

Saliva as a Source of Feline Leukemia Virus Antigen for Diagnosis of Disease

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An enzyme-linked immunosorbent assay (ELISA) for detection of feline leukemia virus (FeLV) p27 in saliva was tested for its accuracy and sensitivity in diagnosing FeLV infections. Saliva and serum samples from 564 clinical cases were tested with a 99.2% specificity. The overall accuracy of the saliva ELISA reactive to the serum ELISA was 97.9%. Experimentally, the ELISA saliva was the least sensitive in diagnosing early FeLV infections. However, the overall accuracy, ease of use, and simplicity of the test support its use as a screening procedure in clinical practice.

Feline leukemia is a horizontally transmitted disease of domestic cats caused by feline leukemia virus (FeLV) (3). Transfer of saliva is the most common means of transmission, with saliva containing between 10^2 to 10^5 infectious particles per ml (1). Clinically, veterinarians need to determine the FeLV status of cats as a diagnosis for numerous disease states associated with FeLV infection (13) and to separate virus-shedding cats from uninfected ones. In addition, development of a successful vaccine for FeLV (7, 14) has created a need for screening for FeLV-infected cats before vaccination. Although good diagnostic tests exist for determining FeLV infections (2, 5, 8), these tests require blood to be drawn and may involve an extended period of time before a diagnosis can be made. The existence of a sensitive enzyme-linked immunosorbent assay (ELISA) to test for FeLV p27 in fluids (4, 8) and the presence of FeLV in the saliva of infected cats suggests the possibility of developing a procedure for testing for FeLV infection with saliva as an antigen source. In this report, we present data from a study of a new FeLV testing system using ELISA technology and saliva as the antigen source.

To demonstrate the effectiveness of the saliva ELISA in diagnosing FeLV disease, we compared the two currently used FeLV testing methods and a newly developed saliva ELISA in a controlled environment and in a clinical situation. Direct comparisons of the three assay methods in an experimental environment used four experimentally infected specific-pathogen-free cats (Table 1). The cats were 8 weeks old at the time they were challenged with an intravenous injection of 0.1 ml of a 20% (wt/vol) FeLV-Rickard tumor homogenate (11). The cats were monitored daily for FeLV infection with three different FeLV testing methods: (i) indirect immunofluorescence assay (IFA) (2, 5), (ii) FeLV antigen presence in serum by ELISA (8), and (iii) FeLV antigen presence in saliva by saliva ELISA. Direct comparisons of the three assay systems showed that the serum ELISA was the first to detect FeLV presence. This has been previously shown by Lutz et al. (8, 9), but these authors suggest that this early detection may be misleading in that detection may be before bone marrow infection and possible virus recovery. Rojko et al. (12) have shown that bone

marrow infection is essential for development of persistent FeLV infection (12). In experimental cats, detection of FeLV by serum ELISA was as much as 8 days before a positive IFA. Upon testing positive by either serum ELISA or IFA, the cats remained positive throughout the experiment. This contrasted with the saliva ELISA, which fluctuated between positive and negative in all four cats until day 23 postchallenge. All tests after day 23 were found to be positive until the conclusion of the experiment at 6 weeks postchallenge. These results corroborate previous results of Lutz et al. (8) showing the serum ELISA to be more sensitive in detecting FeLV, although Jarrett et al. (6) found the serum ELISA to be no more sensitive than IFA. Our results show the saliva test to be less sensitive at detecting early infections than either of the other two tests, but by 4 weeks postchallenge the test gave consistent results that compared with either the IFA or serum ELISA. FeLV, when observed clinically, is usually not diagnosed until after physical signs of the disease are present. In most cases these patients are assumed to have been infected for an extended period of time. These experimental results suggest that, by this time, any of these tests would be adequate for diagnosis.

To test this hypothesis, a study was undertaken to determine the accuracy of the saliva test in clinical situations. A total of 564 cats were tested for FeLV disease with the saliva and serum ELISAs. A comparison of the two ELISAs in cats suspected of FeLV infections (Table 2) showed that the saliva ELISA was effective in diagnosing uninfected animals with a specificity of 99.2% (483 of 487 serum ELISA-negative cases). Four cats tested negative by serum ELISA but positive by saliva. Of these four cats, three were retested at a later date. Two retested negative by both serum and saliva, and one retested positive by both tests. These false-positive results may have resulted from a problem in performing the saliva test procedure or contamination with FeLV p27 from other samples, or FeLV antigens may have been present in the cats' saliva without being present in the serum. Eight cats tested positive by serum but negative by saliva, giving a correlation of 89.6% (69 of 77 serum-positive cats). The eight saliva-negative cats may have been tested during an early stage of the infection process after entrance into oral lymphatic tissue but before salivary gland involvement. Similar results have been reported by Lutz et al. (9)

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TABLE 1. Direct comparison of the three immunoassays

Animal no.	Assay	Test result at postchallenge day:																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	42
3477	Saliva ELISA ^a	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+
	IFA ^b	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Serum ELISA ^c	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3480	Saliva ELISA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	+	+	+	+	+
	IFA	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Serum ELISA	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3481	Saliva ELISA	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-	-	-	+	+	+	+	+
	IFA	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Serum ELISA	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3482	Saliva ELISA	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	-	+	+	+	+	+	+
	IFA	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Serum ELISA	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

^a The test kit (provided by Synbiotics Corp., San Diego, Calif.) consisted of plastic sticks with bulbous ends which were coated with monoclonal antibodies to FeLV p27. The bulbous ends were rinsed with sterile water and inserted along the gums of the cats to cover them with saliva. Each bulbous end was then immersed in 0.5 ml of horseradish peroxidase-labeled p27 antibody, which binds to a different site of p27. After 10 min, the stick was removed and rinsed with sterile water. The stick was then immersed in a chromogenic substrate and incubated for 10 min. The appearance of blue color indicated a positive test.

^b IFA used the procedure of Hardy et al. (2) as modified by Hoover et al. (5).

^c Serum ELISA was performed by the ViraChek ELISA kit (Synbiotics). Serum samples were prepared from each bleed and frozen until use.

and were also observed in all of the experimental cats and in two retested clinical cases. Alternatively, these animals may have been transiently infected before salivary gland involvement, or they may have been in a recovery phase with some FeLV antigen in the serum but not in the saliva. Clinically, these cats would not be considered virus shedders and could potentially eliminate the infection.

To compare the saliva ELISA and IFA, 26 cats that were serum ELISA positive were tested with the IFA and saliva ELISA (Table 3). Of 19 cats that tested similarly for both tests, 15 were positive and 4 were negative. Seven cats tested negative by IFA but positive by saliva and serum ELISA. Although this may appear contradictory, two possible explanations can justify these results. (i) Some FeLV infections are known to cause dramatic anemia in the host, which in turn can cause a false-negative IFA because of the limited number of circulating cells (10). (ii) Lutz et al. (9) have reported localized infections in cats with no viral antigens present on their blood cells but with detectable infections in salivary gland epithelium. Some of these seven cats may have had limited infections involving epithelial cells before bone marrow involvement or after elimination of the virus from the bone marrow. Rojko et al. (12) have shown that during the infection process the oral lymph tissues are the first tissues infected, leading to circulating infectious virus. Bone marrow infection does not occur until 1 or 2 weeks after virus introduction.

Although the saliva ELISA is not as sensitive as either the serum ELISA or IFA at detecting early FeLV infections, our results indicate that it is an effective test for determining

TABLE 3. Comparison of IFA and saliva ELISA results in 26 serum ELISA-positive cats

Saliva ELISA result	No. of IFA results	
	+	-
+	15	7
-	0	4

virus shedding and diagnosing clinical and experimental infections of cats with FeLV. Clinicians are concerned with rapid diagnosis of FeLV involvement in sick cats to formulate a treatment. In addition, cats that shed the virus are the source of FeLV in the natural environment, and removal of FeLV shedders from the uninfected population is the standard procedure for maintaining a virus-free surrounding. This test lends itself to use as a rapid screening procedure for removal of infected cats from multiple-cat households. The experimental results show that a 2-week window exists within which all of the tests gave equivalent results. The specificity of the saliva test (99.2%, based on serum ELISA), the simplicity of the test procedure, and the speed of the results support its use as a screening procedure in clinical practices. In addition, since the test requires no blood sampling, no additional stress is placed on an already ill animal. We propose that the primary use of this assay should be as a screening procedure for the presence of FeLV-shedding cats. This would be for rapid clinical diagnosis or prescreening before vaccination with FeLV vaccine. A confirmatory test, i.e., an IFA or a repeated saliva test, should be performed before FeLV infection can be considered established.

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TABLE 2. Comparison of serum and saliva ELISAs in cats suspected of FeLV infection at four clinical sites^a

Saliva ELISA result	No. of serum ELISA results	
	+	-
+	69	4
-	8	483

^a Sensitivity, 69/77 (89.6%); specificity, 483/487 (99.2%); accuracy, 552/564 (97.9%).

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