



Experimental quantification of the feline leukaemia virus in the cat flea (*Ctenocephalides felis*) and its faeces

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Abstract

Cat fleas (*Ctenocephalides felis*) were fed via artificial membranes and infected with the feline leukaemia virus (FeLV) from cell cultures. After removing the fleas from the blood source, the quantity of virus in the flea and its faeces was measured over a defined period of time. The virus was detectable in the fleas for up to 30 h at room temperature and up to 115 h at 4°C. In the faeces, the amount of virus decreased much more slowly – after 2 weeks half of the initial amount of virus could still be detected. Thus the faeces might be a source of further infections, e.g. for the flea larvae or the cat itself.

Introduction

Feline leukaemia virus (FeLV) belongs to the class of retroviruses and spreads through infected saliva, blood, urine, tears, faeces and also from an infected queen to her kitten during gestation and by nursing. Transmission usually requires intimate contact between cats (Hardy 1975; Essex 1977). The various ways of transmission give rise to the question: is there another possible way of transmission of FeLV, maybe through blood sucking ectoparasites such as fleas, without the need for intimate contact of cats? Fleas are generally known to transmit various pathogens, including bacteria, tapeworms and also viruses (Shepherd and Edmonds 1977; Hinaidy 1991; Mehlhorn 2001). In this study, the vector potential of the cat flea (*Ctenocephalides felis*) for the FeLV was investigated as well as the stability and traceability of FeLV inside the flea and its faeces.

Our early studies showed that the cat flea may transmit the FeLV from one blood sample to another under in vitro conditions (Vobis et al. 2003a, 2003b). Figure 1 shows the previous experimental set-up.

An infection with FeLV can lead to a persistent or transient infection of cats (Hoover et al. 1975; Lutz et al. 1980; Hoover and Mullins 1991). Whereas clinical signs are various and range from fever, diarrhoea and weight loss to cancer, most infected cats die within 4 years after infection (Lutz 1990).

Materials and methods

Fleas were artificially reared and fed via membranes in the “artificial dog” (FleaData, Freeville, USA). The air temperature inside the artificial dog was adjusted to 38°C, leading to a blood temperature of 37°C.

To ensure detection and quantification of viruses with at least intact nucleocapsids, rather than RNA of destroyed viruses, all samples were treated with RNase cocktail (Ambion) before viral RNA preparation. This step eliminates all free RNA in a sample, enabling the measurement of only the RNA protected in the nucleocapsid. To eliminate proviral DNA contamination, all viral RNA preparations were treated with DNase I on-column with the RNase-free DNase kit according to the protocol of the manufacturer (Qiagen). Viral RNA was isolated from blood samples and cell culture supernatant using the QiAamp viral RNA mini kit according to the protocol of the manufacturer (Qiagen).

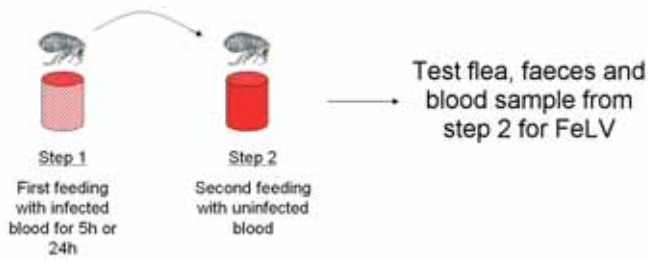


Fig. 1. Transmission experiment. Fleas were fed with feline leukaemia virus (*FeLV*)-infected blood for 5 or 24 h, respectively. Fleas were then transferred to a *FeLV*-free blood source. After 12 h, blood from step 2, fleas and their faeces were tested positive for *FeLV*

Cell culture supernatant was centrifuged at 500 g for 10 min prior to preparation. Preparation of viral RNA from fleas was performed by freezing the fleas in liquid nitrogen and pulverizing them in a steel mortar.

The powder was then mixed with PBS, followed by RNase treatment, and viral RNA preparation was performed according to the protocol of the manufacturer.

Preparation of viral RNA from faeces was performed by mixing 20 mg faeces with PBS and by continued preparation of RNA according to the protocol of the manufacturer. Fleas and blood samples were infected with viruses harvested from cell culture supernatant of *FeLV*-3281 cells producing *FeLV* subtype A. Reverse transcription of viral RNA followed by real-time PCR was performed as a single tube reaction with the QuantiTect SYBR green RT-PCR kit (Qiagen) on a ABI SDS7700 real-time cycler system. The target sequence of the primer pair was a 74-bp conserved fragment of exogenous *FeLV*-A, as previously described (Hofmann-Lehmann et al. 2001).

Results

Figure 2 shows the detection limit of the *FeLV* in cell culture media (used as the reference). A cell culture supernatant of *FeLV*-3281 cells was divided into 17 aliquots. One aliquot was treated with RNase and subsequently frozen at -80°C (initial titre of 100% equivalent to 10^7 virions), and the others were kept at the

defined temperatures. At each point in time, sequence aliquots were taken, treated with RNase and frozen at -80°C until finally analysed.

Figure 3 shows the detection limit of the *FeLV* after being ingested by the cat flea. One hundred and twenty fleas were fed for 24 h with *FeLV*-infected blood and subsequently removed from the blood source. Ten fleas were directly pulverized and viral RNA was isolated (initial amount of 100% equivalent to 2.2×10^6 virions). The other fleas were kept in two populations at 4°C and 22°C , respectively. At defined times, ten fleas per temperature were taken and frozen at -80°C until finally analysed. It was verified that the fleas were still alive when the sample was drawn.

Figure 4 shows the time-based detection limit of the *FeLV* in flea faeces. Approximately four hundred fleas were fed for 24 h with *FeLV*-infected blood. Faeces were removed and 20 mg faeces were directly resolved in PBS and viral RNA was isolated (initial amount of 100% equivalent to 1.4×10^7 virions). The rest of the faeces was kept in two samples at 4°C and 22°C , respectively. At defined times, 20 mg faeces per temperature was taken and frozen at -80°C until finally analysed.

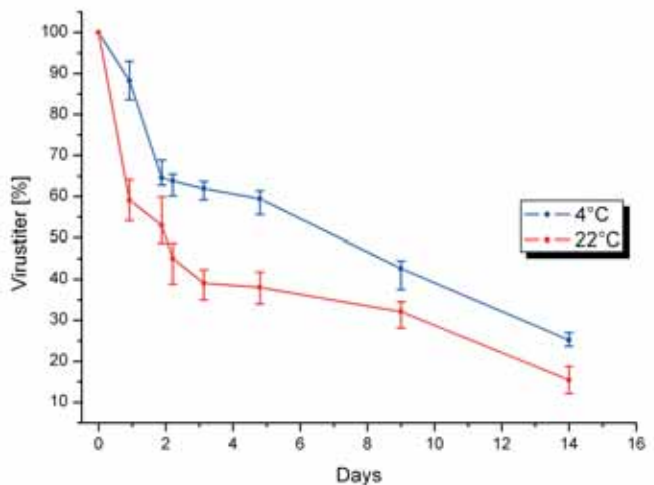


Fig. 2. Time-based detected amounts of the *FeLV* in cell culture media. A value of 100% is equivalent to 10^7 virions. Values are mean values of three independent measurements. Bars indicate highest and lowest amounts measured



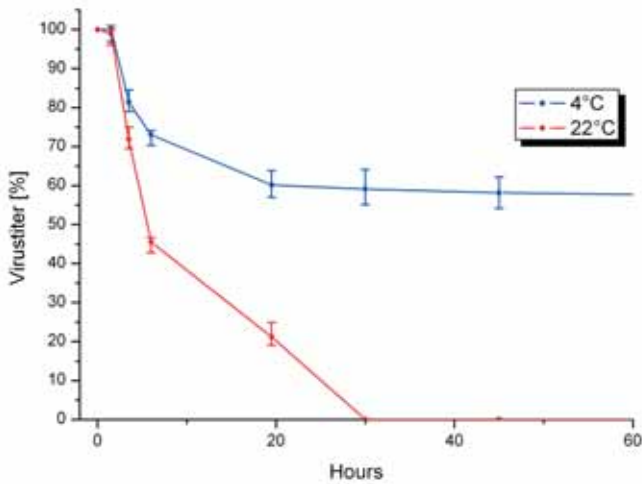


Fig. 3. Time-based detected amounts of the FeLV in the cat flea after being removed from the blood source. After 115 h, 56% of the initial amount of FeLV was still detectable in the fleas when kept at 4 °C (data not shown). A value of 100% is equivalent to 2.2×10^6 virions. Values are mean values of three independent measurements. *Bars* indicate highest and lowest amounts measured

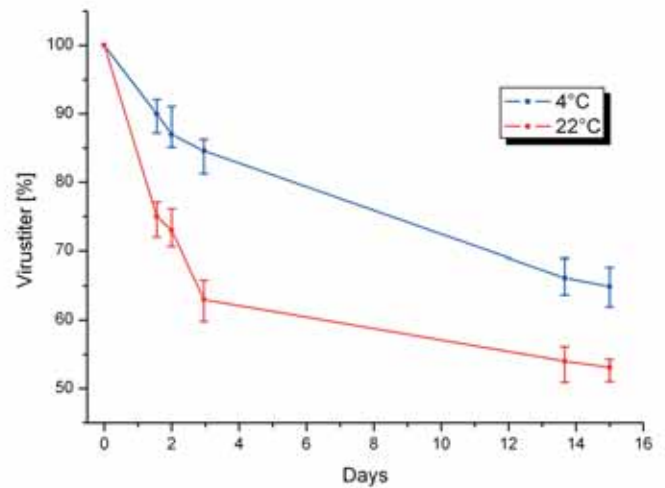


Fig. 4. Time-based detected amounts of the FeLV in cat flea faeces. A value of 100% is equivalent to 1.4×10^7 virions. Values are mean values of three independent measurements. *Bars* indicate highest and lowest amounts measured

To test the ratio of the amount of ingested FeLV and the amount excreted, 150 fleas were fed for 24 h with infected blood. After removal from the blood source, 50 fleas were pulverized and viral RNA was isolated. Initially 100% of the virus is in the fleas and 0% excreted in the faeces. The remaining fleas were kept in petri dishes in two populations of 50 individuals at 22°C. After 20 h, all fleas of population 1 were pulverized and the viral RNA was isolated.

In addition, all faeces were collected and also analysed. The same procedure was performed for population 2 after 30 h. The results are presented in Fig. 5.

Discussion

FeLV is known to be spread from cat to cat via saliva (by licking), blood (scratching or fighting) and all other body fluids (Hardy et al. 1975; Hoover and Mullins 1991). Close and intimate contact between healthy and infected cats is usually required to disperse the virus within a population or household (Essex et al. 1977). The results of our early work showed that there may be alternative means of trans-

mission, rather than close cat-to-cat contact: independent transmission of the FeLV by the cat flea *C. felis* (Vobis et al. 2003a; Vobis et al. 2003b). This is now supported by quantitative data.

Fleas as blood-sucking parasites are important vectors for various pathogens including bacteria, viruses and

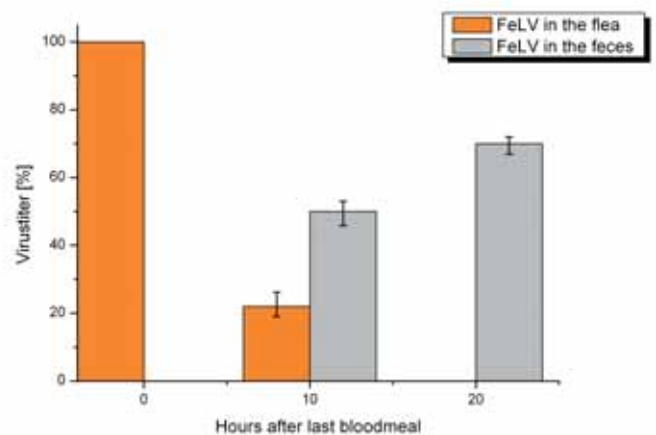


Fig. 5. Time-based detected amounts of the FeLV in 50 cat fleas after removal from the blood source. A value of 100% is equivalent to 1.2×10^7 virions. Values are mean values of three independent measurements. *Bars* indicate highest and lowest amounts measured



even tapeworms (Shepherd and Edmonds 1977; Hinaidy 1991; Mehlhorn 2001). Due to the wide host range of some fleas, pathogens can even be spread across host-range borders, e.g. from rat to man (e.g. *Yersinia pestis* the causative pathogen of the plague). However, the participation of the cat flea in the transmission of viruses is widely unknown. It has been reported that some viruses can persist intact in the flea for at least 24 h (Smetana 1965). The possible transmission by the flea of the FeLV, or the HIV-related feline immunodeficiency virus, is unknown.

Recent studies showed that the cat flea can transmit the FeLV from an infected blood sample to an uninfected one in vitro (Vobis et al. 2003a, 2003b). Although the transmitted amounts seemed to be rather low and were detected in the second round of a nested PCR reaction, they were present nonetheless.

Beside this direct transmission by the flea through the sucking channel or FeLV-contaminated mouthparts, we can now clearly show other possible ways of transmission. If fleas are fed with FeLV-infected blood, the virus is excreted in large amounts with the flea faeces. As Fig. 5 shows, approximately 70% of the ingested virus can withstand passage through the intestine with at least an intact nucleocapsid, and is excreted and spread in the flea faeces. In comparison to the degradation rate of the FeLV in cell culture supernatant (Fig. 2), the nucleocapsid seems to be even more stable in the faeces (Fig. 4). Once the faeces were excreted, the detectable viral amount decreased slowly; 55% of the initial amount could still be detected after 15 days at room temperature. Lower temperatures led to slower degradation rates.

Generally, adult cat fleas of a population produce faeces in high amounts to provide nourishment for the flea larvae. If the high amounts of virus in the faeces are ingested by a larva, it may also become infected.

Our experiments also showed that the FeLV can be detected for a defined time within the flea. Fleas were fed with FeLV-infected blood and subsequently removed from any nutrition source. The virus was detectable for up to 29 h after the last blood meal, when the fleas were kept at room temperature. After 30 h, 70% of the ingested virus was released within the faeces, leading to a loss of only 30% of the initially

ingested virus (see Fig. 5). At a temperature of 4°C the amount of virus exceeded the limit of detection well beyond 115 h. If the metabolism of the fleas is heavily reduced, the result is a persistently infected flea. It may be presumed that excretion is the main limiting factor for the time of detection of the FeLV in the flea at room temperature. Probably, the virus could persist for longer in the fleas at room temperature, if it was not excreted.

Our recent results indicate that the risk of infection of FeLV for a cat may be increasing. It is obvious that the high amounts of the FeLV excreted within the faeces of infected fleas might carry a potential risk of infection. The observed amounts of the FeLV in the flea are also a possible risk factor for cats. Cats are commonly infected with FeLV orally. Swallowing or breaking a flea in two with the teeth leads to direct contact with flea-ingested blood, which may also be considered as source of an infection. Until now, no studies have been undertaken to investigate the possible infection risks for a cat swallowing a FeLV-infected flea.

However, we are aware that retroviruses are unstable in the environment and are very sensitive to heat or drying. Irrespective of whether excreted or ingested, FeLV is still infectious even if only for a short period of time, and this remains a subject for further studies. It is known that 10% of the FeLV is still infectious after 48 h when kept at 22°C in cell culture media, and after 2 h in saliva if it is allowed to dry (Francis et al. 1979).

Even if this time-frame is quite limited, it still offers an opportunity for the FeLV to infect a cat that is in contact with infected fleas or their faeces.

Summarizing, our studies show that the cat flea is a notable vector of viruses. The FeLV may be ingested by the flea and excreted with the faeces, but may also be directly transmitted during blood-sucking. If this route of FeLV infection is more likely in vivo and has any clinical significance, remains, however, unanswered up until now, and there is a need for further experiments which examine re-infection.

While the risk of pathogen transmission via flea bites to both pets and humans is known, our study indicates that fleas must be seriously considered as vectors of





important viruses. This is of even greater importance when considering the transmission of much more stable viruses like caliciviruses or parvoviruses. Effective flea control in dogs and cats also minimizes the risk of the transmission of agents of disease. ●

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