Departement der Nutztierklinik
Veterinärmedizinisches Labor
der Vetsuisse-Fakultät Universität Zürich

Leitung: Prof. Dr. med. vet. Regina Hofmann-Lehmann

Transmission of Feline Leukemia Virus Infection by Provirus Positive Blood

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Stefanie Nesina

Tierärztin
von Poschiavo, GR

genehmigt auf Antrag von

Prof. Dr. med. vet. Regina Hofmann-Lehmann, Referentin
PD Dr. med. vet. Felicitas S. Boretti, Korreferentin

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Contents

1. Zusammenfassung/ Summary 2
2. Introduction 3
3. Materials and methods 7
   3.1 Animals 7
   3.2 Experimental design and blood transfusion 8
   3.3 Clinical examination and sample collection 11
   3.4 Hematology 12
   3.5 Clinical chemistry and serum protein electrophoresis 12
   3.6 Nucleic acid extraction and quantification 13
   3.7 Serological assays 14
   3.8 Isolation of FeLV using QN10S cells 15
   3.9 Bone marrow cell culture 15
   3.10 Necropsy 16
   3.11 Immunohistology 16
   3.12 Statistics 17
4. Results 17
   4.1 Verification of SPF status and blood transfusion 17
   4.2 FeLV provirus, plasma viral RNA and p27 antigen detection 17
   4.3 Clinical and laboratory findings 24
   4.4 Virus isolation from blood and bone marrow 29
   4.5 Outcome of the infection 29
   4.6 Viral shedding and immune response 33
5. Discussion 35
6. References 41
7. Curriculum Vitae ....

1. Zusammenfassung/ Summary

Der Nachweis der felinen Leukämievirus (FeLV) Infektion erfolgt meistens mittels p27 Antigen ELISA. Es gibt jedoch p27-negative Katzen, welche provirus-positiv im Blut sein können. Das Risiko einer FeLV Übertragung durch provirus-positives Blut wurde bisher nicht untersucht. Das Ziel dieser Studie war es, dieses Risiko zu erforschen. Fünfzehn zehn Wochen alte spezifiziert pathogen freie (SPF) Katzenwelpen wurden zufällig in drei Gruppen eingeteilt: Die Katzen der Gruppe A erhielten Blut von einer provirus-positiven Spenderkatze, Gruppe B von einer provirus-positiven und viralen RNA positiven Katze und Gruppe C von einer nicht-infizierten SPF Katze. Nach der Transfusion wurden alle Katzen der Gruppe A und B positiv für Provirus und virale RNA. In der Gruppe A wurden alle Katzen und in der Gruppe B drei Katzen p27-positiv. Die Katzen aus der Kontrollgruppe blieben FeLV-negativ. Bemerkenswerterweise wurden die Katzen der Gruppe A später provirus-positiv als diejenigen der Gruppe B; sie zeigten jedoch einen schlechteren Infektionsausgang (höhere FeLV-Bürden) als Gruppe B. Zwei Katzen der Gruppe A wurden persistierend infiziert. Unsere Resultate zeigen erstmals, dass FeLV durch Bluttransfusion mit provirus-positivem, antigen-negativem Blut auf naive Empfänger übertragen werden kann. Aufgrund unserer Resultate empfehlen wir Blutspender-Katzen mittels PCR auf FeLV Provirus zu testen.

Cats with suspicion of feline leukemia virus (FeLV) infection are commonly tested for antigenemia by p27 ELISA. However, many p27 negative cats test FeLV provirus positive in the blood. So far, the risk of a FeLV transmission *via* provirus-positive blood has not been evaluated. The aim of this study was to explore this risk. Fifteen ten-week old specified pathogen free (SPF) kittens were randomly assigned to three groups: five cats (group A) received blood from a FeLV provirus positive cat, five cats (group B) were transfused with blood from a provirus and viral RNA positive cat and five control cats (group C) received SPF blood. All cats in groups A and B turned provirus and plasma viral RNA positive post transfusion. Moreover, all cats in group A, and three cats in group B became p27 positive. All cats in group C stayed FeLV negative. Remarkably, cats in group A turned FeLV positive later than cats in group B but had a graver infection outcome (higher FeLV loads); two cats in group A became persistently infected. Our results demonstrate for the first time that FeLV infection can be transmitted via blood transfusion of blood from FeLV provirus positive, antigen negative cats to naïve recipients. Based on our results we highly recommend screening blood donors for FeLV provirus by PCR prior to blood transfusion.

2. Introduction

The feline leukemia virus (FeLV) was discovered in 1964 and belongs to the family of gammaretroviridae (Jarrett, Crawford et al. 1964). FeLV is naturally occurring worldwide in domestic cats and some related small felids (Hoover and Mullins 1991), (Leutenegger, Hofmann-Lehmann et al. 1999). Retroviral infections are of veterinary as well as human medical interest as an important animal model for AIDS and tumor research (Onions 1985; Miyazawa 2002). FeLV infection can induce both

cytoproliferative (tumors) and cytosuppressive diseases (immunodeficiency, and myelosuppression) with fatal outcome (Post JE 1980).

For FeLV infection a variety of outcomes has been demonstrated. They are influenced by different host and virus factors, such as age and the immune system status of the cat as wells as the strain, subtype and dose of the virus (Hoover and Mullins 1991). The spectrum of different infection outcomes is classified into: abortive (no virus detection after exposure), regressive (p27 negative, provirus positive after or without transient antigenemia), progressive (provirus positive, plasma viral RNA positive, persistently p27 positive, virus isolation positive) and atypical infection (seroconversion, provirus negative, p27 negative) (Torres, Mathiason et al. 2005; Hofmann-Lehmann, Cattori et al. 2008; Major, Cattori et al. 2010). In addition, latent non-productive infection characterized by the absence of viremia and the persistence of the virus in the bone marrow can be identified in cats following regressive infection (Post JE 1980; Rojko, Hoover et al. 1982; Madewell and Jarrett 1983). Latent infections are usually resolved within a few months of exposure to FeLV but have been detectable in some cats up to 30 months after infection (Pacitti and Jarrett 1985). While persistently infected cats usually succumb to FeLV-associated diseases (anemia, immunosuppression, and neoplasia), cats with regressive infections overcome viremia usually after a few weeks and remain provirus positive (Hofmann-Lehmann, Cattori et al. 2007). The clinical importance of the provirus-positive status is still unclear, it may well be essential for solid protection and long-lasting maintenance of protective immunity (Hofmann-Lehmann, Cattori et al. 2008). On the other hand, reactivation of the infection with developing of FeLV associated diseases might occur as had been observed for at least some FeLV antigen-negative, provirus positive cats (Post JE 1980; Rojko, Hoover et al. 1982; Madewell and Jarrett 1983; Hofmann-Lehmann, Cattori et al. 2007; Helfer-

Hungerbuehler, Cattori et al. 2010). PCR is necessary to detect provirus-positive cats, while p27 antigen can be determined by ELISA and was found to be a marker for viremia in most but not all cats (Jarrett, Golder et al. 1982). The proviral DNA is integrated into the cat's genome, so it is unlikely to be cleared throughout the infection. In provirus positive but p27 negative cats no replicating virus is detectable using virus isolation. However, FeLV may retain the potential for replication in provirus positive cats for many years (Helfer-Hungerbuehler, Cattori et al. 2010). In addition, plasma viral RNA could be detected in the absence of antigenemia in some provirus positive cats (Cattori, Tandon et al. 2006; Hofmann-Lehmann, Tandon et al. 2006; Hofmann-Lehmann, Cattori et al. 2007). Thus, at least a part of the proviral DNA in these cats was transcriptionally active (Hofmann-Lehmann, Cattori et al. 2008). In cats with regressive infection only certain blood subsets, such as $CD4^+$ and B lymphocytes, are provirus-positive compared to persistently infected cats were provirus was found in all leukocyte subsets (Pepin, Tandon et al. 2007; Cattori, Pepin et al. 2008). The prevalence of provirus positive but p27 negative cats varies among different investigated cat populations and was found to be between 1.2% (Englert, Lutz et al. 2012) and 10% (Hofmann-Lehmann, Huder et al. 2001).

FeLV is transmitted horizontally, mainly via saliva from persistently infected cats and vertically from infected queens to their kittens. The virus is also detectable in different body fluids, such as feces, urine, milk and blood (Francis, Essex et al. 1977; Pacitti, Jarrett et al. 1986; Cattori, Tandon et al. 2009; Gomes-Keller, Gonczi et al. 2009). Therefore, blood sucking parasites and blood transfusions should also be considered as potential infection routes (Hardy, Old et al. 1973). Therapeutic blood transfusions of blood from FeLV viremic cats can cause iatrogenic transmission of FeLV with the high risk of developing FeLV associated diseases (Hardy, Old et al. 1973), especially

because blood transfusion recipients may be highly susceptible to infections due to pre-existing severe clinical conditions and possible immunosuppression. Common causes for blood transfusion in cats are hemorrhage (trauma, peri- or postoperative bleeding, gastrointestinal bleeding, abdominal neoplasia, coagulopathies, thrombocytopenia), lack of erythropoesis and primary immune-mediated hemolytic anemia (Barfield and Adamantos 2011). Clinical cases of intense flea burden, hepatic necrosis or neonatal isoerythrolysis are causing anemia in kittens so that a blood transfusion is required for therapy (Barfield and Adamantos 2011). In veterinary medicine fresh whole blood is most commonly used for blood transfusions, composed of red blood cells, white blood cells, platelets, the coagulations factors, albumin and immunoglobulins (Chiaramonte 2004; Godinho-Cunha, Ferreira et al. 2011). Blood transfusions containing fresh whole blood is indicated when several blood components are required as coagulation effect, severe thrombocytopenia or acute hemorrhage occurs (Lanevschi and Wardrop 2001; Chiaramonte 2004; Godinho-Cunha, Ferreira et al. 2011).

So far, the risk of a FeLV transmission with provirus-positive blood has not been evaluated. However, intradermal inoculation plasmid DNA carrying the FeLV-A provirus (Rickard/FRA) led to productive FeLV infection (Chen, Bechtel et al. 1998). Therefore, whole blood including white blood cells carrying the FeLV provirus might be infectious, when transfused to a susceptible cat.

With the growing importance of feline blood transfusions, the public awareness increases, and in turn the demand for high-quality blood products (Kaufman 1992). Blood banks are already present in North America and it is likely that in future feline blood products will be available in other countries (Barfield and Adamantos 2011). Therefore, the screening management of blood donors needs to be employed and updated to further guarantee blood safety. To date, for FeLV the screening of the blood

for FeLV antigen by ELISA is recommended prior to transfusion (Wardrop, Reine et al. 2005). So far, no data was available concerning the risk of a potential transmission of a productive FeLV infection via provirus positive blood.

The aim of this study was to test the hypothesis that FeLV infection can be transmitted via blood transfusion from a FeLV provirus-positive but antigen negative blood donor to a naïve recipient.

3. Materials and methods

3.1 Animals

Fifteen, 8 weeks-old SPF male kittens (blood transfusion recipients) and three castrated adult male SPF cats (blood donors) at the age of one year (Liberty Research, Waverly, NY, USA) were included in this study. Animals were kept in a confined university facility under barrier condition and optimal ethological and hygienic conditions, as previously described (Museux, Boretti et al. 2009; Geret, Riond et al. 2011). All experiments were performed according to the Swiss law and were officially approved by the veterinary office of the canton of Zurich (160/2010). Prior to the start of the experiment, each cat was clinically examined, blood and plasma samples, conjunctival, oropharyngeal, and rectal swabs were collected and tested to verify the cat's SPF status, as previously described (Museux, Boretti et al. 2009). In addition, serum samples were tested for antibodies to feline calicivirus, feline herpes virus and feline parvovirus by immunofluorescence assay, as described (Hofmann-Lehmann, Fehr et al. 1996).

3.2 Experimental design and blood transfusion

Experimental setup

After arrival, the kittens were adapted to the new environment and the care persons for two weeks. During this period the cats were trained for easy handling and blood collections without anesthesia. The kittens were randomly assigned to three groups (A, B, and C) of five kittens. At the age of ten weeks the 15 kittens received the blood transfusion (see also below). The cats in group A received blood from a FeLV provirus-positive, plasma viral negative, and p27 negative cat; cats in group B were transfused with blood from a provirus-positive, plasma viral RNA positive and p27 negative cat and cats in the control group C received blood from a FeLV-negative naïve SPF cat. Each kitten received a total volume of 10 ml of whole blood (Table 1).

Blood typing

The blood type of each cat was determined prior to the experiment using EDTA anticoagulated blood and a commercial gel column technique (ID-Gel Test Feline A + B Typing; DiaMed AG, Cressier sur Morat, Switzerland).

Cross match

A standard saline-agglutination crossmatching procedure was performed at 37 °C with blood from all recipients and blood donors prior to the blood transfusion (Wardrop 2010). The blood from each recipient cat was crossmatched with the blood from the corresponding blood donor.

Table 1: Experimental setup, recipients and transfused blood

Group	Recipient cats	Transfused blood: Volume (ml)*	Provirus	Viral RNA	p27
A	HBW2 HBZ3 HCC1 HCC4 HCD1	10	positive	negative	negative
B	HBU1 HBW1 HBZ1 HBY1 HCC3	10	positive	positive	negative
C	HBU2 HCD2 HCC2 HBZ2 HBV1	10	negative	negative	negative

* Volume per recipient

Blood collection for blood transfusion and baseline analysis

The three adult blood donor cats (GBX3, GCN4, GCN5; Table 2) were anesthetized with 10 mg/kg ketamin (Narketan®, Vétoquinol AG, Belp, Switzerland) and 0.1 mg/kg midazolam (Dormicum®, Roche Pharma AG, Reinach, Switzerland) intramuscularly. Then, 50 ml of blood were collected into a syringe containing 7 ml of the anticoagulants citrate phosphate dextrose adenine (CPDA-1, Fenwal, Lake Zurich, IL, USA). The collected blood from every blood donor was subsequently distributed into five syringes each containing finally 10 ml of anticoagulated blood (one syringe for each cat). In addition, blood was collected at the same time for baseline analysis performing complete hematology, clinical chemistry, quantitative real-time TaqMan PCR for FeLV provirus, RT-PCR for plasma viral RNA and p27 ELISA (see below). Subsequently, the donor cats received a substitution of warmed 50 ml Ringer-Lactat solution (Fresenius

Kabi, Stans, Switzerland) to prevent hypovolemia; the cats were monitored until recovery from anesthesia.

Characterization of the transfused blood

The cats in groups A and B (GCN4), received blood from two experimentally FeLV-A/Glasgow-1 infected cats, GBX3 and GCN4, respectively (Table 2). These two cats had been exposed to FeLV at the age of 19-21 weeks, as described previously (Bösch 2012). Briefly, each cat was inoculated intraperitoneally with cell culture supernatant containing 0.8 x 10^6 focus forming units (FFU) of FeLV-A/Glasgow-1 (Jarrett, Laird et al. 1973) in a volume of 1 ml. In week 6 after the first exposure, the cats received a second intraperitoneal virus challenge with 1.7 x 10^6 FFU of FeLV-A/Glasgow-1 in a volume of 1.8 ml cell culture supernatant. The two FeLV-infected cats underwent a regressive infection with undetectable antigenemia. At the time of blood collection for the blood transfusion they were 42 weeks after experimental infection (Bösch 2012). The blood donor for group C was a naïve adult SPF cat (GCN5); (Table 2).

Table 2: Characterization of the transfused blood: quantification of the FeLV loads

Group of recipient cats	Donor cats	FeLV provirus (copies/cell)*	FeLV plasma viral RNA (copies/ml plasma)	p27	Virus isolation
A	GBX3	0.002	negative	negative	negative
B	GCN4	0.031	175,464	negative	negative
C	GCN5	negative	negative	negative	negative

* Calculated using the feline albumin copy numbers determined by real time PCR

Blood transfusion

The kittens were anesthetized with Ketamin/Midazolam (10 mg/kg; 0.1 mg/kg, Vétoquinol AG, Roche Pharma AG) intramuscularly. Prior to the blood transfusion, 10 ml of whole blood was collected from the kittens for baseline analysis and to prevent circulatory volume overload. Thereafter, 10 ml of the blood from the respective blood donor was transfused slowly to each recipient cat (Tables 1 and 2). During and after the blood transfusion the kittens were monitored for heart rate, respiratory rate, temperature, clinical signs of urticaria and vomiting potentially associated with a transfusion reaction or anesthesia incident.

3.3 Clinical examination and sample collection

Throughout the observation period weekly clinical examinations including monitoring of the general condition, the body temperature and palpation of the lymph nodes were performed by veterinarians. In addition, the general condition of the cats was monitored daily by a veterinarian and the animal care takers. EDTA anticoagulated blood samples and oral swabs were collected prior to and at the day of the blood transfusion (week 0) and weekly thereafter for 15 weeks post transfusion. A bone marrow aspirate was collected from each recipient cat as described below in week 17 post transfusion. From the two FeLV infected blood donor cats a bone marrow collection was performed in week 18 post transfusion (week 60 post infection). All recipient and blood donor cats stayed in the facility after the end of this study and were monitored continuously by a veterinarian. Clinical examination, hematology, blood chemistry analyses and p27 ELISA were performed at regular intervals for 65 weeks.

3.4 Hematology

White blood cell differential and complete hemograms were performed using a Sysmex XT-2000iV (Sysmex Corporation, Kobe, Japan) (Riond, Weissenbacher et al. 2011). Diff-Quick-stained (Medion Diagnostics, Dudingen, Switzerland) smears were evaluated by light microscopy for leukocyte differentiation. In a case of severe anemia manual reticulocytes (RET) counts were performed by enumerating the RETs as a percentage of 1,000 mature red blood cells RBCs using a standard method based on Brilliant Cresyl Blue (SIGMA-ALDRICH, Steinheim, Germany) stained blood smears (Cossandi and Maggiora 1952). Only the aggregated RETs, which represent the more immature cells, characterized by large clumps or strands of precipitated nucleoprotein (Perkins, Grindem et al. 1995), were counted. Absolute values of RETs were calculated by multiplying the microscopically determined fraction of RETs by the RBC count from the Sysmex XT-2000iV (Sysmex Corporation).

3.5 Clinical chemistry and serum protein electrophoresis

Clinical chemistry was performed using a Cobas Integra 800 system (Roche Diagnostics, Rotkreuz, Switzerland) at the time point of the blood transfusion (week 0; prior to the transfusion) and in case of the appearance of clinical signs. Serum biochemistry analysis included bilirubin, glucose, blood urea nitrogen, creatinine, protein, albumin, cholesterol, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lipase, sodium, chloride, potassium, phosphorus and calcium.
Serum protein electrophoresis was performed in one cat in week 8 with hyperproteinemia (HCC1, group A) using a semi-automated agarose gel electrophoresis system (Hydragel-Hydrasis 7 Protein kit, Sebia PN 4100, Issy-les-Moulineaux,France) according to the procedure described by (Riond, Wenger-Riggenbach et al. 2009).

3.6 Nucleic acid extraction and quantification

Proviral DNA

Genomic DNA was extracted from 100 µl buffy coat that was obtained from 1 ml of EDTA anticoagulated blood using the QIAamp Blood Kit (Qiagen, Hombrechtikon, Switzerland) following the manufacturer's recommendations. DNA was eluted in 100 µl of elution buffer and the samples were stored at -20°C until further use. Negative extraction controls, consisting of 100 µl of phosphate-buffered saline (PBS) were tested in parallel to monitor for cross-contamination. Total FeLV provirus loads were quantified by TaqMan PCR (U3 region), as described (Tandon, Cattori et al. 2005) using 5 µl of TNA and the following modifications: the TaqMan® Fast Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was used in an ABI 7500 sequence detection system (Applied Biosystems). The thermocycling conditions consisted of an initial denaturation of 20 s at 95 °C, followed by 45 cycles of 95 °C for 3 s and 60 °C for 45 s. Negative and positive PCR controls were included with each run. Provirus loads were normalized to feline albumin copy numbers (Helfer-Hungerbuehler, Widmer et al. 2013).

Plasma viral RNA

Total nucleic acid (TNA) was extracted from 200 µl EDTA plasma using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Diagnostics). TNA was eluted in 90 µl of elution buffer and stored at -20°C until PCR testing was performed. During all extractions, negative controls consisting of 200 µl of PBS were concurrently prepared with each batch of samples. TNA was analyzed by real-time TaqMan RT-PCR, as described previously (Tandon, Cattori et al. 2005), using 5 µl of TNA and the SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (Invitrogen, Basel,

Switzerland) on an ABI 7500 sequence detection system (Applied Biosystems). Negative and positive PCR controls were concurrently included with each run. Plasma viral RNA loads were calculated as copies per milliliter of blood.

Viral RNA in saliva

The oral swabs were processed as described using 200 µl of PBS to extract TNA from the saliva swabs (Gomes-Keller, Tandon et al. 2006), and 5 µl of TNA was analyzed by real-time TaqMan PCR for plasma viral RNA, as previously described (Tandon, Cattori et al. 2005).

3.7 Serological assays

EDTA-anticoagulated plasma samples were analyzed for the presence of p27 antigen and for antibodies to FeLV whole virus as follows: the p27 antigen was determined using a sandwich ELISA, as previously described (Lutz, Pedersen et al. 1983). Results are represented as percentages of a defined positive control (plasma sample from a persistently FeLV-infected cat), which was considered 100%. Samples reaching > 4 % of the positive control signal were considered positive (Hofmann-Lehmann, Huder et al. 2001). Anti-FeLV whole virus antibodies were measured by ELISA using 500 ng of gradient purified antigen per well (FeLV produced in FL-74 cells), as described previously (Lutz, Pedersen et al. 1980). The plasma samples were diluted 1:100 and the goat anti-cat IgG (Jackson, Immunoresearch Laboratories Inc., Baltimore, USA; 0.8 mg/ml) were used in a dilution of 1:3,000.

3.8 Isolation of FeLV using QN10S cells

Heparin plasma samples for virus isolation were collected in week 5 post transfusion. Isolation of FeLV was performed as described (Jarrett and Ganiere 1996) with the following modifications: 4 x 10^4 QN10S cells were seeded per well, using a 12-well cluster plate (TPP; 24-well tissue culture plate, TPP Techno Plastic Products AG, Trasadingen, Switzerland). Cells were cultivated in 1 ml of cell culture medium supplemented with 4 µg/ml Polybrene (SIGMA-ALDRICH, Buchs, Switzerland). The cell culture medium consisted of Dulbecco's MEM containing HEPES buffer, 10% fetal calf serum (FCS; BioConcept, Allschwil, Switzerland), 1 mM sodium pyruvate, 2 mM L-glutamine, 400 U/ml penicillin, and 400 µg/ml streptomycin (Invitrogen, GIBCO Paisley, Scotland, UK). The QN10S cells were incubated with 200 µl of lithium heparin anticoagulated feline plasma. After two hours, the supernatant was replaced with fresh medium. Development of cytopathic effects were monitored daily. At day 7, the p27 antigen was quantified in the cell culture supernatant using a sandwich ELISA (Jarrett and Ganiere 1996).

3.9 Bone marrow cell culture

A bone marrow aspirate was collected of the proximal humerus from each recipient cat at week 17 post transfusion under general anesthesia, as described previously (Museux, Boretti et al. 2009). In addition, a bone marrow aspirate was collected using identical procedures from each of the two FeLV-infected blood donors 18 weeks after the blood for the blood transfusion was collected (week 60 after experimental infection).
The bone marrow aspirate was prepared for cell culture, as described (Madewell and Jarrett 1983), with the following modifications: the bone marrow was collected into 3.5 ml of transport medium: RPMI 1640 cell culture medium (SIGMA-ALDRICH, Buchs,

Switzerland), 200mM L-glutamine, 400 U/ml penicillin, 400 µg/ml streptomycin (Invitrogen) and 10% of FCS (BioConcept). Crandell feline kidney (CrFK) cells (20,000 cells/ml) were cultured in a 24 well-plate (TPP Techno Plastic Products AG). The CrFK cells were tested for the absence of FeLV using PCR prior to the start for the experiment. Negative controls (cells with medium) were concurrently cultured on each plate. At the day of bone marrow collection the CrFK cells were 75% confluent. The bone marrow cells were resuspended in medium and co-cultured with the CrFK cells. The cell culture medium contained: RPMI, Mc Coys 5A (SIGMA-ALDRICH), 200 mM L-glutamine, 400 U/ml penicillin, 400 µg/ml streptomycin (Invitrogen), 10% FCS (BioConcept) and hydrocortisone 1 µg/ml (SIGMA-ALDRICH). The cultures were maintained for 12 weeks and half of the medium was replaced every three days, when supernatant was collected for p27 ELISA.

3.10 Necropsy

Four of the cats (GCN4, HBU1, HCC3 and HBW2) had to be euthanized for humane reasons and underwent necropsy and histopathological examination. Tissues for histology were fixed in 10% buffered formalin and processed by standard procedures. Samples concurrently taken for further analyses were snap-frozen in liquid nitrogen and stored at -80°C until further use.

3.11 Immunohistology

Tissue sections were tested to identify B and T cells using a CD3 T-cell marker (M7254, DAKO) and the B-cell marker for CD79 (M7051, DAKO; RB-90-13-P, Labvision, Thermo Fisher Scientific, Fremont, USA).

3.12 Statistics

Statistical analyses were performed using GraphPad Prism for Windows, Version 4.03 (GraphPad software, San Diego, CA). Changes over time (same group, two different time points) were tested using the Wilcoxon signed rank test for paired samples (pw). Frequencies were compared using the Fisher's exact test (pF). Differences between group A and B were compared using the Mann-Whitney U-test (pM). A p-value < 0.05 was considered to be statistically significant. Reference ranges for hematological and clinical chemistry parameters, as well as serum protein electrophoresis, were determined using identical methods as described above and blood samples from 58 clinically healthy cats. The reference ranges are given as 5% and 95% quantiles.

4. Results

4.1 Verification of SPF status and blood transfusion

The SPF status was confirmed prior to the start of the experiment; all cats were found to be negative for all tested infections. All cats included in the study were of blood type A and the recipient cats were identified to be compatible with the donors in the cross match. During and after the blood transfusion no adverse reactions in all blood donor and recipient cats were observed.

4.2 FeLV provirus, plasma viral RNA and p27 antigen detection

<u>FeLV provirus</u>

All cats in groups A and B became FeLV provirus positive; all cats in the negative control group C remained negative (Figures 1 and 3). The cats in group B turned significantly earlier FeLV provirus positive than the cats in group A: in week one post transfusion all cats in group B were already provirus positive, while none of the cats in

group A was positive (pF = 0.0079; Figure 1). In group A, four cats (HBZ3, HCC1, HCC4, HCD1) became FeLV provirus positive two weeks post transfusion (Figure 1). One cat (HBW2) in group A turned provirus positive for FeLV in week three post transfusion (Figure 1). The proviral loads in groups A and B showed significant differences in weeks 1, 2, 7 to 12 and week 15 post transfusion. In the first two weeks post transfusion the proviral loads were significantly higher in group B than in group A (Figure 3b, pM = 0.0079, 0.0317), in accordance that the cats in group B turned earlier positive than the cats in group A. However, in weeks 7 to 12 (pM = 0.0317, 0.0159, 0.0317, 0.0159, 0.0317, 0.0079) and week 15 (pM = 0.0317) the proviral loads became significantly lower in the cats in group B compared to those in group A (Figure 3a).

Plasma viral RNA

All cats in groups A and B turned also plasma viral RNA positive; in the control group C, none of the cats was plasma viral RNA positive at any time point (Figure 1 and 4). In three cats in group A (HCC1, HCC4, HCD1) plasma viral RNA could be detected in the first week post transfusion. The cat HBZ3 turned plasma viral RNA positive in week 2 and in cat HBW2 plasma viral RNA was detectable in week 4 post transfusion (Figure 1). All cats in group B became plasma viral RNA positive in the first week post transfusion (Figure 2). Three cats in group B had plasma viral loads below the detection limit by week 15 post transfusion, while all cats in group A stayed plasma viral RNA load positive (Figure 1). The plasma viral RNA loads were significantly higher in cats in group B compared to those in group A at week 1 post transfusion (pM = 0.0079, Figure 4b). However, subsequently, the cats in group B had lower plasma viral RNA loads than the cats in group A (significantly lower in weeks 4 and 7 to 11 ($pM \leq 0.0317$), (Figure 4a).

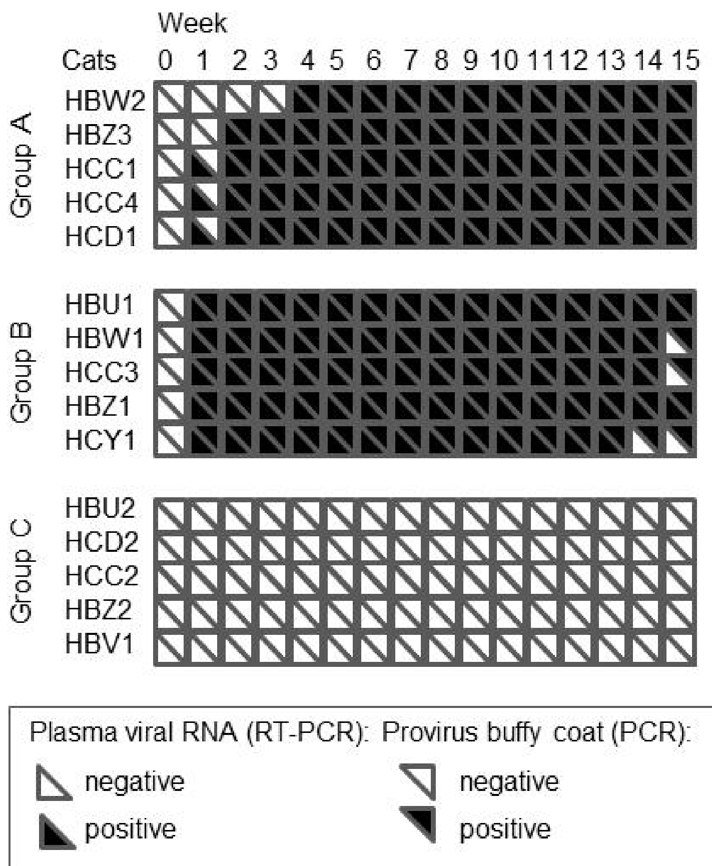


Figure 1: Outcome of FeLV infection in the recipient cats post transfusion. Plasma viral RNA and provirus results from blood in the five cats in group A (a), the five cats in group B (b) and the five cats in the negative control group C (c). In Group B provirus was detectable significantly earlier (at week 1) than in group A (pF = 0.0079).

P27 antigen

In group A, all cats were p27-positive at some time points post transfusion (Figure 2). Two of five cats in group A became persistently infected and showed remarkably high p27 values (up to 88%); the three other cats in this group developed a transient viremia with lower p27 loads reaching up to 10% (Figure 5a). Three of five cats in group B showed a transient viremia with maximal p27 values of 7.9% (Figure 2 and 5b); while two cats in group B remained negative for p27 antigen (< 4%) during the 15 weeks post

transfusion (Figure 1 and 5b). Moreover, all cats in the negative control group C stayed p27 antigen negative during the observation period of 15 weeks (Figure 2 and 5c). There was no significant difference concerning the time point, when cats in groups A and B became p27 positive. However, 4 weeks post transfusion, significantly more cats were p27-positive in group A compared to group B (pF = 0.0476; Figure 2). Thereafter, no significant differences were found anymore concerning presence or absence of antigenemia between groups A and B. When analyzing the p27 loads, significant differences were found in weeks 8 and 9: cats in group A had significantly higher p27 loads at these time points than cats in group B (pM = 0.0317).

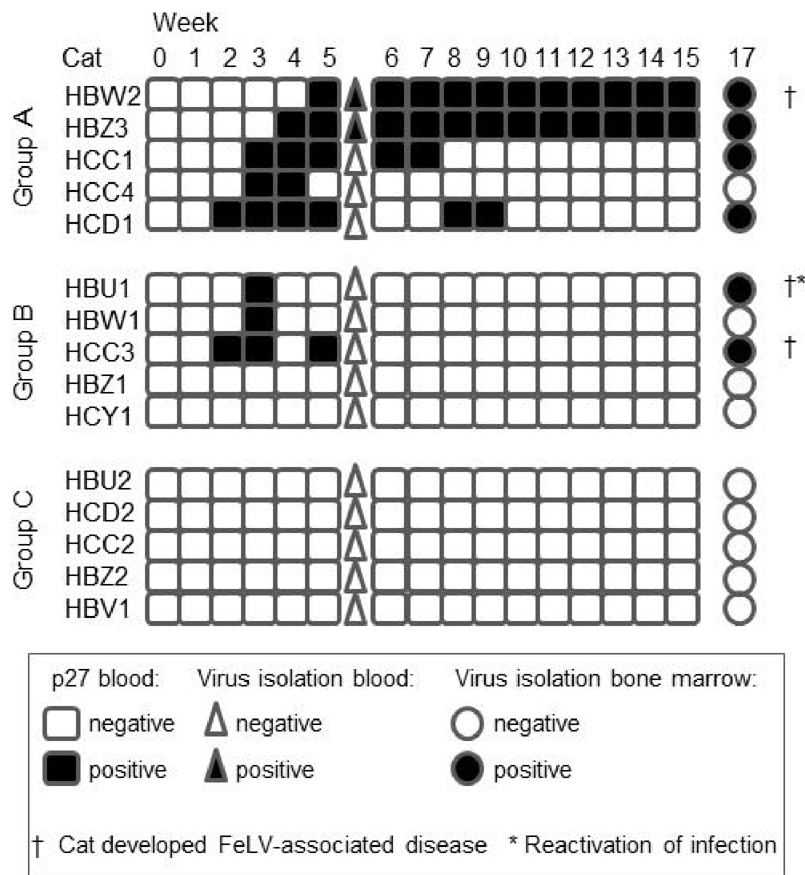


Figure 2: Outcome of FeLV infection in the recipient cats post transfusion. P27 results from the blood, virus isolation from the blood and virus isolation from the bone marrow of the five cats in group A (a), the five cats in group B (b) and the five cats in the

negative control group C (c). Four weeks post transfusion, significantly more cats were p27-positive in group A compared to group B (pF = 0.0476).

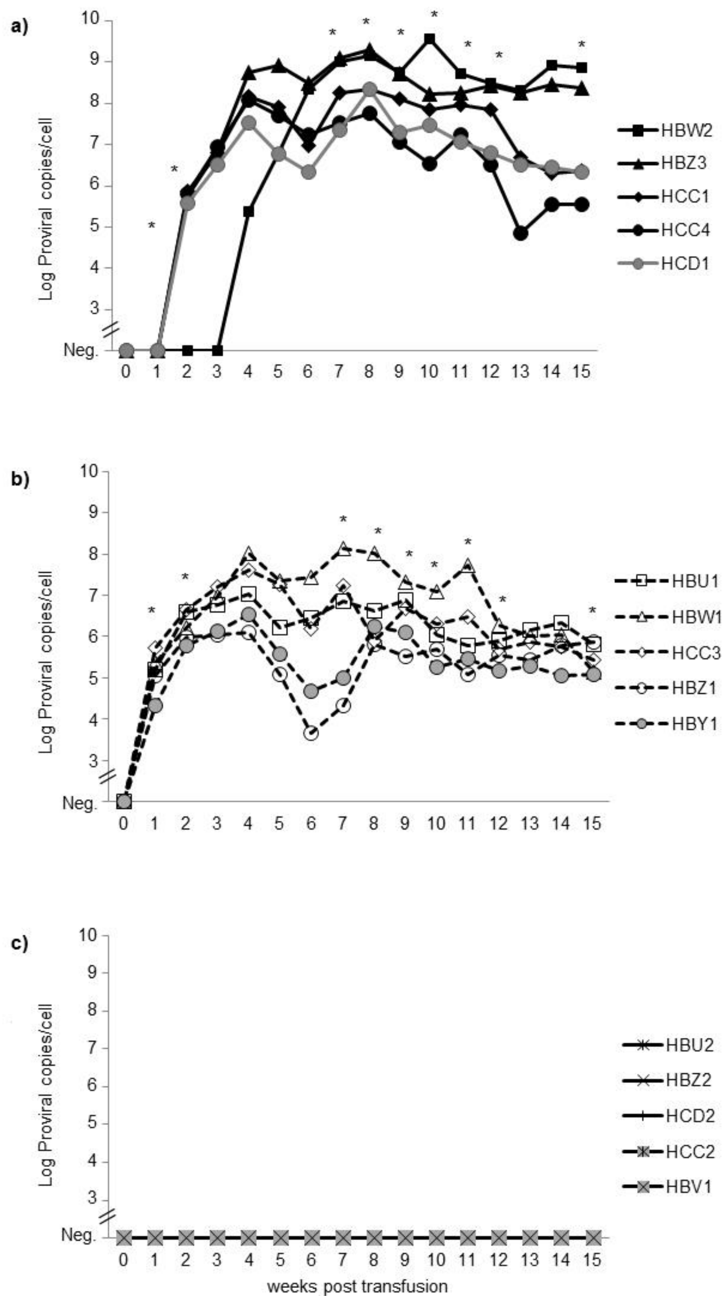


Figure 3: Time course of FeLV infection in the recipient cats. Proviral load of the five cats in group A (a), the five cats in group B (b) and the five cats of the negative control group C (c). The proviral loads are given as proviral copies/cell. The cats in

group B had significantly higher proviral loads in weeks 1 and 2, but lower loads in weeks 4, 7 to 12 and 15 (pM < 0.05; indicated with an asterisk).

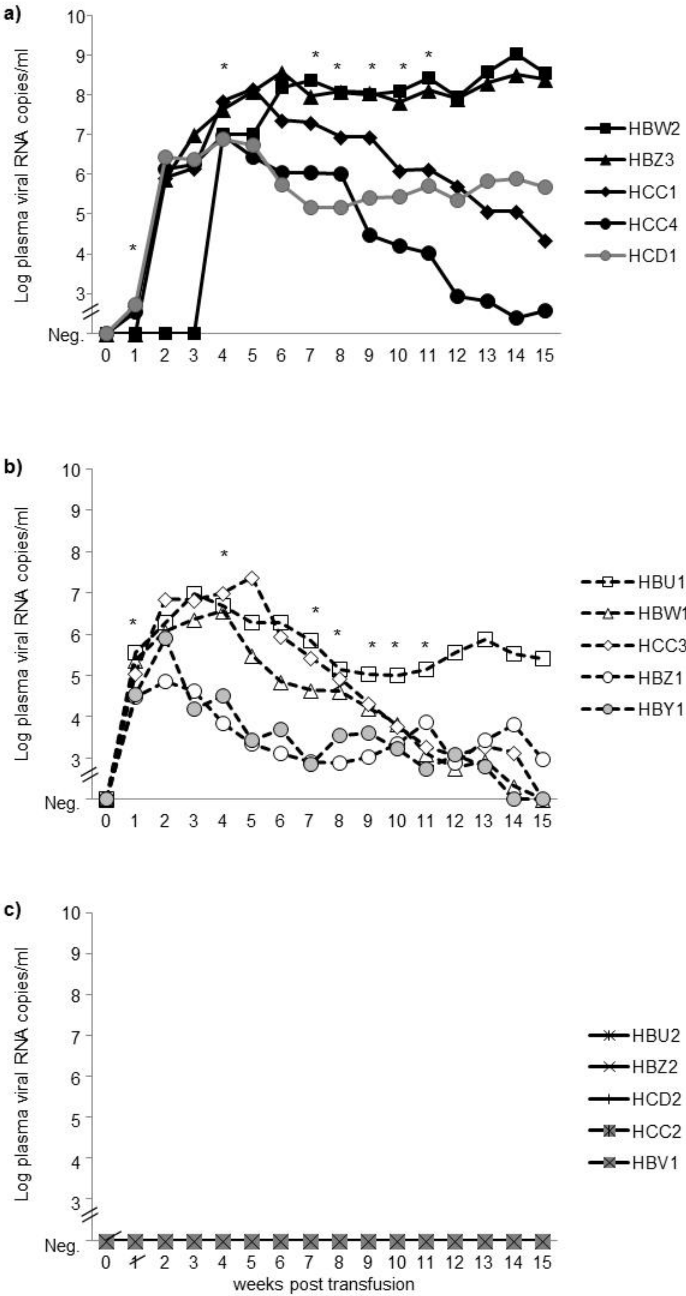


Figure 4: Time course of FeLV infection in the recipient cats. Plasma viral RNA load of the five cats in group A (a), the five cats in group B (b) and the five cats in the negativecontrol group C (c). The plasma viral RNA loads are given as copies/ml. The

cats in group B had significantly higher viral RNA loads in week 1, but lower loads in weeks 7 to 11 (pM < 0.05; indicated with an asterisk).

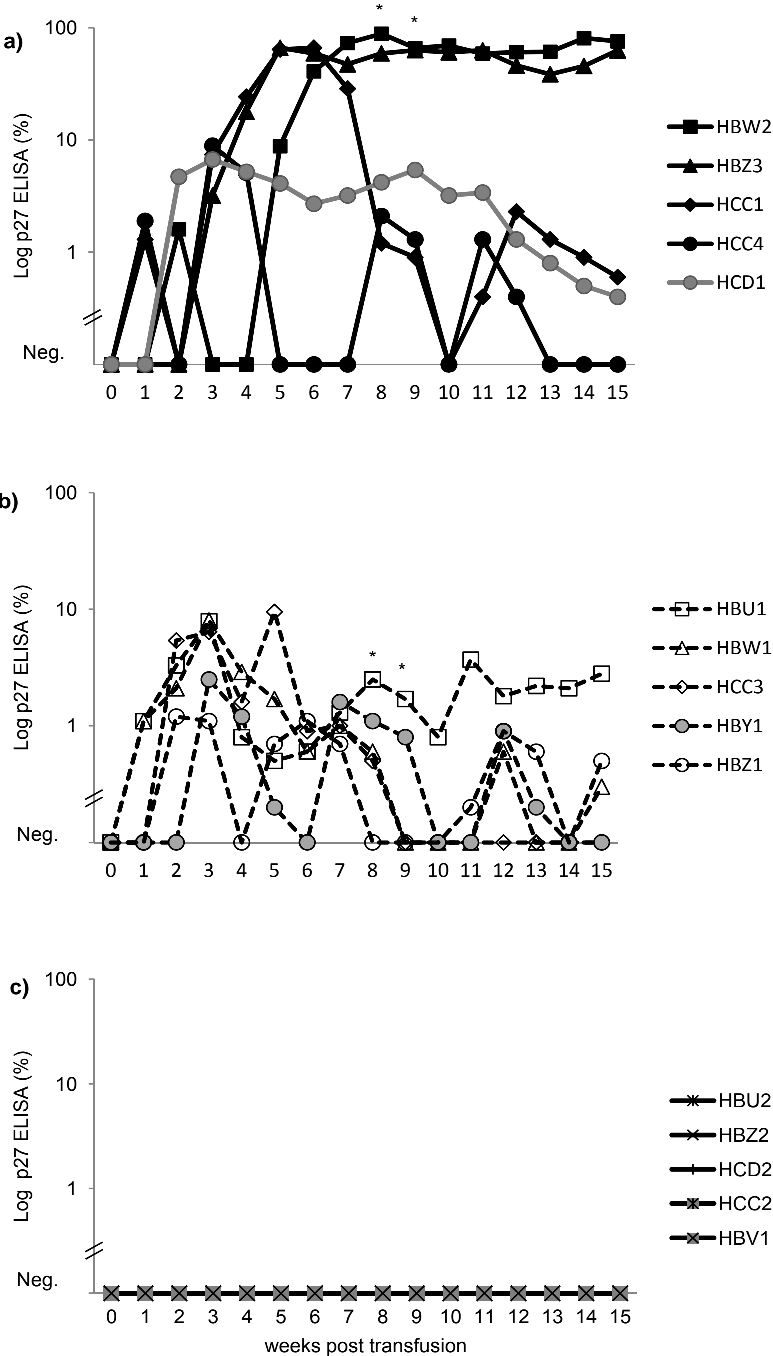


Figure 5: Time course of FeLV infection in the recipient cats. P27 antigen load of the five cats in group A (a), the five cats in group B (b) and the five cats in the negative control group C (c). The p27 loads are given as percentage of a positive control run on

each plate. Significantly higher p27 loads were found in cats in group A compared to cats in group B at weeks 8 and 9 (pM = 0.0317; indicated by an asterisk).

4.3 Clinical and laboratory findings

In the clinical examination, two cats in group A (HBZ3, HCC1) and one cat in group B (HBU1) showed elevated body temperatures at some time points during the experiment. Cat HBZ3 had a body temperature of 39.8°C in week 6 and cat HCC1 had a temperature of 40.1 °C in week 8. In these two cats, anorexia and mild lethargy were observed during fever depression. The two cats, HCC1 and HBZ3 were treated once daily peroral with meloxicam a nonsteroidal drug (Metacam® 0.5 mg/ml cats ad us. vet., orale suspension, Boehringer Ingelheim GmbH, Basel, Switzerland) and a total amount of 100 ml of Ringer-Lactat (Fresenius Kabi) was infused intravenously in a constant rate of 4 ml/kg/h until full recovery. After two days, the cats recovered well and the treatment could be stopped. In group B, one cat, HBU1, showed an increased body temperature of 39.6 °C in week 3, but did not show any further sings.

In total, five cats from groups A and four cats from group B developed a general lymphadenomegaly of the mandibular, cervicalis superficialis and popliteal lymph node. In contrast, in the negative control group C all five cats showed normal lymph node size throughout the observation period (Table 3).

In hematology, three cats in group A showed values outside the reference range. The PCV of cat HBZ3 in group A decreased from 28% at week 0 (reference range for kittens at the age of 8-20 weeks 33%) to 23% at week 6 and increased again to 33% (within normal range) at week 15. After week 3 this cat also demonstrated intermittent neutropenia of as low as 0.2 x 10^3/µl (reference range 2.32 x 10^3 -10.01 x 10^3) until week 13, when neutrophils returned to normal. In week 4, the PCV of cat HBW2

decreased from 31% to 27% and persisted around 29% until week 15. Moreover, cat HCC1 had PCV values below the reference range with 24% in weeks 7 and 9; the PCV returned to within the reference range at week 11. In addition, in group B a trend (pw = 0.062) could be observed in that the lymphocytes of all these cats decreased from week 0 to 1 post transfusion; however, all values remained in the lower region of the reference range (Figure 6).

Furthermore, during weeks 8 and 9 post transfusion, hyperproteinemia (99 g/l; reference range 64-80 g/l) and hypoalbuminemia (21 g/l; reference range 30-40 g/l) were noticed in the clinical chemistry of cat HCC1 in group A. Serum protein electrophoresis analysis revealed hyperbetaglobulinemia and polyclonal hypergammaglobulinemia (Figure 7).

Table 3: Lymph node evaluation of the 15 recipient cats throughout the observation period.

Group/ Cats		Lymph node size(-/++++)*															
	week	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	HBW2	-	-	-	-	-	-	++	++	+++	++	++	++	++	++	+	+
	HBZ3	-	-	-	-	-	+	+	+++	++	++	+	+	+	+	+	-
	HCC1	-	-	-	-	-	++	+++	++++	++++	+++	+++	++	++	++	+	+
	HCC4	-	-	-	-	-	++	++	+	-	-	-	-	-	-	-	-
	HCD1	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
B	HBU1	-	-	-	-	-	+++	+	+	+	+	+	-	-	-	-	-
	HBW1	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	HCC3	-	-	-	-	-	++	+++	++	+	+	+	+	-	-	-	-
	HBZ1	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	HBY1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	HBU2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	HCC2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	HCD2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	HBZ2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	HBV1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Grade assessment: normal lymph node size = -, 1 cm diameter = +, 2 cm diameter = ++, 3 cm diameter = +++, ≥ 4 cm diameter = ++++. The diameter specifications are depicted from the mean value of the mandibular, cervicalis superficialis and popliteal lymph nodes.

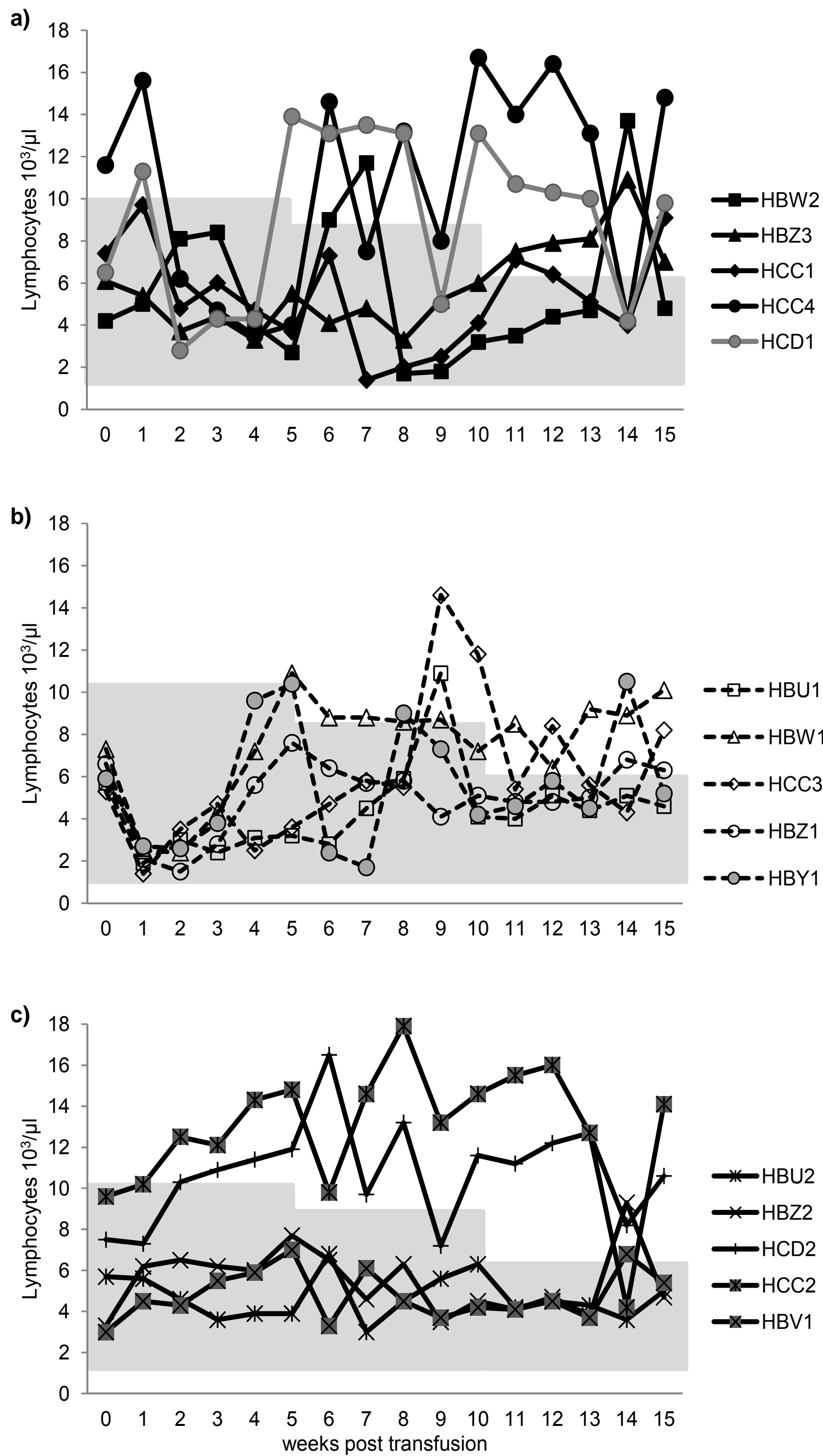
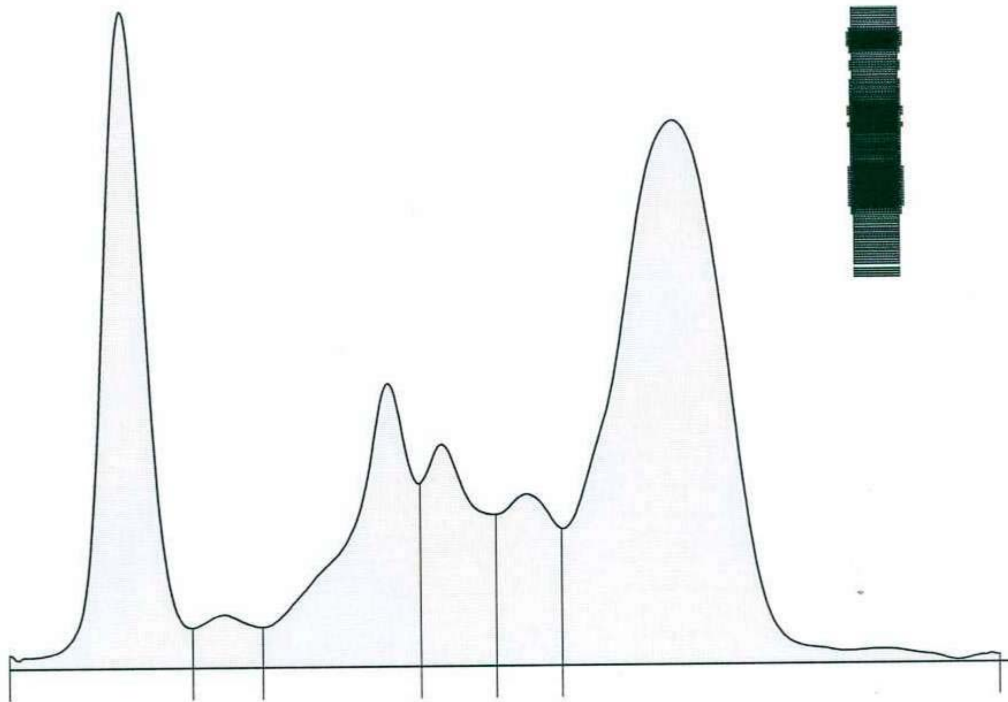


Figure 6: Lymphocyte counts during the experiment. Five cats in group A (a), the five cats in group B (b) and the five cats in the negative control group C (c). Reference range in kittens is marked with a gray zone.



Serum protein electrophoresis

Fractions	%	Ref. %	Conc.	Ref. Conc.
Albumin	20.5		19.7	
Alpha 1	2.1		2.0	
Alpha 2	14.6		14.0	
Beta 1	9.0		8.6	
Beta 2	6.8		6.5	
Gamma	47.0		45.1	

A/G **0.26** T. P.: **96** g/l

Figure 7: Serum protein electrophoresis from cat HCC1.

4.4 Virus isolation from blood and bone marrow

Two persistently infected cats (HBW2, HBZ3) were found to be positive in the virus isolation from blood. All the other cats including the two FeLV infected blood donors remained negative in the virus isolation from blood on the selected time point.

Latent virus infection in the bone marrow defined as replicating virus upon prolonged *in vitro* culture in the presence of hydrocortisone was observed in six recipient cats (HBW2, HBZ3, HCC1, HCD1 in group A and HBU1, HCC3 in group B). All five cats in the negative control group C were confirmed to be negative in the virus isolation from blood and bone marrow (Table 4). In addition, the blood donor for group B was found positive for latent infection, 18 weeks after the blood for the blood transfusion had been collected and 60 weeks after FeLV infection).

4.5 Outcome of the infection

Recipient cats: The cat HBU1 in group B developed after transient viremia a reactivation of the FeLV infection and had to be euthanized due to severe dyspnea in week 26 post transfusion (Table 4). A lymphoblastic leukemia (49 x 10^3 cells/µl with 75% lymphoblasts; Figure 8)) and a p27 load of 113% were detected in blood analysis. In the histopathological examination a $CD3^+$ T-cell lymphoma of the precordial mediastinum, the mesenteric lymph nodes, the liver and the bone marrow was found in this cat (Figure 9). In the immunohistology of the bone marrow 25% of lymphoblast cells ($CD3^+$) were found.

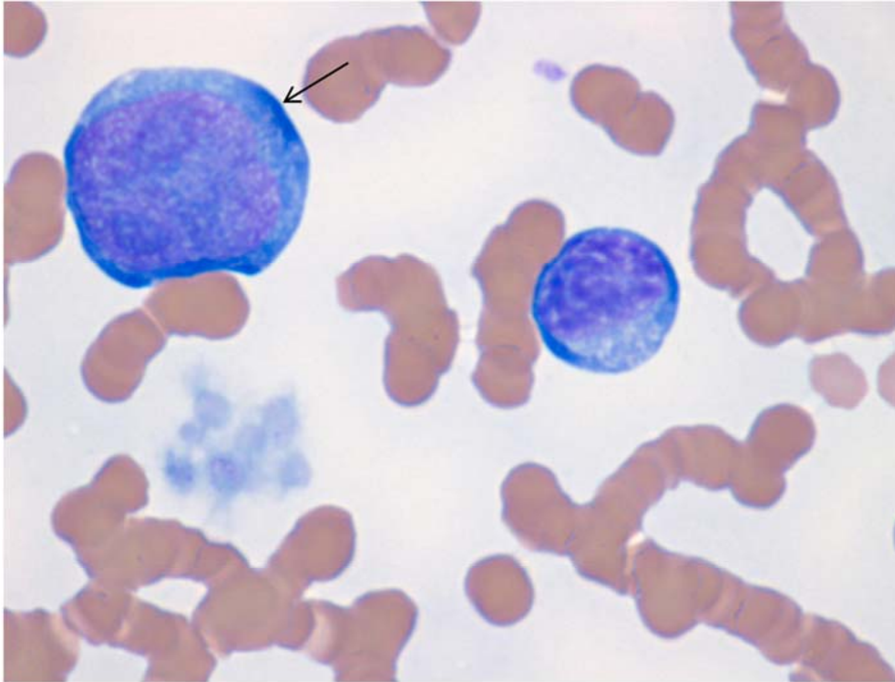
Furthermore, cat HCC3 in group B had to be euthanized at week 28 post transfusion with pleural effusion due to a $CD3^+$ T-cell lymphoma of the precordial mediastinum (Table 4). Additionally, also in the kidney and the lumbar lymph node a $CD3^+$ T-cell lymphoma was found during the histopathologic examination. In this case no FeLV

reactivation was observed – the cat had remained p27 negative - and all blood parameters including complete hematology and clinical chemistry were within the reference range.

In week 31, one of the two persistently infected cats in group A, HBW2, had to be euthanized with severe non-regenerative anemia with a PCV of 7% and reticulocytes < 0.1 (reference range 0-1.0). At the time of euthanasia the p27 antigen load was 114%.

Blood donors: One of the two transiently FeLV-infected blood donor cats (GCN4, blood donor for group B) reactivated the infection and succumbed to FeLV associated disease at the time point of the bone marrow collection in week 18 post transfusion (60 weeks p.i.; Table 4). Clinically, the cat showed severe dyspnea and was found to have pleural effusion. Upon necropsy and immunhistological examination a $CD3^+$ T-cell lymphoma of the mediastinum was found. In the hematology no abnormalities were noted but a very high p27 load in the plasma of 132% of the positive control was measured. The second FeLV-infected blood donor cat (GBX3, blood donor for group A was still healthy and remained provirus positive and p27-negative 65 weeks after blood transfusion (107 week post infection).

a)



b)

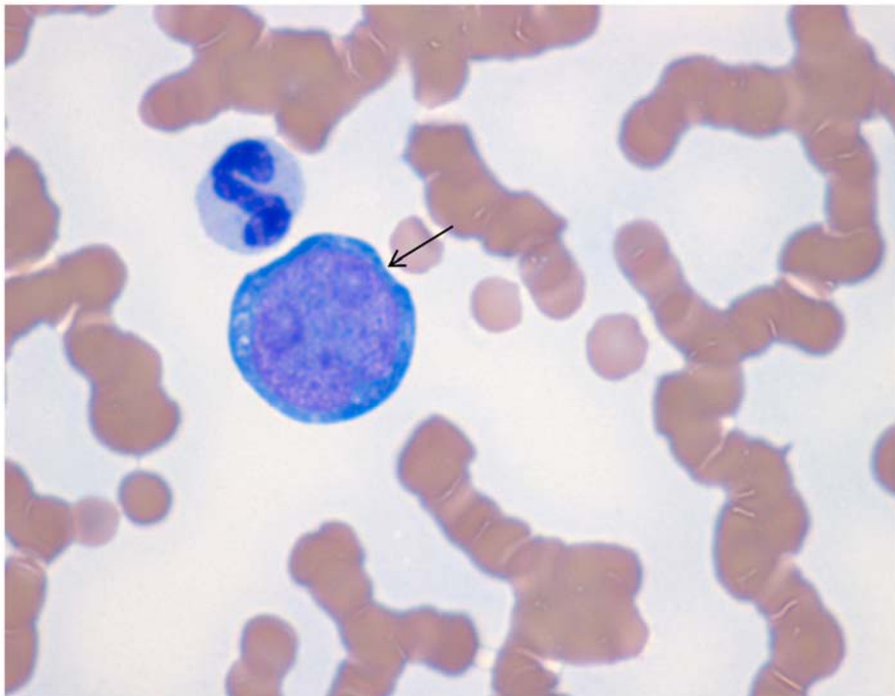


Figure 8: Cat HBU1 with a lymphoblastic leukemia. Lymphoblast cells are marked with an arrow. a) The picture displays a large lymphoblast with moderate amounts of basophilic cytoplasm and a large, round nucleus with fine chromatin pattern and several large, indistinct nucleoli visible. There is also a medium-sized lymphocyte with moderate

amounts of pale basophilic cytoplasm and round nucleus with coarse chromatin pattern. b) The picture shows a medium-sized to large lymphoblast with small amounts of basophilic cytoplasm and a large, round nucleus with fine chromatin pattern and two prominent round nucleoli.

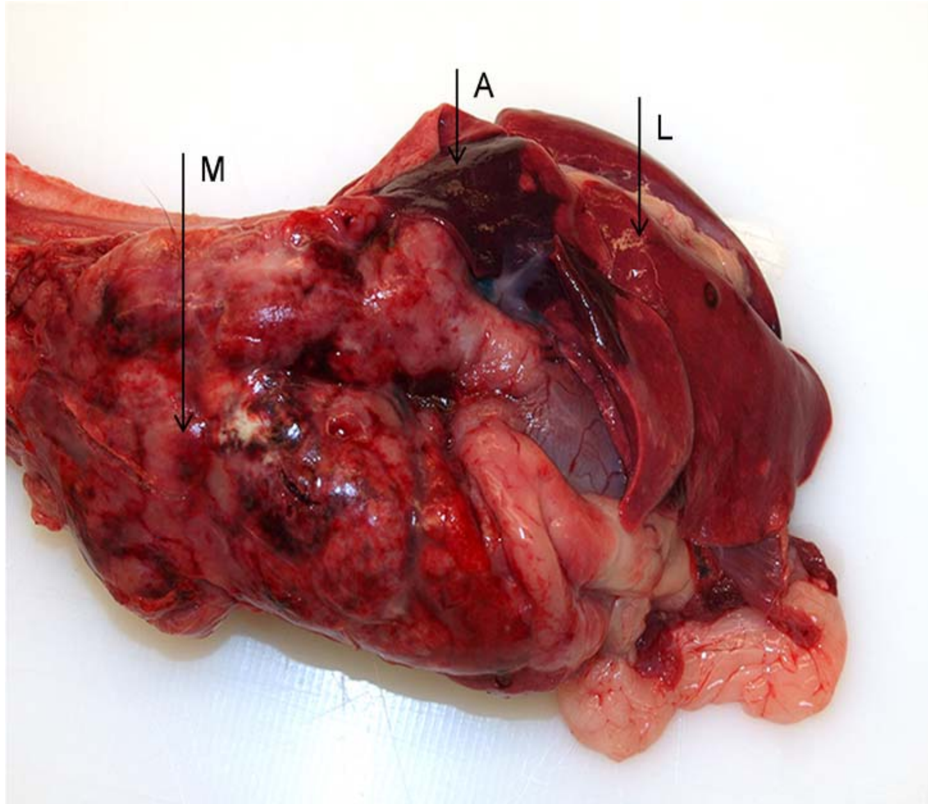


Figure 9: Cat HBU1 precordial T-cell-Lymphoma: A firm, slightly nodular, whitish mass (M) with multiple confluent hemorrhages, measuring approximately 12 x 5 cm replaces the precordial mediastinum and obscures physiological structures (thymus, sternal lymph nodes). The lung (L) is collapsed and shows partial atelectasis (A) due to profound pleural effusion.

Table 4: Outcome of the infection with results from virus isolation from blood and bone marrow cell culture and clinical outcome of the cats

			VI B	VI BM	Outcome of infection*
		Week	**5**	**17**	
Blood Donors **Group**	**A**	GBX3	-	-	provirus- positive, healthy
	B	GCN4	-	+	† week 60 reactivation: thymic T-cell lymphoma
Recipients	**A**	HBW2	+	+	† week 31: non-regenerative anemia
		HBZ3	+	+	viremic, healthy
		HCC1	-	+	provirus-positive, healthy
		HCC4	-	-	provirus-positive, healthy
		HCD1	-	+	provirus-positive, healthy
	B	HBU1	-	+	† week 26 reactivation: multicentric T-cell lymphoma
		HBW1	-	-	provirus-positive, healthy
		HBZ1	-	-	provirus-positive, healthy
		HCC3	-	+	† week 28 no reactivation: thymic T-cell lymphoma
		HBY1	-	-	provirus-positive, healthy
	C	HBU2	-	-	uninfected control, healthy
		HCD2	-	-	uninfected control, healthy
		HCC2	-	-	uninfected control, healthy
		HBZ2	-	-	uninfected control, healthy
		HBV1	-	-	uninfected control, healthy

VI B = Virus isolation from blood
VI BM = Virus isolation from bone marrow
* Infection outcome of the cats in the study was determined until week 65 post transfusion

4.6 Viral shedding and immune response

Viral RNA loads in saliva could be detected by real-time RT-PCR in all cats in groups A and B (Table 5). Particularly high saliva viral RNA loads were found in the two p27 positive cats, HBW2 and HBZ3. All infected cats with the exception of the two viremic

cats (HBW2, HBZ3) developed antibodies to FeLV whole virus (Table 5). In Group C all cats remained negative for FeLV.

Table 5: Viral RNA loads in saliva detected by real-time RT-PCR and antibodies to FeLV whole virus measured by FL-74 ELISA

		viral RNA loads in saliva*				Antibodies to FeLV	Outcome
Group	**Week**	**0**	**3**	**5**	**7**	**15**	
A	HBW2	0	0	321	48,632	-	viremic
	HBZ3	0	2	0	76,074	-	viremic
	HCC1	0	4	2	1,143	+	provirus- positive
	HCC4	0	0	15	150	+	provirus- positive
	HCD1	0	143	858	0	+	provirus- positive
B	HBU1	0	17	23	0	+	provirus- positive
	HBW1	0	8	16	0	+	provirus- positive
	HCC3	0	2	12	226	+	provirus- positive
	HBZ1	0	0	4,714	17	+	provirus- positive
	HBY1	0	3	0	2	+	provirus- positive
C	HBU2	0	0	0	0	-	negative
	HCD2	0	0	0	0	-	negative
	HBZ2	0	0	0	0	-	negative
	HCC2	0	0	0	0	-	negative
	HBV1	0	0	0	0	-	negative

* Copy numbers are depicted per PCR reaction

5. Discussion

Our results demonstrated for the first time that FeLV infection can be transmitted via blood transfusion from FeLV provirus positive, antigen negative cats to naïve recipients. As little as approximately 20,000 proviral copies in 10 ml of blood led to productive FeLV infection in the recipient cats. Our results extend earlier results, where proviral FeLV DNA in the form of plasmid DNA injected subcutaneously led to infection in recipient cats (Chen, Bechtel et al. 1998). Productive infection after intramuscular and intradermal injection of a plasmid carrying proviral DNA had also been reported for a feline lentivirus, the feline immunodeficiency virus (FIV) (Rigby, Hosie et al. 1997; Sparger, Louie et al. 1997), as well as for macaques inoculated intramuscularly with simian immunodeficiency virus (SIV) plasmid DNA (Liska, Khimani et al. 1999). Our study has direct biological relevance and clinical importance: from our results we concluded that there is an imminent risk of FeLV transmission via blood transfusion of provirus-positive blood, which needs to be considered in clinic.

In the cats that received blood carrying proviral DNA and viral RNA (group B), provirus was detected in the first week post transfusion and one to three weeks prior cats that received only blood with proviral DNA (group A). In addition, cats in group B had higher plasma viral RNA loads in week 1 and higher proviral loads in weeks 1 and 2 after the transfusion compared to cats in group B. However, by week 4 after transfusion the picture had changed and more cats were p27 positive in group A compared to group B and viral RNA loads were higher in group A compared to group B. Subsequently, cats in group A had higher viral RNA, proviral and p27 loads compared to cats in group B at several time points particularly after week 7 post transfusion. Overall, in group A all cats became FeLV antigen positive and two cats developed persistent infection, while in group B only three cats became transiently antigenemic. A possible explanation for the

less grave infection outcome in group B could be a more efficient detection of FeLV by the immune system of cats in group B due to the presence of viral RNA and higher proviral DNA loads in the transfused blood compared to group A. In contrast, in group A the provirus in the donor cells may have been hidden from the immune system of the recipient and no viral RNA was present that could have been easily detected early on. Thus, the proviral DNA could have undetected infected on this way the host and integrate to its genome. However, of note, the infection outcomes, i.e. the number of persistently infected cats and of cats developing FeLV-associated disease, were not significantly different between the two groups, potentially due to small numbers.

Symptoms and changes in laboratory parameters compatible with a natural FeLV infection were observed in the recipient cats, such as elevated body temperature, lymphadenomegaly, anorexia, lethargy and abnormal hematology parameters (anemia, neutropenia). The lymph node size was related to the grade of FeLV infection in that lymphadenomegaly was more distinct and longer present in cats in group A than in group B, and it was especially pronounced in the two viremic cats in group A. Three cats in group A showed a mild decrease of the PCV that persisted for several weeks. Two of these anemic cats were viremic and one cat showed transiently high p27 antigen levels, though this cat resolved antigenemia thereafter. Neutropenia ($0.2 \times 10^3/\mu$) occurred in only one of the viremic cats. In the cats in group B, the lymphocyte counts decreased in the first week post transfusion, when all cats of this group turned plasma viral RNA and provirus positive. Thus, the decrease of the lymphocytes seemed associated with the time point of viral infection in the kittens. Clinical disease of FeLV infection is characterized by leucopenia, lymphopenia and variable degrees of anemia (Hoover and Mullins 1991). A decrease of white blood cells and red blood cells was described for viremic cats. However, hematologic abnormalities are mild, absent and

mostly transiently in cats that do not have viremia but are only provirus positive. The decrease of the red and white blood cells appears due to a suppression of the myeloid and erythroid stem cells in the bone marrow (Pedersen, Theilen et al. 1977).

Replicating virus in the blood could be detected in the two antigenemic cats five weeks post transfusion confirming viremia in these cats. In addition 17 weeks after transfusion, replicating virus could be isolated from the bone marrow from six recipient cats by cell culture assay using hydrocortisone. This included the two viremic cats in group A, but also four non-antigenemic cats, where the virus isolation from bone marrow was indicative for the presence of latent infection. The positive results from cell culture assays further confirmed the replication capacity of FeLV in these six cats after infection via blood transfusion of proviral DNA. In addition, all eight cats with regressive infection developed antibodies to FeLV whole virus; this further supports that in all these cats minimal viral replication took place after transfusion. Subsequently, three out of ten recipient cats succumbed to FeLV associated diseases within 31 weeks post transfusion. The cats suffered from anemia and lymphoma, which are commonly regarded as FeLV associated diseases (Hoover and Mullins 1991). Thus, the infection outcome and disease spectrum in the recipient cats mirrors that of a natural FeLV infection.

Two of the provirus-positive cats, one blood donor and one recipient cat, showed reactivation of FeLV infection by recurrence of antigenemia. This further emphasizes the importance of provirus-positive cats. Provirus-positive cats should be recognized by PCR because, subsequent to reactivation, they may pose an exposure risk for unprotected cats within the population. In the recipient cat, plasma viral RNA could be detected at each time point investigated prior to reactivation. This would further support our hypothesis that viral RNA in the blood may be associated with subsequent

reactivation of the infection (Hofmann-Lehmann, Cattori et al. 2007). For the blood donor cat no samples were available for RT-PCR. The reactivation and subsequent development of lymphoma in the blood donor cat raised the question whether collection of the large volume of blood increased the risk of reactivation of FeLV infection in the blood donor.

In the saliva of all recipient cats viral RNA was detected post transfusion to varying degrees. High loads of saliva viral RNA were mainly noted in the viremic cats but FeLV RNA was also observed in cats with regressive infection. This confirms our earlier observation (Cattori, Tandon et al. 2009) that during early infection up to 15 weeks after FeLV exposure, saliva viral RNA does not seem to be a marker for antigenemia/viremia as had been demonstrated for long-term infected cats (Gomes-Keller, Tandon et al. 2006).

In small animal clinics, transfusion of blood products is a frequent necessity and has become more sophisticated with increased access to blood components, knowledge of blood types, cross-matching requirements, the purchase of blood bank products and external donor programs (Lanevschi and Wardrop 2001). Although blood transfusions are potentially life-saving procedures they carry some risks, such as transfusion reactions and especially the potential of transmitting infections (Chiaramonte 2004). Transmission of retroviral infections through blood products are already known in humans since the first described cases of human deficiency virus 1 (HIV-1) following blood transfusion in hemophilic patients in 1982 (CDC 1982). After numerous HIV-1 transmissions via blood transfusion, the screening of human blood donors was conducted by immunoassays to detect viral antibodies or antigens (Stramer, Glynn et al. 2004). In the late 1980’s, alarming reports of immunosilent HIV (Farzadegan, Polis et al. 1988; Imagawa, Lee et al. 1989) showing that up to 10% of infected HIV-1 persons may

be PCR positive and antibody-negative (immunosilent) carriers were disproved; they were found to be due to PCR contamination (Imagawa and Detels 1991; Roy, Damato et al. 1993). Detection of early HIV-1 infection during the window period defined as the time period between infectivity and the detectability of infection by the immunoassays, was performed by p24 antigen screening and recommended by the Food and Drug Administration (FDA) in the United States in 1996 (Fiebig, Wright et al. 2003; Stramer, Glynn et al. 2004). Later on, the screening of blood donors for HIV-1 tested by nucleic acid testing was implemented in a number of developed countries to improve blood safety based on the data that HIV-1 RNA screening is more sensitive and able to detect infection earlier in the window period than p24 antigen testing (Busch, Kleinman et al. 2000; Fiebig, Wright et al. 2003; Stramer, Glynn et al. 2004). However, anti-HIV-1 and anti-HIV-2 immunoassay or HIV combination antigen-antibody immunoassays are recommended to minimize the risk of HIV infection through the route of blood transfusion by the World Health Organization (WHO). In a previous study 1 per 3.1 million donors screened was confirmed to be positive for HIV-1 RNA, therefore blood donation testing by nucleic acid testing was useful to prevent iatrogenic transmission of HIV-1 (Stramer, Glynn et al. 2004). In lentiviral infection it is unknown whether proviral DNA is detectable in the absence of viral RNA. However, for FeLV the incidence of proviral DNA without the detection of viral RNA is well described (Hofmann-Lehmann, Huder et al. 2001; Torres, Mathiason et al. 2005; Hofmann-Lehmann, Tandon et al. 2006). A significant number up to 10% of provirus positive cats has been described in the cat population of Switzerland (Hofmann-Lehmann, Huder et al. 2001).

Young cats were chosen for this experiment because they are highly susceptible to FeLV infection compared to adult cats (Hoover, Olsen et al. 1976) and they therefore rather represent the situation of blood transfusion recipients than healthy adult cats

would. We concluded that blood transfusions of an antigen negative but provirus positive blood donor may exhibit a potential risk of transmitting FeLV to adult cats. Although, older cats become less susceptible to FeLV, at high challenge doses they can still be infected. In previous studies efficient natural and experimental infection of adult cats was demonstrated (Grant, Essex et al. 1980; Lehmann, Franchini et al. 1991). Moreover, recipients of blood transfusions may be particularly prone to infections due to preexisting severe clinical conditions and possible immunosuppression, therefore the risk of infection may also be high in adult cats. By performing the commonly used blood donor screening assay (detection of p27 antigen) both FeLV infected blood donors would not have been detected as a potential risk. We highly recommend to screen all blood donors, in addition to p27 ELISA, by FeLV provirus-PCR. The use of this screening method will prevent inadvertent transmission of FeLV and should be performed prior to each blood transfusion. With the results from this study and the growing importance of feline blood banks in the future, blood safety for feline patients need to be improved (Wardrop, Reine et al. 2005). Therefore, general recommendations for the feline blood donor screening need to be adjusted.

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