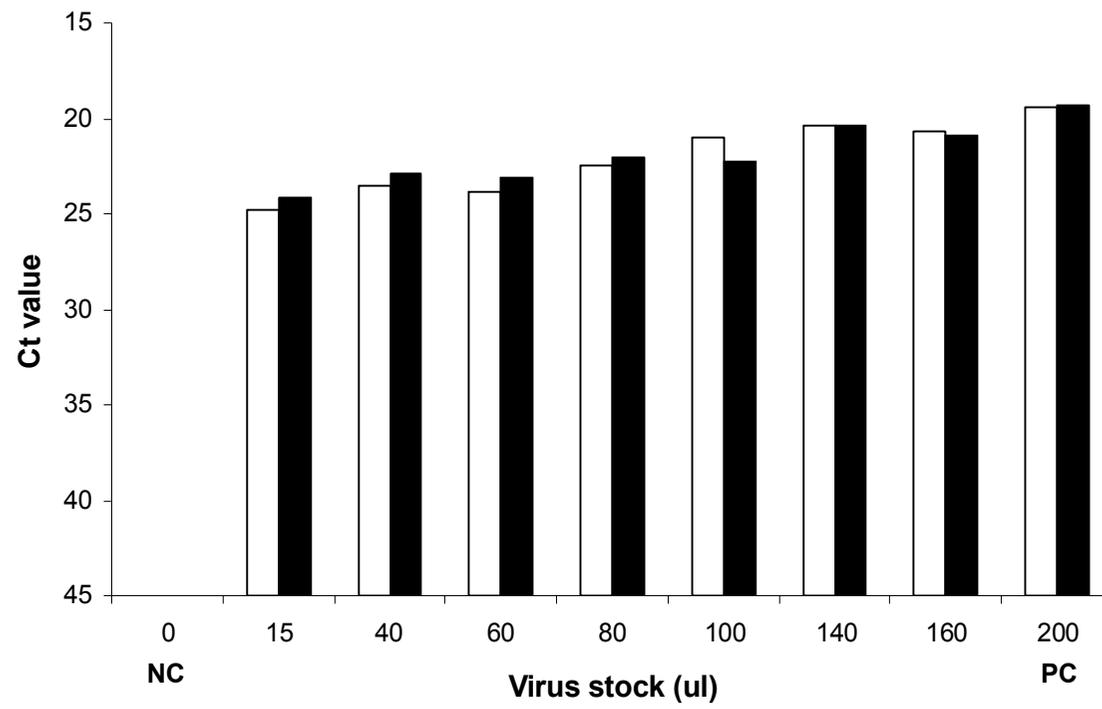
731

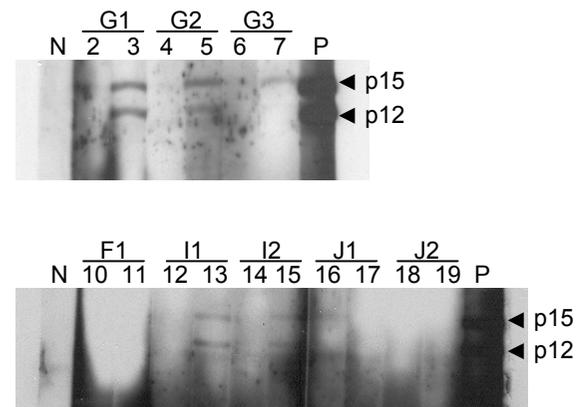
732 FIG. 4. FeLV virus Isolation from feces and plasma from experimentally infected cats  
733 at days 10 and 18 post-inoculation using QN10S cell cultures. Results are expressed  
734 as Ct value 45 (considered to be negative) minus Ct values measured obtained by  
735 real-time RT-PCR done in total nucleic acids extracted from QN10S cell culture

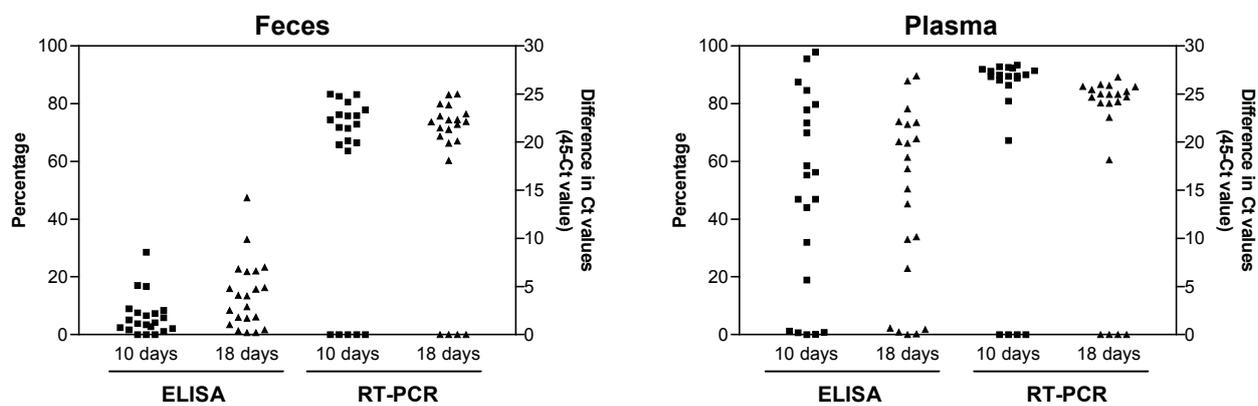
736 supernatant after inoculation; and as percentage obtained by p27 ELISA performed  
737 using cell culture supernatant as sample, in comparison to a positive control (FL74  
738 feline lymphoblastoid cell culture supernatant), which was considered to be 100%.  
739 The four cats found to be negative in RT-PCR and p27 ELISA were identical.  
740

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## Tables

TABLE 1. Detection of FeLV DNA and RNA in rectal swabs, plasma and blood samples, and detection of p27 in plasma from FeLV infected cats collected one year after experimental infection

| Cat | Provirus <sup>a</sup> | Plasma RNA <sup>b</sup> | Fecal RNA <sup>c</sup> | Fecal DNA <sup>c</sup> | Plasma p27 <sup>d</sup> | Plasma IgG <sup>e</sup> |
|-----|-----------------------|-------------------------|------------------------|------------------------|-------------------------|-------------------------|
| 8   | 7.66                  | 9.24                    | 5.86                   | 4.93                   | 33.64                   | 0.248                   |
| 9   | 7.13                  | 8.95                    | 5.02                   | 3.34                   | 27.51                   | 0.600                   |
| 10  | 6.76                  | 8.91                    | 4.76                   | 3.58                   | 42.83                   | 1.303                   |
| 14  | 5.36                  | 7.18                    | 4.58                   | 2.95                   | 15.95                   | 3.731                   |
| 16  | 6.68                  | 8.77                    | 5.78                   | 3.63                   | 52.49                   | 2.007                   |
| 17  | 6.99                  | 8.90                    | 5.90                   | 3.41                   | 45.09                   | 1.655                   |
| 18  | 4.06                  | 5.55                    | 3.19                   | 0.13                   | 11.10                   | 5.083                   |
| 21  | 7.79                  | 9.51                    | 5.75                   | 3.22                   | 42.78                   | 0.221                   |
| 24  | 2.82                  | nd                      | nd                     | nd                     | < 5.00                  | 3.862                   |
| 27  | 6.89                  | 9.07                    | 5.54                   | 3.78                   | 46.13                   | 0.745                   |
| 28  | 7.01                  | 8.83                    | 5.36                   | 3.45                   | 32.60                   | 0.483                   |
| 31  | 6.89                  | 8.76                    | 4.96                   | 3.78                   | 33.30                   | 1.834                   |
| 33  | 6.90                  | 8.90                    | 5.34                   | 2.98                   | 47.34                   | 2.434                   |
| 38  | 7.23                  | 8.92                    | 5.24                   | 3.70                   | 29.71                   | 1.469                   |
| 39  | 7.11                  | 8.98                    | 5.10                   | 3.19                   | 39.25                   | 1.414                   |
| 41  | 6.87                  | 8.58                    | 5.32                   | 3.34                   | 51.45                   | 1.807                   |
| 42  | 6.94                  | 8.72                    | 5.31                   | 2.89                   | 33.12                   | 1.524                   |
| 43  | 7.23                  | 9.22                    | 5.22                   | 3.87                   | 51.62                   | 0.331                   |
| 47  | 6.99                  | 8.63                    | 5.93                   | 3.65                   | 53.12                   | 1.462                   |
| 53  | 4.60                  | 6.00                    | 3.81                   | 2.69                   | 22.78                   | 4.828                   |
| 54  | 6.85                  | 8.40                    | 5.33                   | 3.14                   | 60.87                   | 2.924                   |
| 56  | 6.90                  | 8.66                    | 5.62                   | 4.06                   | 62.54                   | 1.110                   |
| 63  | 7.70                  | 9.63                    | 6.53                   | 3.96                   | 33.47                   | 0.234                   |
| 68  | 3.17                  | nd                      | nd                     | nd                     | < 5.00                  | 2.931                   |

nd, not detectable, that is below the lower detection limit of the assay (1 copy/PCR reaction for provirus in blood and for fecal DNA; 4.93 log<sub>10</sub> copies/ml for plasma RNA; 3.16 log<sub>10</sub> copies/reaction for fecal RNA).

<sup>a</sup> copies (log 10)/10<sup>6</sup> cells

<sup>b</sup> copies (log 10)/ml of plasma

<sup>c</sup> copies (log 10)/reaction

<sup>d</sup> values are expressed in percentage in comparison to a positive control (FL74 feline lymphoblastoid cell culture supernatant), which was considered to be 100%.

<sup>e</sup> values are expressed as ratios of the mean optical density (OD) in comparison to the mean OD of samples collected from the same cats before infection (n=24) and processed in an identical manner. Ratios  $\leq 1$  are considered negative for the presence of FeLV-specific antibodies.

TABLE 2. Correlation between different parameters characterizing FeLV infection and FeLV-RNA and DNA levels in rectal swabs

| Variable                          | Correlation with fecal viral load<br>(log <sub>10</sub> FeLV RNA copies/reaction) |                      | Correlation with fecal viral load<br>(log <sub>10</sub> FeLV DNA copies/reaction) |                      |
|-----------------------------------|---|----------------------|---|----------------------|
|                                   | Spearman's r <sub>s</sub> <sup>e</sup>  | P value <sup>f</sup> | Spearman's<br>r <sub>s</sub> <sup>e</sup>   | P value <sup>f</sup> |
|                                   | Plasma FeLV RNA <sup>a</sup>  | 0.626                | 0.0027  | 0.612                |
| Provirus <sup>b</sup>             | 0.551   | 0.0082               | 0.664   | 0.0014               |
| Plasma p27 <sup>c</sup>           | 0.662   | 0.0015               | 0.534   | 0.0105               |
| Plasma anti-FeLV IgG <sup>d</sup> | -0.592  | 0.0045               | -0.719  | 0.0006               |

<sup>a</sup> Detection of FeLV RNA sequences by real-time RT-PCR in plasma samples;

<sup>b</sup> Detection of FeLV DNA sequences by real-time PCR in whole blood;

<sup>c</sup> Concentrations of p27 in plasma samples were measured by ELISA assay;

<sup>d</sup> Levels of anti-FeLV IgG in plasma samples were determined by ELISA assay;

<sup>e</sup> Spearman's r<sub>s</sub>, Spearman's correlation coefficient;

<sup>f</sup> P-values < 0.05 were considered as statistically significant;

TABLE 3. Detectable anti-FeLV antibodies against gradient-purified whole FeLV and its proteins in cats exposed to feces from FeLV persistently infected cats

| Cat | ELISA <sup>a</sup> |         | WB <sup>b</sup> |     |
|-----|--------------------|---------|-----------------|-----|
|     | Week 18            | Week 26 | p12             | p15 |
| F1  | 0.674              | 0.720   | -               | -   |
| G1  | 2.682              | 2.808   | +               | +   |
| G2  | 1.411              | 1.811   | +               | +   |
| G3  | 1.235              | 1.426   | -               | +   |
| I1  | 1.593              | 1.537   | +               | +   |
| I2  | 1.321              | 1.612   | +               | +   |
| J1  | 1.216              | 1.162   | -               | -   |
| J2  | 0.918              | 1.194   | -               | -   |

<sup>a</sup> Anti-FeLV IgG in plasma samples were determined by ELISA. Values are expressed as ratios of the mean optical density (OD) in comparison to the mean OD of samples collected from the same cat before FeLV exposition and processed in an identical manner. Ratios  $\leq 1$  are considered negative for the presence of FeLV-specific antibodies;

<sup>b</sup> Anti-FeLV IgG measured by WB analysis. Responses defined as (+) for a positive reaction, and (-) for a negative reaction to either p12 and/or p15. Responses to other FeLV proteins could not be detected in our assay. WB analysis was performed at week 18 of exposition; plasma samples collected at week 0 (no exposition to FeLV-infected feces) tested negative for the presence of anti-FeLV antibodies.