Feline Leukemia Virus: Survival Under Home and Laboratory Conditions

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Feline leukemia virus maintained its infectiousness in cell culture medium at 37°C or lower for at least 48 h. However, virus in saliva or medium was inactivated within a few hours if allowed to dry.

Feline leukemia virus (FeLV) infection of cats is a classic example of a horizontally transmitted, oncogenic virus of outbred mammals. Horizontal transmission of FeLV has been well documented by serological comparisons of exposed and unexposed cat populations (2, 8–10), by investigations of clusters of cases (1, 8), and by prospectively following infected cat populations (1, 2, 9, 10, 15).

In a previous paper, we reported that saliva appeared to be the primary source of FeLV responsible for the community spread of this naturally occurring oncogenic virus. The implications of that finding were: (i) cat-to-cat transmission is primarily via saliva, and (ii) healthy pet cats that excrete FeLV expose humans to high concentrations of a potentially oncogenic agent (6).

To our knowledge, the heat stability of this virus in saliva and tissue culture media has not been determined. In this paper, we extend our previous findings and report the stability of this virus once released into the environment. These studies included salivary FeLV survival on a dry surface similar to surfaces found in homes and FeLV survival in laboratory culture medium to simulate conditions in research laboratories.

Saliva was collected from a cat whose FeLV titer had remained consistently between 10^3 and 10^4 focus-forming units (FFU) per ml for almost a year. After injecting a narcotizing dose of ketamine hydrochloride, we put one drop of atropine on the cat’s tongue and collected drooled saliva in an iced petri dish. Samples of saliva were made and kept frozen at −90°C until tested.

For survival of FeLV in culture medium, we used standardized supernatant virus collected from a feline lymphoma cell line (F422) that produced FeLV-A in titers of 10^2 to 10^5 FFU/ml (14).

To test for FeLV survival in saliva, we smeared 0.1 ml of saliva over a 20-mm diameter cover slip and then allowed it to stand at room temperature (24°C and 24% relative humidity) inside a laminar flow biohazard hood. At predetermined times, including zero time, cover slips were removed and placed in centrifuge tubes containing 1.2 ml of culture medium (McCoy 5A with 15% fetal bovine serum, penicillin 100 IU/ml, streptomycin 100 μg/ml, and amphotericin, 0.25 μg/ml). After being mixed, the specimens were kept frozen at −90°C until tested.

To test for virus survival in cell culture medium, we made dilutions of our stock cell-free F422 supernatant in closed plastic tubes (NUNC®, Vanguard International, Red Bank, N.J.), and these tubes were subjected to various temperatures (4, 24, 37, and 56°C). At zero time and subsequent determined times, the tubes were removed and frozen at −90°C until tested.

Titers of infectious FeLV were determined by a modified method of Fischinger et al. (5, 6). Briefly, triplicate samples were diluted appropriately in cell culture medium (as described above) and placed over day-old, diethylaminoethyl dextran-treated CCC81 monolayers in six-well cluster plates (Falcon Plastics, Oxnard, Calif.). Foci were read directly at day 13, and titers were reported in FFU.

Titers of infectious salivary FeLV dropped rapidly in saliva which was allowed to dry on glass cover slips at room temperature. Saliva originally containing 10^3 to 10^4 FFU per ml dropped to 10^2 to 10^3 FFU before zero time specimens could be taken and, within 3 h, contained fewer than 10 FFU/ml (Fig. 1). Identical loss of infectious virus was observed for FeLV from a nonsaliva source of virus (F422 supernatant) when it was allowed to dry (data not shown). Levels of infectious FeLV in media dropped to fewer than 10 infectious units within 60 min after the drying occurred.

In contrast to the rapid decay of infectiousness observed for dry virus, cell-free FeLV in cell culture medium appeared quite stable. Except for an initial decline in titer before the collection of the first temperature-exposed sample, there...
was little loss of infectious virus at 4°C up to 48 h. There was slightly more loss at 22 and at 37°C, but survival was still prolonged. At 56°C, however, there was a rapid loss of infectiousness—dropping below detectable levels within 3 to 4 h (Fig. 2).

Transmission of FeLV from an excretor cat to other cats probably occurs through close direct contact between cats presumably during face-to-face contact. Two lines of evidence suggest that minimal transmission of FeLV occurs via aerosols. First, FeLV excretor cats are asymptomatic. Unless they are infected with a secondary respiratory agent, they do not manifest the coughing and sneezing necessary to produce aerosols. Second, there is evidence that uninfected cats caged apart in the same room with FeLV excretor cats do not become infected (9). Conversely, when cats are allowed to interact freely while residing in the same room essentially all develop serological evidence of infection within 5 months (2, 9, 15).

In this report, we document the potential for transfer of infectious FeLV from the saliva of an excretor cat to a recipient via environmental surfaces. Although the infectiousness of salivary FeLV declines relatively rapidly on dry surfaces, the 30- to 60-min interval during which significant amounts of FeLV can still be detected may be adequate to transfer the agent. This might occur, for example, when several cats are eating from the same dish. The habitual licking characteristic of cats probably represents the primary transmission mechanism, but the relative stability of FeLV suggests that transmission could also occur through the sharing of common food and water containers. In addition to FeLV transmission to other cats, owners of infected pet cats may be exposed to high levels of FeLV depending on their handling characteristics and social interactions with cats.

In the laboratory, the potential for human infection seems to be considerable. Virus survival results presented here for FeLV are similar to those reported for murine retroviruses (12) and could be typical for retroviruses as a whole. Unlike the oncogenic murine retroviruses, however, the FeLVs grow readily in cultured human cells (4). The stability of FeLV in laboratory media at room temperature and at 37°C makes it necessary to protect humans from direct contact with virus-containing fluids in the laboratory. As stipulated by National Institutes of Health guidelines (1), laboratory workers must be protected by the use of biohazard hoods from inhaling aerosols of culture fluids, and all discarded labware should be soaked in virocidal solutions and autoclaved before being removed from the laboratory.

The need for these precautions is predicated on the theoretical risk posed by FeLV for humans. The extent, if any, of that risk has yet to be established. Although one recent report identified antibody to FeLV reverse transcriptase on the surface of cells from chronic myelogenous leukemia patients (16), more extensive epidemiological studies are necessary before accurate recommendations based on potential risk of FeLV for humans can be made (3). Two other reports failed to find free serum antibodies to different FeLV proteins in numerous human samples (7, 11). All that we say at this point is that FeLV is present and potentially hazardous

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**Fig. 1. FeLV survival in saliva allowed to dry on glass cover slips at room temperature (24°C). Each dot represents the mean of triplicate portions from a single sample.**

**Fig. 2. FeLV survival in cell culture medium at 4, 22, 37, and 56°C. Each dot represents the mean of three aliquots of the same sample.**
to humans who either work in FeLV research laboratories or own FeLV-excreting pet cats.

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LITERATURE CITED