Hemostatic Disorders in Feline Immunodeficiency Virus-Seropositive Cats

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The hemostatic function of 40 feline immunodeficiency virus (FIV) seropositive and 8 FIV and feline leukemia virus (FeLV) seropositive cats was evaluated and compared with reference values from 30 clinically healthy cats. The FIV-positive cats were divided into 3 groups: group I included asymptomatic carriers; group II comprised sick FIV-infected cats with illnesses not likely to influence the hemostatic system; and group III included FIV-positive cats with diseases potentially associated with coagulopathies. Platelet counts in FIV/FeLV-infected cats were significantly lower than in healthy cats ($P < .003$), whereas the differences in the 3 groups of FIV-positive cats were variable (group I, $P = .009$; II, $P = .05$; III, $P = .09$). Thrombocytopenia ($<145,000$ platelets/ μL) was present in 4 FIV-positive and 3 FIV/FeLV-positive cats. Platelet aggregation induced by collagen (0.5 and 0.25 $\mu\text{g/mL}$), adenosine diphosphate (ADP) (1 and 0.6 $\mu\text{mol/L}$), and thrombin (0.4 and 0.25 IU/mL) was not significantly different from that of healthy cats. The plasma coagulation system was evaluated by measuring one-stage prothrombin time (OSPT), activated partial thromboplastin time (APTT), thrombin time, fibrinogen concentration, coagulation factor assays, fibrinogen and fibrin degradation products (FDP), and plasma exchange test. The OSPT was similar in FIV-seropositive cats and in the healthy control group. Cats with FIV infection, however, had markedly shorter clotting times than healthy cats when using a modified test system

($P < .05$). In all groups of FIV-infected cats and in those with FIV/FeLV infection, APTT measured with 2 different commercially available tests, and a modified plasma assay was markedly prolonged compared with healthy cats (APTT1 and 2:3 modification: $P < .01$; APTT2: $P < .05$ except group III). In 22 of 40 cats with FIV and in 5 of 8 cats with FIV/FeLV infection, plasma samples were beyond the reference range. The thrombin time was also significantly prolonged in cats with FIV and FIV/FeLV infection ($P < .01$); values in 17 of 40 FIV-positive cats were above reference range. The mean fibrinogen concentration of cats with FIV and FIV/FeLV infection was higher than in the healthy control group ($P < .001$). Factor VIII activity of 4 cats with FIV infection was 1.5 times higher than that of healthy cats. Factor XII activity of 3 cats from a group of 20 cats with prolonged APTT was between 20% and 35%. Factor IX and XI activities ranged between 70% and 120%. The markedly prolonged APTT in 2 FIV-positive cats could be shortened considerably in a plasma exchange test using 20% feline pooled plasma. The alterations in the coagulogram of FIV-seropositive cats were not related to a clinical stage or concurrent diseases. A definite explanation of the distinct disorder within the intrinsic plasma coagulation system in FIV-infected cats was not found.

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Hemostatic disorders can occur in the course of different viral infections in cats.¹⁻⁶ Disseminated intravascular coagulation (DIC) is a complication of feline infectious peritonitis virus (FIPV) and feline panleucopenia virus infections.¹⁻⁴ Feline leukemia virus (FeLV) infection can cause thrombocytopenia, and seldom thrombocythemia.^{5,6}

After the discovery of the feline immunodeficiency virus (FIV) in 1987, its virologic features, pathogenesis, and clinical and pathological behavior have been well described.⁷⁻³⁹ Reports on hemostatic disorders associated with FIV infection are rare; moderate thrombocytopenia during the disease was identified in some FIV-positive cats.³⁷⁻³⁹

The purpose of this study was to characterize the primary and secondary hemostatic abnormalities in FIV-infected cats.

Materials and Methods

Cats and Sample Collection

Cats were considered healthy based on a normal physical examination, a hemogram within the reference range, and a negative enzyme linked immunosorbent assay (ELISA) for FIV and FeLV. Healthy cats were presented to the clinic for vaccination or neutering. Blood was collected from the jugular or cephalic vein from 30 healthy cats, 40 FIV-positive cats and 8 cats with concurrent FIV and FeLV infection. Table 1 lists age, breed, and gender of the cats evaluated. A small aliquot of blood (0.5 mL) was collected in a tube containing edetic acid (EDTA) for platelet counting and hemogram. The remainder of the sample (6 mL) was added to a tube containing 3.8% sodium citrate (blood/anticoagulant = 10:1) for aggregation studies and coagulation assays.

FIV and FeLV serology was performed using a commercially available ELISA test (Cite Combo, IDEXX Laboratories, Westbrook, ME). Cats with less intense color development in the ELISA were either tested repeatedly (twice in an interval of 2 months), or the diagnosis was confirmed by western blot.⁴⁰ The FIV- and FeLV-seropositive cats were client-owned patients of the Clinic of Small Animals, and were thus in different stages of infection (Table 2). FIV-positive cats were divided in three groups: group I included asymptomatic cats, Group II comprised FIV-seropositive cats with illnesses not likely to induce hemostatic abnormalities (eg, gingivitis, diarrhea, skin disease), and group III included FIV-infected cats with concurrent diseases that may be associated with coagulopathies (uremia, liver disease, neoplasia, etc). Platelet-rich plasma (PRP) for platelet aggregation was obtained by centrifuging citrated blood at 80g and 20°C in a centrifuge (Beckman GPKR, Beckman Instruments, Munich) for 10 minutes. Centrifugation for 15 minutes at 2500g resulted in platelet-poor plasma (PPP). PRP was diluted with PPP to a platelet number of 300,000/ μL . In case of a lower platelet count, undiluted PRP was used for platelet aggregation. Until testing, aliquots of frozen PPP were stored at -70°C .

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Table 1. Distribution of Breed, Gender, and Age of the Cats Evaluated

Cats	No. Healthy (%)	No. FIV Seropositive (%)	No. FIV/FeLV Positive (%)
No.	30 (100)	40 (100)	8 (100)
Breed			
Domestic Shorthair	26 (87)	38 (95)	7 (87.5)
Persian	2 (7)	0 (0)	1 (12.5)
Siamese	1 (3)	2 (5)	0 (0)
Norwegian Forest	1 (3)	0 (0)	0 (0)
Gender			
Male	13 (43)	11 (27.5)	3 (37.5)
Male, neutered	0 (0)	18 (45)	3 (37.5)
Female	17 (57)	7 (17.5)	2 (25)
Female, neutered	0 (0)	4 (10)	0 (0)
Age (y)			
Mean	1.8	6.1	3.8
Median	1.0	6.3	4.0
Range	0–3.0	0.3–11.5	0.5–7
25th percentile	1.0	3.5	0.5
75th percentile	2.0	8.5	6.5

After incubation in a water bath for 30 minutes at 37°C, the glass tube for quantification of fibrinogen and fibrin degradation products (FDP) was centrifuged at 1500g for 10 minutes, and the serum was stored at –70°C until further testing.

Platelet Counts

A 20- μ L capillary tube was filled with EDTA-anticoagulated blood and placed in a Thrombo plus (Sarstedt, Nümbrecht, Germany) tube after 2 minutes; the platelets were then counted in a hemacytometer within 3 hours of sample collection.

Platelet Aggregation Studies

Platelet aggregation studies were performed with an Automated Platelet Aggregation and Coagulation Tracer (APACT) (Labor, Ahrensburg, Germany) within 3 hours of venipuncture. PRP was adjusted to 100% light transmission and PPP to 10% transmission. PRP (200 μ L) was incubated for 30 seconds at 37°C and then aggregation was started by adding 10 μ L of an agonist. The following agonists were used: collagen (0.25 and 0.5 μ g/mL; Kollagenreagens Horm, Hormon-Chemie, Munich, Germany), Adenosinediphosphate (ADP) (0.6 μ mol/L; 1 μ mol/L; Sigma Diagnostics, St. Louis, MO) and thrombin (0.25 and 0.4 IU/mL; Test-Thrombin, Behringwerke, Marburg, Germany).

Table 2. Clinical Stages of FIV Infection in Cats

Clinical Stage	No.	Clinical Signs in Cats in This Study
Acute phase	0	
Asymptomatic carrier	7	Lymphadenopathy, 3; Femoral fracture, 3; tibial fracture, 1
AIDS-Related Complex	27	Upper respiratory tract infection, 6; renal failure, 5; skin disease, 4; gingivitis, 3; liver disease, 2; diarrhea, 2; cystitis, 2; vomiting, 1; neurological disorder, 1; neoplasia, 1
AIDS phase	6	Neoplasia, 2; anemia, 2; liver disease, 1; renal failure, 1

(Clinical staging according to Ishida and Tomoda.²⁸)

Coagulation Tests

One-stage prothrombin time (Quick time, OSPT) and activated partial thromboplastin time (APTT) were measured with reagents of different manufacturers in healthy and FIV-seropositive cats (OSPT reagent 1 [OSPT1; Calcium-Thromboplastin; Behringwerke, Marburg, Germany (thromboplastin extract from human placentas)], APTT reagent 1 (APTT1; Pathromtin; Behringwerke, Marburg, Germany (lipid extract from human placentas with kaolin)); OSPT reagent 2 (OSPT2; Hepato Quick; Boehringer Mannheim, Mannheim, Germany) and APPT reagent 2 (APTT2; PTT-Reagenz; Boehringer Mannheim, Mannheim, Germany [cephalin and kaolin]). Additionally, plasma was diluted with diethylbarbiturate acetate (DBA) buffer solution (Behringwerke, Marburg, Germany) 1:3 and 1:4 for OSPT1, and 2:3 for APTT1.

Thrombin time (TT; Thrombin-Reageuz; Boehringer Mannheim, Mannheim, Germany) and fibrinogen concentration (Fibrinogen-Reagenz, Boehringer Mannheim, Mannheim, Germany) were measured following the manufacturer's recommendation with a Schnitger and Gross coagulometer (Amelung, Lemgo, Germany).

Factor Assay. Factors VIII and XII (Boehringer Mannheim, Mannheim, Germany), and IX and XI (Behringwerke, Marburg, Germany) activities were measured in FIV-positive cats after 1:40 dilution with DBA or imidazole buffer (Behringwerke, Marburg, Germany) and compared with a standard curve of pooled plasma from 10 healthy cats.

Fibrinogen and Fibrin Degradation Products (FDP test; Boehringer Mannheim, Mannheim, Germany). Serum was serially diluted from 1:1 to 1:64 with glycine buffer. Ten-microliter aliquots of diluted serum and latex suspension were gently mixed on a test card and evaluated for agglutination 2 minutes later. The highest dilution with clear agglutination was considered as the agglutination titer.

Plasma Exchange Test. Citrated plasma (0.2 mL) from FIV-infected cats with highly prolonged APTT were replaced by 0.04, 0.10, and 0.16 mL of pooled plasma of healthy cats and the APTT determined.

Statistical Analysis. The nonparametrical Kruskal-Wallis analysis of variance was used to compare the platelet count and the coagulogram (screening tests, fibrinogen) of 3 groups of FIV-seropositive cats, FeLV/FIV-infected cats, and healthy controls.⁴¹

Table 3. Coagulation Parameters in Healthy Cats

	No. of Cats	Mean $\pm$ SD	Median	Reference Range ($\bar{X} \pm 2s$)
Platelets ($10^3/\mu\text{L}$)	30	357 ± 106	270	145–569
OSPT1 (s) ^a	30	8.5 ± 0.6	8.6	7.3–9.7
OSPT1 1:3 modification (s) ^b	30	12.6 ± 1.5	11.0	9.6–15.6
OSPT1 1:4 modification (s) ^c	30	14.8 ± 2.0	12.6	10.8–16.8
OSPT2 (s) ^d	14	21.4 ± 3.1	21.8	15.2–27.6
APTT1 (s) ^e	30	16.8 ± 1.5	21.2	13.8–19.9
APTT1 2:3 modification (s) ^f	30	22.7 ± 3.1	37.6	16.5–28.9
APTT2 (s) ^g	17	12.0 ± 0.7	14.9	10.6–13.4
TT (s)	30	16.3 ± 1.1	17.8	14.1–18.5
Fibrinogen (mg/dL)	30	168 ± 57	415	54–282

^a OSPT1 (Calcium-Thromboplastin), undiluted plasma.

^b OSPT1, 1:3 plasma dilution with DBA buffer solution.

^c OSPT1, 1:4 plasma dilution with DBA buffer solution.

^d OSPT2, (Hepato Quick), undiluted plasma.

^e APTT1 (Pathromtin), undiluted plasma.

^f APTT1, 2:3 plasma dilution with DBA buffer solution.

^g APTT2 (PTT-Reagenz), undiluted plasma.

Abbreviations: APTT, activated partial thromboplastin time; DBA, diethylbarbiturate acetate; OSPT, one-stage prothrombin time.

Differences among the 3 groups of FIV-positive cats were evaluated with the Kruskal-Wallis test as well. *P* values < .05 were considered significant.

Results

Tables 3 and 4 summarize the results of the coagulograms and platelet counts in clinically healthy, FIV- and FIV/FeLV-infected cats.

Platelet counts in FIV/FeLV-positive cats were lower than in the control group (*P* = .003), whereas in the FIV-seropositive cats (group I, *P* = .09; II, *P* = .05; III, *P* = .09) they were variable. Thrombocytopenia without evidence of spontaneous bleeding was present in 4 FIV-infected (10%; 2 upper respiratory signs, 1 neoplasia, 1 dermatitis) and 3 FIV/FeLV-infected cats (37.5; liver disease, dermatitis and fever).

Platelet aggregation could be induced in healthy and FIV-positive cats by the same agonists. The mean maximal aggregation and aggregation gradients were similar in FIV-infected cats and in healthy ones (Table 5).

The OSPTs were not significantly different in healthy and FIV-positive cats with the 2 tests evaluated (OSPT1, 2). The modified OSPT1 differed in FIV-seropositive cats from the control depending on the dilution (1:3 dilution: group I, *P* = .05, II, *P* = .006, III, *P* = .05; 1:4 dilution: group I, *P* = .023; II, *P* = .002; III, *P* = .007).

The APTT was significantly prolonged in all groups of FIV-infected cats (APTT1 group I, *P* = .007; II, *P* = .001; III, *P* = .004; 2:3 dilution: group I, *P* = .006; II, *P* = .003; III, *P* = .005) and in FIV/FeLV-seropositive cats (APTT1 *P* = .001, 2:3 dilution *P* = .007) (Table 4). The APTT1 values were beyond the reference range in 22 FIV-positive cats.

Five plasma samples of FIV/FeLV seropositive cats were also beyond the reference range for APTT1.

With the second APTT test, significant differences from the control group were observed in groups I (*P* = .004), II (*P* = .020), and in FIV/FeLV-infected cats (*P* = .029).

The TT was also prolonged in FIV- (group I, *P* = .008; II, *P* = .001; III, *P* = .002), and FIV/FeLV-positive cats (*P* = .042) when compared with the healthy controls (Tables 3 and 4).

Fibrinogen concentration was elevated over reference range in 27 FIV-seropositive (67.5%) and 5 FIV/FeLV-seropositive cats (62.5%), so that the results remarkably differed from those of the healthy ones (*P* < .001) (Tables 3 and 4). Seventeen FIV-infected (42.5%) and 4 FIV/FeLV-infected cats (50%) had fibrinogen concentrations above 400 mg/dL. The results of the platelet counts and the hemostasis screening tests did not vary considerably among the 3 groups of FIV-infected cats, and the cause of the underlying disease did not seem to have an influence on the hemostatic system (Table 4).

Coagulation factor assays (factors VIII, IX, XI, and XII) were performed in 20 FIV-seropositive cats with prolonged APTT (APTT1: mean $\pm$ SD = 44.3 ± 30.9 s; APTT1 2:3 dilution: mean $\pm$ SD = 53.9 ± 27.9 s; APTT2: mean $\pm$ SD = 17.5 ± 9.8 s; TT: mean $\pm$ SD = 19.2 ± 4.7 s). Factor VIII activity was higher than 150% in 4 of 20 FIV-infected cats (mean factor VIII activity in all FIV-seropositive cats: 123%). Mean factor XII activity was 84%; 3 FIV-positive cats had 20 to 35% factor XII activity. Factor IX and XI activity in 10 FIV-positive cats ranged between 70% and 120%.

The highest agglutination titer of fibrinogen and fibrin degradation products in 10 FIV-positive cats with prolonged APTT was 1:16 (reference range below 1:8).

A plasma exchange test performed in 2 cats with prolonged APTT resulted in a clear shortening of the coagulation time (APTT1) after adding 20% feline pooled plasma. Addition of more pooled plasma led to a successive normalization of the coagulation time. Plasma exchange after incubation for 1 hour yielded similar results.

Discussion

Reports on coagulation disorders during the course of FIV infection in cats are rare, probably because hemorrhagic diatheses occur infrequently. Only Grindem et al¹⁴ briefly described bleeding disorders in FIV-seropositive cats. An FIV-positive cat with leukopenia and thrombocytopenia associated with gastrointestinal bleeding was described by Friend et al.³⁹

Published reference ranges for platelet counts in normal cats are variable.^{42–48} The reference range in our study in 30 healthy cats (145,000 to 569,000/ μL) is similar to those reported by others.^{42,43} However, other investigators have reported ranges of 300,000 to 800,000 platelets/ μL .^{44–48}

Thrombocytopenia plays an important role in various infectious diseases^{1,2,5} and also occurs in cats with FIV infection.^{37,38} In our study, 4 of 40 FIV-seropositive cats (10%) were thrombocytopenic (<145,000/ μL). According to Shel-

Table 4. Coagulation Parameters in FIV- and FIV/FeLV-Seropositive Cats

	FIV-Seropositive Cats (Group I)*			FIV-Seropositive Cats (Group II)†			FIV-Seropositive Cats (Group III)‡			FIV/FeLV-Seropositive Cats§	
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	Mean ± SD	Median	Range	Mean ± SD	Median
APTT (s)	309 ± 150	7	188–633	284 ± 100	20	105–490	278 ± 108	13	33–453	181 ± 127	8
APTT (s)	8.6 ± 0.8	8.6	7.6–9.9	8.7 ± 0.5	8.6	7.6–9.5	8.8 ± 0.7	8.5	8.0–10.4	8.8 ± 0.5	8.9
APTT modification (s)	11.4 ± 1.4	11.0	9.8–13.8	11.4 ± 1.0	11.2	9.3–13.1	11.6 ± 1.4	11.4	9.7–13.9	11.8 ± 0.9	11.9
APTT modification (s)	12.6 ± 1.6	12.6	10.6–14.9	13.0 ± 1.3	12.8	10.3–15.4	12.9 ± 1.7	12.7	10.6–15.8	13.4 ± 1.3	13.3
APTT modification (s)	21.6 ± 1.8	21.8	19.3–23.6	22.3 ± 4.8	20.6	18.8–31.9	19.4 ± 2.0	20.0	15.3–21.5	21.4 ± 1.5	21.6
APTT modification (s)	33.7 ± 28.8	21.2	13.8–96.6	24.7 ± 15.3	20.2	13.8–83.2	35.8 ± 27.6	22.0	14.6–98.5	37.5 ± 31.8	23.0
APTT modification (s)	44.4 ± 28.3	37.6	21.7–101.5	33.3 ± 16.9	26.4	19.0–86.7	46.3 ± 32.8	32.6	18.8–119.1	45.4 ± 29.6	32.5
APTT modification (s)	16.4 ± 5.0	14.9	12.4–25.1	13.4 ± 1.8	13.9	10.6–16.9	15.4 ± 5.1	14.5	9.8–24.4	13.9 ± 3.0	13.1
APTT modification (s)	17.7 ± 1.0	17.8	15.7–19.2	19.8 ± 4.1	18.6	15.4–31.8	19.7 ± 4.4	18.3	12.8–28.7	18.5 ± 4.0	19.0
APTT modification (s)	362 ± 119	415	221–495	353 ± 172	354	131–840	460 ± 322	382	188–1,416	390 ± 161	383

APTT, activated partial thromboplastin time; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; OSPT, one-stage prothrombin time.

*FIV-Seropositive Cats (Group I), undiluted plasma.

†FIV-Seropositive Cats (Group II), plasma dilution with DBA buffer solution.

‡FIV-Seropositive Cats (Group III), plasma dilution with DBA buffer solution.

§FIV/FeLV-Seropositive Cats, plasma dilution with DBA buffer solution.

||FIV/FeLV-Seropositive Cats, plasma dilution with DBA buffer solution.

¶FIV/FeLV-Seropositive Cats, plasma dilution with DBA buffer solution.

|||FIV/FeLV-Seropositive Cats, plasma dilution with DBA buffer solution.

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|||||FIV/FeLV-Seropositive Cats, plasma dilution with DBA buffer solution.

||||||FIV/FeLV-Seropositive Cats, plasma dilution with DBA buffer solution.

Table 5. Platelet Aggregation in Healthy and FIV-Seropositive Cats

Aggregation Agonist	No. of Cats		Aggregation-Maximum (%)		Aggregation-Gradient (%/min)	
	Healthy	FIV Seropositive	Healthy	FIV Seropositive	Healthy	FIV Seropositive
Collagen (0.5 µg/mL; Kollagenreagens Horm)	13	16	89.9	87.5	108	103.3
Collagen (0.25 µg/mL; Kollagenreagens Horm)	3	8	90.4	86.5	90.3	96.7
Adenosinediphosphate 1 µmol/L	6	12	89.2	85.6	125.3	109.5
Thrombin (0.4 IU/mL; Test-Thrombin)	5	6	97.8	93.0	91.3	80.9

Abbreviation: FIV, feline immunodeficiency virus.

ton et al³⁸ the prevalence of thrombocytopenia in FIV-positive cats is 6%. A mild thrombocytopenia (>100,000 and <150,000 platelets/µL) was found by other investigators in 16% of FIV-infected cats.³⁷ A third report only mentioned one thrombocytopenic cat among 46 FIV-seropositive cats.²⁹ In only one cat, thrombocytopenia was associated with a spontaneous bleeding disorder.³⁹

Thrombocytopenia appears to be more frequent in cats with concurrent FIV and FeLV infection (11%),³⁸ probably because FIV/FeLV-positive cats have a 77-fold increase in the risk for developing myeloproliferative diseases,²⁶ which in turn results in thrombocytopenia due to myelophthisis. In our study, 3 of 8 FIV/FeLV-seropositive cats (37.5%) were thrombocytopenic.

In addition to platelet count, platelet function is vital for primary hemostasis. Platelets from clinically healthy cats aggregate well after adding collagen, ADP, arachidonic acid, or thrombin, but they do not respond to ristocetin, kaolin, or epinephrine.⁴⁹⁻⁵⁵

Thrombocytopathies occur in the course of various feline diseases, such as liver disease, leukemia, and Chédiak-Higashi syndrome.⁵⁵⁻⁵⁷ However, in our study platelet aggregation in FIV-seropositive cats did not differ from that of healthy cats.

The OSPT evaluates the extrinsic and common pathways of the plasma coagulation system. The results of our study reveal that the reference ranges for the 2 tests (OSPT1 and 2) we used to measure OSPT clearly differ.

It has been shown that an 80% plasma dilution leads to significant prolongation of the OSPT.⁴⁴ In an earlier study in our laboratory, plasma diluted with DBA buffer to 1:3 and 1:4 led to a smoother standard curve and improved the readability of the assay in healthy cats.⁶¹

An alteration in the extrinsic pathway of the coagulation system in FIV-positive cats was detected by the modified OSPT1 test. However, the unmodified OSPT1 test yielded similar results in FIV-infected and healthy cats. Unfortunately, a definite explanation for these discrepancies is not possible with the diagnostic tests currently available for the cat.

The published reference ranges for the APTT vary considerably when using the same test system but different devices.^{45,60} Gentry and Cooper⁶⁰ evaluated 4 reagents and found remarkable differences. However, the authors regarded all of the reagents tested as useful in the diagnosis of

severe coagulation disorders.⁶⁰ Both reagents evaluated in our study resulted in different reference ranges (14 to 20 seconds for APTT1 and 11 to 14 seconds for APTT2).

The use of plasma dilutions is recommended when evaluating the APTT in cats.⁴⁴ To improve sensitivity compared with undiluted plasma in healthy cats, the plasma sample in our study only needed to be diluted in a ratio of 2:3 for APTT1.

In vitro alterations in the intrinsic coagulation system were detected in 55% of FIV-infected cats in our study. They were characterized by a prolongation of the APTT, which was similar for both test reagents (APTT1, 2) and for the modified test assay of APTT1, which is more sensitive than APTT2. However, dilution did not provide additional benefit when evaluating the APTT.

The TT evaluates the generation of fibrin from fibrinogen after the addition of thrombin. It is influenced by heparin, FDPs, and severe dysfibrinogenemia or hypofibrinogenemia.^{2-4,62} The reference ranges for the TT vary considerably, depending on the thrombin concentration used. Our reference ranges were similar to those published in the literature.^{42,63}

The fibrinogen concentration quantified using the method of Clauss^{42,45,65,66} yielded reference ranges of 54 to 282 mg/mL. This reference range is similar to that published by other authors (50 to 310 mg/dL).^{47,65} Hyperfibrinogenemia has not been previously reported in FIV- and FIV/FeLV-seropositive cats. Yet, this is common in cats with inflammatory diseases.^{2,67-69} Because the Clauss method only detects functional, clottable fibrinogen, it is unlikely that dysfibrinogenemia accounted for the prolonged TT.⁷⁰

Isolated prolongation of the APTT may be caused by a clotting factor deficiency in the intrinsic pathway. Deficiencies of factors VIII, IX, XI, and XII have been reported in cats.^{63,64,71-82} Hemophilia A (factor VIII deficiency) is associated with a marked prolongation of the APTT.⁸⁰ However, in this study F VIII activity was elevated (>120%) in 13 of the 20 FIV-positive cats (65%) in which it was evaluated. This is likely a reflection of the fact that F VIII is an acute phase reactant, and its plasma activity increases in the course of inflammatory processes.⁸³

Factor IX deficiency (hemophilia B) is also associated with a prolongation of the APTT in the cat,⁷⁸ but a particularly marked prolongation of this screening test occurs in

combined factors IX and XII deficiencies (>100 s).^{81,82} Factor IX-activity of the FIV-seropositive cats evaluated was within the reference ranges and did not cause the abnormality in the hemostatic system.

Previous reports suggest that F XII deficiency is relatively common in healthy cats.^{63,71} However, in most of these, F XII activity is below 5%. Therefore, the F XII activity of 20% and 30% in 3 FIV-positive cats in this study is not likely to explain the prolongation of the APTT.

Circulating FDPs appear to be common in cats with DIC.^{2,4} High concentrations of circulating FDPs (>100 $\mu\text{g/dL}$) may cause prolongation of the APTT in humans.^{84,85} It is unlikely that the concentration of FDPs in FIV-infected cats in this study was a consequence of DIC. Moreover, these concentrations were probably not high enough to effect the APTT.^{42,45}

A plasma exchange test was performed in 2 FIV-seropositive cats with markedly prolonged APTT to elucidate this coagulation disorder. In humans, circulating inhibitors of coagulation occur in various diseases.⁸⁶⁻⁸⁹ Progressive inhibitory antibodies that exert an effect after 1 to 4 hours of plasma incubation are distinguished from direct inhibiting agents with immediate action.⁸⁶ They can inhibit single coagulation factors (eg, F VIII or IX) or cause prolongations of the APTT or OSPT. The antibodies, usually immunoglobulin (Ig)G or IgM, interfere with phospholipids involved in the coagulation process. However, despite the prolonged clotting times, spontaneous bleeding occurs only when concurrent thrombocytopenia or factor deficiency exist.⁸⁹

There is only one report of a coagulation disorder induced by circulating inhibitors to F XI in a cat.⁷⁴ Mixing of normal pooled plasma with the patient's plasma (with and without incubation for 1 hour) resulted in a slow normalization of the APTT, and gradual increases of F XI activity. From these results, the authors concluded that an inhibitory immunoglobulin may have been present.⁷⁴ In our study, the result of the plasma exchange test revealed that already a 20% to 50% mixture of patients with feline pool plasma corrects the prolonged APTT. Therefore, the presence of inhibiting antibodies is relatively unlikely. Based on the reports in the literature and on our own results, there is no satisfying explanation for the abnormalities in the intrinsic pathway of the plasma coagulation system in FIV-infected cats.

References

- Kraft W, Gerbig T, Geyer S. Thrombozytopenie bei Feliner Infektiöser Panleukopenie. *Kleintierpraxis* 1980;25:129-134.
- Weiss RC, Dodds WJ, Scott FW. Disseminated intravascular coagulation in experimentally induced infectious peritonitis. *Am J Vet Res* 1980;41:663-a671.
- Hiller D, Kraft W. Fibrin- und Fibrinogenspaltprodukte (FSP) bei Feliner Infektiöser Panleukopenie. *Kleintierpraxis* 1985;30:279-284.
- Boudreaux MK, Weiss RC, Cox N, et al. Evaluation of anti-thrombin-III activity as a coindicator of disseminated intravascular coagulation in cats with induced feline infectious peritonitis virus infection. *Am J Vet Res* 1989;50:1910-1913.
- Boyce JT, Kociba GJ, Jacobs RM, et al. Feline leukemia virus induced thrombocytopenia and macrothrombocytosis in cats. *Vet Pathol* 1986;23:16-20.
- Juchem R, Pause B. Megakaryozytenleukose bei drei Katzen. *Tierärztl Prax* 1987;15:195-200.
- Pedersen NC, Ho EW, Brown ML, et al. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 1987;235:790-793.
- Pedersen NC, Yamamoto JK, Ishida T, et al. Feline immunodeficiency virus infection. *Vet Immunol Immunopathol* 1989;21:111-129.
- Yamamoto JK, Sparger E, Ho EW, et al. Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats. *Am J Vet Res* 1988;49:1246-1258.
- Ackley CD, Yamamoto JK, Levy N, et al. Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus. *J Virol* 1990;64:5652-5655.
- Hosie MJ, Robertson C, Jarrett O. Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom. *Vet Rec* 1989;125:293-297.
- Bennett M, McCracken C, Lutz H, et al. Prevalence of antibody to feline immunodeficiency virus in some cat populations. *Vet Rec* 1989;124:397-398.
- Cohen ND, Carter CN, Thomas MA, et al. Epizootologic association between feline immunodeficiency virus infection and feline leukemia virus seropositivity. *J Am Vet Med Assoc* 1990;197:220-225.
- Grindem CB, Corbett WT, Ammerman BE, et al. Seroepidemiologic survey of feline immunodeficiency virus infection in cats of Wake County, North Carolina. *J Am Vet Med Assoc* 1989;194:226-228.
- Gruffydd-Jones TJ, Hopper CD, Harbour DA, et al. Serological evidence of feline immunodeficiency virus infection in UK cats from 1975-76. *Vet Rec* 1988;123:569-570.
- Moraillon A. Feline immunodepressive retrovirus infections in France. *Vet Rec* 1990;126:68-69.
- Furuya T, Kawaguchi Y, Miyazawa T, et al. Existence of feline immunodeficiency virus infection in Japanese cat population since 1968. *Jpn J Vet Sci* 1990;52:891-893.
- Lutz H, Egberink H, Arnold P, et al. Felines T-lymphotropes Lentivirus (FTLV): Experimentelle Infektion und Vorkommen in einigen Ländern Europas. *Kleintierpraxis* 1988;33:455-459.
- Kölbl S, Schuller W. Serologische Untersuchungen zum Vorkommen des feline Immunodefizienzvirus (FIV) bei Katzen in Österreich. *Wien Tierärztl Mschr* 1989;76:185-189.
- Neu H, Moennig V, Leidinger K, et al. Erste Ergebnisse über die Verbreitung FIV- (FTLV-)seropositiver Katzen in Deutschland und Interpretation der Ergebnisse. *Prakt Tierarzt* 1989;70:38-44.
- Witt CJ, Childs JE, Bishop BD, et al. Historical assessment of the prevalence of lentivirus infection in domestic cats. *Lab Anim Sci* 1988;38:512.
- Witt CJ, Moench TR, Gittelson AM, et al. Epidemiologic observations on feline immunodeficiency virus and toxoplasma gondii coinfection in cats in Baltimore, Md. *J Am Vet Med Assoc* 1989;194:229-233.
- Ishida T, Washizu T, Toriyabe K, et al. Detection of feline T-lymphotropic lentivirus (FTLV) infection in Japanese domestic cats. *Jpn J Vet Sci* 1987;50:39-44.
- Ishida T, Washizu T, Toriyabe K, et al. Feline immunodeficiency virus infection in cats of Japan. *J Am Vet Med Assoc* 1989;194:221-225.
- Shelton GH, Waltier RM, Connor SC, et al. Prevalence of feline immunodeficiency virus and feline leukemia virus infections in pet cats. *J Am Anim Hosp Assoc* 1989;25:7-12.
- Shelton GH, Cotter SM, Gardner MB, et al. Feline immuno-

deficiency virus and feline leukemia virus infections and their relationship to lymphoid malignancies in cats: A retrospective study (1968–1988). *J Acquired Immune Defic Syndr* 1990;3:623–630.

27. Pedersen NC, Torten M, Rideout B, et al. Feline leukemia virus infection as a potentiating cofactor for the primary and secondary stages of experimentally induced feline immunodeficiency virus infection. *J Virol* 1990;64:598–606.

28. Ishida T, Tomoda I. Clinical staging of feline immunodeficiency virus infection. *Jpn J Vet Sci* 1990;52:645–648.

29. Hopper CD, Sparkes AH, Gruffydd-Jones TJ, et al. Clinical and laboratory findings in cats infected with feline immunodeficiency virus. *Vet Rec* 1989;125:341–346.

30. Hosie MJ, Sparkes A, Hopper CD. Feline immunodeficiency virus. In *Practice* 1989;11:87–95.

31. Neu H. FIV(FTLV)-Infektion der Katze: 11 Fälle—Beitrag zur Epidemiologie, klinischen Symptomatologie und zum Krankheitsverlauf. *Kleintierpraxis* 1989;34:373–392.

32. Lutz H, Lehmann R, Winkler G, et al. Das Feline Immunschwäche Virus in der Schweiz: Klinik und Epidemiologie im Vergleich mit dem Leukämie- und dem Coronavirus. *Schweiz Arch Tierheilk* 1990;132:217–225.

33. Remington KM, Cheesebro B, Wehrly K, et al. Mutants of feline immunodeficiency virus resistant to 3'-azido-3'-deoxythymidine. *J Virol* 1991;65:308–312.

34. North TW, North GT, Pedersen NC. Feline immunodeficiency virus, a model for reverse transcriptase-targeted chemotherapy for acquired immune deficiency syndrome. *Antimicrob Agents Chemother* 1989;33:915–919.

35. Egberink H, Borst M, Niphuis H, et al. Suppression of feline immunodeficiency virus infection in vivo by 9-(2-phosphonomethoxyethyl)adenine. *Proc Natl Acad Sci USA* 1990;87:3087–3091.

36. Dieth V, Lutz H, Hauser B, et al. Pathologische Befunde bei mit Lentiviren infizierten Katzen. *Schweiz Arch Tierheilk* 1989;131:19–25.

37. Yamamoto JK, Hansen H, Ho EW, et al. Epidemiological and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. *J Am Vet Med Assoc* 1989;194:213–220.

38. Shelton GH, Linenberger ML, Grant CK, et al. Hematologic manifestations of feline immunodeficiency virus infection. *Blood* 1990;76:1104–1109.

39. Friend SCE, Birch CJ, Lording PM, et al. Feline immunodeficiency virus: prevalence, disease associations and isolation. *Aust Vet J* 1990;67:237–243.

40. Lutz H, Arnold P, Hübscher U, et al. Specificity assessment of feline T-lymphotropic lentivirus serology. *J Vet Med B* 1988;35:773–778.

41. Sachs L. *Statistische Methoden: Planung und Auswertung*, 6th ed. Berlin, Germany: Springer-Verlag; 1988:187–191.

42. Killingsworth CR, Weiss DJ, Eyster GE, et al. Screening coagulation tests in the cat: Reference values based on direct venipuncture and catheterized samples. *Vet Clin Pathol* 1985;14:19–23.

43. Pfeil R, Genschow E. Untersuchung zur elektronischen Thrombozytenzählung bei Katze und Hund. *Kleintierpraxis* 1987;32:119–122.

44. Greene CE, Tsang VC, Prestwood AK, et al. Coagulation studies of plasmas from healthy domesticated animals and persons. *Am J Vet Res* 1981;42:2170–2177.

45. O'Rourke L, Feldman BF, Ito RK. Coagulation, fibrinolysis, and kinin generation in adult cats. *Am J Vet Res* 1982;43:1478–1480.

46. Weiser MG, Kociba GJ. Platelet concentration and platelet volume distribution in healthy cats. *Am J Vet Res* 1984;45:518–522.

47. Jain NC, ed. *Schalm's Veterinary Hematology*, 4th ed. Philadelphia, PA: Lea & Febiger, 1986:466–486.

48. Kirk RW, ed. *Current Veterinary Therapy X. Small Animal Practice*. Philadelphia, PA: Saunders; 1989.

49. Allen DG, Johnstone JB, Crane S. Effects of aspirin and propranolol alone and in combination on hemostatic determinants in the healthy cat. *Am J Vet Res* 1985;46:660–663.

50. Greene CE. Effects of aspirin and propranolol on feline platelet aggregation. *Am J Vet Res* 1985;46:1820–1823.

51. Helenski CA, Ross JN. Platelet aggregation in feline cardiomyopathy. *J Vet Intern Med* 1987;1:24–28.

52. MacMillan DC, Sim AK. A comparative study of platelet aggregation in man and laboratory animals. *Thrombos Diathes Haemorrh* 1970;24:385–394.

53. Mills DCB. Platelet aggregation and platelet nucleotide concentration in different species. *Symp Zool Soc Lond* 1970;27:99–107.

54. Tschopp TB. Aggregation of cat platelets in vitro. *Thrombos Diath Haemorrh* 1970;23:601–620.

55. Hart S, Nolte I. Zur Thrombozytenaggregation der Katze. *Tierärztl Prax* 1991;19:413–418.

56. Davenport DJ, Breitschwerdt EB, Caracostas MC. Platelet disorders in the dog and cat. Part I: Physiology and pathogenesis. *Compend Contin Educ Pract Vet* 1982;4:762–772.

57. Catalfamo JL, Dodds WJ. Hereditary and acquired thrombopathias. *Vet Clin North Am Small Anim Pract* 1988;18:185–192.

58. Feldman BF. Disorders of platelets. In: Kirk RW, ed. *Current Veterinary Therapy*. Philadelphia, PA: Saunders; 1989:457–464.

59. Cowles BE, Meyers KM, Wardrop KJ, et al. Prolonged bleeding time of Chediak-Higashi cats corrected by platelet transfusion. *Thrombos Haemost* 1992;67:708–712.

60. Gentry PA, Cooper ML. Reagent dependent variability of plasma clotting times in the cat. *Feline Pract* 1979;9:33–37.

61. Hart S, Nolte I. Schwierigkeiten bei der Gerinnungsdiagnostik der Katze. In: 35. Jahrestagung der Fachgruppe "Kleintierkrankheiten" der DVG, Giessen, 1989:332–338.

62. Greene CE, Meriwether E. Activated partial thromboplastin time and activated coagulation time in monitoring heparinized cats. *Am J Vet Res* 1982;43:1472–1477.

63. Lewis JH. Comparative hematology: Studies on cats including one with factor XII (Hageman) deficiency. *Comp Biochem Physiol* 1981;68A:355–360 (abstr).

64. Hoffman L, Sperling K, Dodds WJ. Acquired hemostatic problems in a cat with factor XII deficiency. *Feline Pract* 1986;16:25–27.

65. Lütze G, Kutschmann K. Untersuchungen zum plasmatischen Gerinnungssystem der Hauskatze. *Berl Münch Tierärztl Wschr* 1991;104:53–57.

66. Clauss A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. *Acta Hematol* 1957;17:237–246.

67. Schalm OW. Plasma protein: Fibrinogen ratios in routine clinical material from cats, dogs, horses, and cattle—Part III. *Calif Vet* 1970;24:6–10.

68. Pedersen NC. Feline infectious peritonitis: something old, something new. *Feline Pract* 1976;6:42–51.

69. Sutton RH. The value of a single haematological examination in the diagnosis of disease in the cat. *J Small Anim Pract* 1980;21:339–345.

70. Lane DA, Scully MF, Thomas DP, et al. Acquired dysfibrinogenemia in acute and chronic liver disease. *Br J Haematol* 1977;35:301–308.

71. Didisheim P, Hattori K, Lewis JH. Hematologic and coagu-

lation studies in various animal species. *J Lab Clin Med* 1959;53: 866-875.

72. Green RA, White F. Feline factor XII (Hageman) deficiency. *Am J Vet Res* 1977;38:893-895.

73. Kier AB, Bresnahan JF, White FJ, et al. The inheritance pattern of factor XII (Hageman) deficiency in domestic cats. *Can J Comp Med* 1980;44:309-314.

74. Feldman BF, Soares CJ, Kitchell BE, et al. Hemorrhage in a cat caused by inhibition of factor XI (plasma thromboplastin antecedent). *J Am Vet Med Assoc* 1983;182:589-591.

75. Littlewood JD. Haemophilia A (factor VIII deficiency) in the cat. *J Small Anim Pract* 1986;27:541-546.

76. French TW, Fox LE, Randolph JF, et al. A bleeding disorder (von Willebrand's disease) in a Himalayan cat. *J Am Vet Med Assoc* 1987;190:437-439.

77. Johnstone IB, Morton JC, Allen DG. Factor VIII deficiency in a cat. *Can Vet J* 1987;28:671-673.

78. Brooks MB, Dodds WJ. Factor IX deficiency (Hemophilia B) in two male domestic Short-Hair cats. *J Am Anim Hosp Assoc* 1989;25:153-155.

79. Dodds WJ. Hemostasis. In: Kaneko JJ ed. *Clinical Biochemistry of Domestic Animals*. San Diego, CA: Academic Press; 1989: 274-315.

80. Cotter SM, Brenner RM, Dodds WJ. Hemophilia A in three unrelated cats. *J Am Vet Med Assoc* 1978;172:166-168.

81. Dillon AR, Boudreaux MK. Combined factors IX and XII deficiencies in a family of cats. *J Am Vet Med Assoc* 1988;193:833-834.

82. Boudreaux MK, Dillon AR. The effect of danazol treatment on factor IX deficiency in cats. *Vet Clin Pathol* 1989;17:84-85.

83. Kitchens CS. Concept of hypercoagulability: A review of its development, clinical application and recent progress. *Semin Thrombos Hemost* 1985;11:293-315.

84. Marder VJ, Martin SE, Francis CW, et al. Consumptive Thrombohemorrhagic Disorders. In: Colman RW, Hirsh J, Marder V, Salzman EW, eds. *Hemostasis and Thrombosis*, 2nd ed. Philadelphia, PA: Lippincott; 1987:975-1015.

85. Wenzel E, Holzhüter H, Muschietti F, et al. Zuverlässigkeit des Fibrinogen- (Fibrin-)Spaltproduktnachweises im Plasma mit Thrombinkoagulase-, Reptilase- und Thrombin-Gerinnungszeit. *Dtsch med Wschr* 1974;99:746-756.

86. Lechner K. Acquired inhibitors in nonhemophilic patients. *Haemostasis* 1974;3:65-93.

87. Schleider MA, Nachmann RL, Jaffe EA, et al. A clinical study of the lupus anticoagulant. *Blood* 1976;48:499-509.

88. Green D, Lechner K. A survey of 215 nonhemophilic patients with inhibitors to factor VIII. *Thrombos Haemost* 1981;45: 200-203.

89. Shaulian MD, Shoenfeld Y, Berliner S, et al. Surgery in patients with circulating lupus anticoagulants. *Int Surg* 1981;66:157-159.