

Plasma Electrophoretogram in Feline Immunodeficiency Virus (FIV) and/or Feline Leukaemia Virus (FeLV) Infections

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Summary

The electrophoretogram of 89 cats, including those infected by feline immunodeficiency virus (FIV+), feline leukaemia virus (FeLV+) and non-infected, showed statistically significant differences in several of the fractions. FIV+ cats had very high protein values (mean, 8.10 g/dl), mostly because of hypergammaglobulinemia (mean, 2.81 g/dl) as compared with non-infected animals and FeLV+. In addition, in these FIV+ animals, the albumin/globulins ratio (A/G) was very low (mean, 0.72). Statistically significant differences in A/G and α 2-globulin fraction were observed in FeLV+ group (A/G mean, 0.88 ± 0.08 ; α 2-globulin, mean, 0.84 ± 0.07 g/dl) when compared with non-infected group (A/G mean, 1.06 ± 0.08 ; α 2-globulin mean, 0.68 ± 0.04 g/dl). The α 1-globulin fraction was higher in double infected animals (FIV and FeLV positive, F-F) (3.55 g/dl), than in FeLV+ or FIV+ cats (3.10 and 3.07 g/dl respectively), but no statistical conclusions may be drawn from this fact because of the low number of F-F animals. This technique may help to assess the initial clinical status of retrovirus-infected cats, and the clinical course of these chronic diseases, specifically during and after suitable therapy.

Introduction

Feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) induce slow developing diseases, characterized by long asymptomatic periods, which could be even longer than 4 years (especially in FIV infection) (Burkhard and Dean, 2003). During these long periods, no clinical signs by definition are observed, but the virus is undoubtedly undergoing replication and inducing changes in the immune system and a progressive decline in the immune function, which later lead to overt disease.

Feline immunodeficiency virus is a very important pathogen of the cat; the prevalence varies according to the geographical area, but in some parts of the world it can be as high as 44% (Arjona et al., 2000). The clinical course of feline immunodeficiency associated with FIV has been divided into five stages, in a way similar to HIV-1 infection with which it shares many homologies (Bendinelli et al., 1995; Hartmann, 1998).

Feline leukaemia virus produces disease usually only after prolonged latency. FeLV-associated syndromes are typically divided into proliferative diseases (lymphoma or leukaemia) or degenerative diseases (Rojko and Hardy, 1994). Within these

degenerative diseases, the feline acquired immunodeficiency syndrome induced by some variants of FeLV (FeLV-FAIDS) is usually fatal, and also very similar to human AIDS (Hardy and Essex, 1986). Immunodeficiency is produced because FeLV replicates in the cells of the immune system, producing a dramatic decrease in the populations of lymphocytes and granulocytes. More cats die from FeLV-induced immunodeficiency than from proliferative diseases (Hardy and Essex, 1986).

Double infected cats (FIV and FeLV+), likely because both processes may induce impairment of the immune system, have more severe clinical signs than those infected with a single virus (Courchamp et al., 1997).

In both FIV and FeLV infections, when clinical signs are observed, they are by no means pathognomonic. In addition, no pattern from the analyses routinely performed (hemogram and biochemical profile) may be clearly linked to these retroviral diseases. This is a big handicap in determining the present clinical state of the animal and in predicting its evolution.

The electrophoretogram is an indicator of the variety of changes that may occur in the serum proteins during the course of a disease. Interpretation of electrophoretograms is based on densitometric measurements of intensity of staining of protein bands on cellulose acetate strips. The serum separates into four fractions: albumin, alpha (α) globulins, beta (β) globulins and gammaglobulins (Willard et al., 1999). Each of the fractions includes a variety of proteins, which amongst other indications, help to infer the immune response of the animal, particularly, in immune-mediated or chronic diseases (Kajikawa et al., 1999).

In feline medicine several authors have reported the predictive value of the electrophoretogram, especially for the diagnosis of feline infectious peritonitis (FIP) (Shelly et al., 1988; Paltrinieri et al., 2001; Addie and Jarrett, 2006). In this disease, an altered proteinogram of the peritoneal effusion is pathognomonic of wet FIP; cats with clinical signs such as anorexia, weakness, wasting, etc. along with ocular and/or neurological signs and elevated serum globulin levels should be suspected of having dry FIP. The albumin/globulin ratio (A/G) is also a useful diagnostic tool, especially when measured in the effusion. An A/G ratio <0.4 is associated with FIP (Sparkes et al., 1994).

The electrophoretogram has also been proposed as a diagnostic and prognostic tool in other species. It is routinely

used in canine leishmaniosis in endemic areas, and, although it may not be considered specific, adequately interpreted it may aid in the clinical diagnosis (Miró and Fraile, 1999).

The present study was designed to determine whether the electrophoretogram can be a useful complementary test to evaluate the variations in plasma proteins. The information that this test offers, combined with other analytical (hemogram and biochemical profile) and immunological (CD4:CD8 ratio) parameters would allow to evaluate the clinical status of the patients. These preliminary data may aid in the prognosis or possible clinical evolution, and improve the clinical follow-up of feline retroviral cases.

Materials and Methods

Animals

A total of 89 cats, which were either infected by FeLV and/or FIV or non-infected were included in the trial. Of the 89 cats, 26 were naturally infected by FIV (29.2%), 30 by FeLV (33.7%), four by both viruses (4.5%) and 29 were non-infected (32.6%) (Table 1). Most of the cats were brought by their owners to one of four private veterinary clinics or to the Veterinary School Clinical Hospital, either because they had symptoms suggestive of feline retrovirolosis or just for routine controls. Exclusion criteria included pregnant or lactating females, or cats exhibiting severe renal, hepatic or cardiac diseases. Owners were informed about the study. Many of the cats had been recently found on the street or adopted from a feline community.

The study started in January 2002, and ended in July 2004. The animals were examined by the practitioners by an exhaustive physical exam, and their clinical histories were recorded. One of the authors (GM) monitored the clinical trials and supervised the uniformity in the rating of the clinical signs. Tests performed included blood (blood smears examination), faeces (faecal flotation and sedimentation), skin (scrapings) and hair (dermatophyte culture). Pathologies

examined for were presence of intestinal parasites (protozoa and nematodes), blood parasites (such as *Mycoplasma haemofelis*) and ectoparasites (flea and *Otodectes* infestations).

Sampling

Animal handling, treatment, reagent manipulations and data collection were all carried out in compliance with the guidelines of Good Clinical Practice, and Good Laboratory Practice of the Animal Welfare Committee of the Veterinary Clinical Hospital and of the University.

A blood sample from each animal was obtained by cephalic or jugular puncture. Some of the animals needed to be sedated as a result of their aggressiveness using Domitor® (medetomidine; Pfizer, Madrid, Spain). Blood was distributed between a tube with Lithium heparin, and a tube with EDTA. Blood samples were sent immediately by express mail to Veterinary School in Madrid to complete the transport and cell processing within 24 h post-bleeding.

Detection of retroviral infection

Immediately upon arrival at the Department of Animal Health in the Veterinary School, the heparinized sample was divided into aliquots. One of them was centrifuged and the plasma was separated from the cellular fraction. The presence of FeLV p27CA and antibodies against FIV p24 was determined using the commercial Snap Combo® (Idexx Laboratories, Inc., Westbrook, ME, USA). The serological results were later confirmed by a nested PCR designed by our group, which uses the amplification of endogenous retroviral sequences as internal control (Arjona et al., 2006). In brief, DNA was extracted from 200 µl of blood, using DNeasy Tissue kit (QiaGen, Valencia, CA, USA). The DNA was amplified by a pair of primers which are common to FIV and FeLV and hybridize in the *pol* region, rendering a product of 1325 bp for FIV and 490 bp for FeLV. This product was re-amplified using

	FeLV-infected	FIV-infected	F-F infected	Non-infected
Number	30 (33.7)	26 (29.2)	4 (4.5)	29 (32.6)
Sex, $n = 76$ ($P < 0.01$)				
Females, $n = 36$	16 (44.44)*	8 (22.22)	1 (2.78)	11 (30.55)
Males, $n = 40$	5 (12.50)*	16 (40.00)	3 (7.50)	16 (40.00)
Neutered, $n = 40$	7 (17.50)	20 (50.00)*	3 (7.50)	10 (25.00)
Non-neutered, $n = 20$	11 (55.00)	5 (25.00)*	1 (5.00)	3 (15.00)
Age, $n = 72$				
Average (years)	3.0	4.0	3.6	4.2
Range (years)	(0.33–10)	(0.15–12)	(0.5–6)	(0.66–9)
< 1 year	8 (44.44)	4 (22.22)	1 (5.56)	5 (27.78)
1–5 years	9 (25.00)	16 (44.44)	2 (5.56)	9 (25.00)
5–10 years	4 (25.00)	3 (18.75)	1 (6.25)	8 (50.00)
> 10 years	0	2	0	0
Breed, $n = 76$ ($P < 0.05$)				
Common European	16 (23.23)*	22 (32.35)*	3 (4.35)	27 (39.71)
Persian	4 (80.00)*	0*	0	1 (20.00)
Siamese	0	2 (66.67)	1 (33.33)	0
Clinical signs ^a , $n = 72$				
Yes	11 (44.00)	12 (48.00)	2 (8.00)	0
No	9 (19.15)	7 (14.89)	2 (4.26)	29 (61.70)

Table 1. Distribution of the animals for which sex, age, breed and symptoms were reported, according to these characters and the virus infection

*Differences statistically significant between groups (Kruskal–Wallis test; $P < 0.05$).

^aClinical signs included those reported in Table 2.

ND, not determined; FeLV, feline leukaemia virus; FIV, feline immunodeficiency virus.

Values in parentheses are expressed in percentage.

primers specific for FIV and for FeLV, resulting in a product 1138 bp for FIV, 306 bp for FeLV and 257 bp for the endogenous retroviruses.

Detection of *Toxoplasma* and coronavirus infection

Toxoplasma gondii-specific antibodies were detected by the indirect immunofluorescence antibodies test (Verhofstede et al., 1987). A titre of $\geq 1:80$ was considered positive. The evaluation of the antibodies against feline enteric coronavirus and FIP was performed using an ELISA (Ingezim Peritonitis[®]; Ingenasa, Madrid, Spain). Diagnosis was based on the result of this evaluation along with clinical examination.

Hemogram and biochemical parameters

Haematological and biochemical tests, as well as the electrophoretograms of plasma proteins, were run in the Laboratory of Biopathology of the Veterinary Clinical Hospital (Veterinary School of Madrid). A complete blood count of the EDTA tubes was performed using an automated haematology analyzer (Sysmex F-800 Microcellcounter, Sysmex Corp., Kobe, Japan). Differential white blood cells (WBC) counts were performed in blood smears using a Romanowsky stain. Urea, creatinin and alanine aminotransferase (ALT) were assayed on plasma heparinized samples by dry chemistry analyser (Reflotron; Boehringer-Mannheim, Mannheim, Germany). Sodium (Na^+), chloride (Cl^-) and potassium (K^+) were measured on a Microlyte 3 + 2 Ion Selective Analyser (Kone, ThermoClinical LabSystems, Espoo, Finland).

Electrophoretic separation of plasma proteins

Cellulose acetate strips were immersed in veronal buffer (diethyl barbituric acid, 3.12 g/l; sodium diethylbarbiturate, 17.1 g/l; pH 8.6) and blotted lightly between sheets of filter paper to remove excess buffer. Plasma samples were applied on the strips with a twin wire applicator. The strips were then placed in the electrophoresis tank (BioSystems, Atom, Barcelona, Spain) and a constant voltage of 200 V and a current of 3.5 mA/strip was applied for 35 min. The strips were stained with amido black for 10 min. The excess stain was removed by washing the strips in a solution of acetic acid and methanol (47.5% methanol, 5% acetic acid). The strips were then scanned on a Helena Optiscan (Helena Laboratories, Beaumont, TX, USA) densitometer.

Analysis of CD4/CD8 by flow cytometry

Mouse anti-feline CD8 labelled with rhodamine and mouse anti-feline CD4 labelled with fluorescein (Southern Biotechnology Associates, Inc., Birmingham, AL, USA) (1 μl of each) were added to 0.25 ml of heparinized blood sample and allowed to label the T cells for 30 min on ice. Red blood cells were lysed with 6 ml of a commercial buffer (BD FACS Lysing Solution, BD Biosciences, San Jose, CA, USA), and WBC were washed twice with phosphate buffered saline (PBS) and resuspended in 30 μl PBS. Labelled cells were fixed (500 μl PBS, 1% formaldehyde, 0.1% sodium azide) and then analysed in a flow cytometer (Becton Dickinson FAC-Scan, San Jose, CA, USA). In our hands, the CD4:CD8 ratio of normal clinically healthy cats is ≥ 1.4 .

Statistical analysis

The Statgraphics Plus 5.1 for Windows program was used to analyse and compare the data. Results from the four groups were compared with one another using a one-way ANOVA. As most results were found to have a non-normal distribution and the sample size was small, the non-parametric Kruskal-Wallis test was applied to determine statistical differences (at a confidence level of 95%, $P < 0.05$) between the groups.

Results

Eighty-nine cats were tested in total. None of them was infected by *Toxoplasma*. The serological and PCR results agreed in all cases, and there were no discordant results. The sex, age, breed and presence of clinical signs, when available, are shown in Table 1. The clinical signs reported by the clinicians are listed in Table 2.

Differences were observed in the electrophoretogram of FIV+, FeLV+, double-infected (F-F) and non-infected cats. These differences were statistically significant for serum total proteins, α_2 - and gammaglobulins, and the albumin/globulin ratio (A/G), and a combination of them could be used to differentiate animals infected by either one or both of the retroviruses.

Statistically significant differences ($P < 0.01$) were seen in the mean values of total serum protein between FIV+ and FeLV+, and FIV+ and non-infected cats (Fig. 1, Tables 2 and 3). No differences were seen for total protein as regards to age or sex. However, a statistically significant difference was seen regarding breed (mean 7.58 g/dl for Common European, 6.42 g/dl for Persian and 8.53 g/dl for Siamese respectively; $P < 0.05$). The low number of Siamese and Persian cats included in the study made it difficult to draw conclusions concerning the differences in breeds.

Significant differences were observed in the A/G between non-infected and FeLV+ animals and between non-infected and FIV+ cats (Fig. 1, Table 4). The FIV+ cats had the lowest A/G values compared with the non-infected group, due mostly to a higher increase in immunoglobulins (Table 3), as the other parameters remained normal or even lower than in non-infected cats.

With respect to the concentration of gammaglobulins, statistically significant differences were seen in our study between FIV+ and FeLV+, and FIV+ and non-infected cats ($P < 0.0001$) (Table 3). The hypergammaglobulinemia observed in FIV-infected cats was of a polyclonal nature in all cats and it probably corresponded to an increase of immunoglobulin (Ig) G, as the β -globulins fraction (where IgM migrates) was not observed to be altered (Table 3). As regards FeLV, on average, animals were found to be slightly hypergammaglobulinemic (1.98 g/dl). However, most of the FeLV+

Table 2. Major clinical signs reported by practitioners

General symptoms	Lethargy, bad hygiene due to behavioural changes, general amyotrophy, weight loss, anorexia, fever, generalized lymphadenopathy
Specific clinical signs	Poliarthritis, constipation, vomiting, diarrhoea, dyschesia, stomatitis-gingivitis, respiratory distress, rhinotracheitis, dermatitis
Associated diseases	Dermatophytosis, otocariosis, fleas

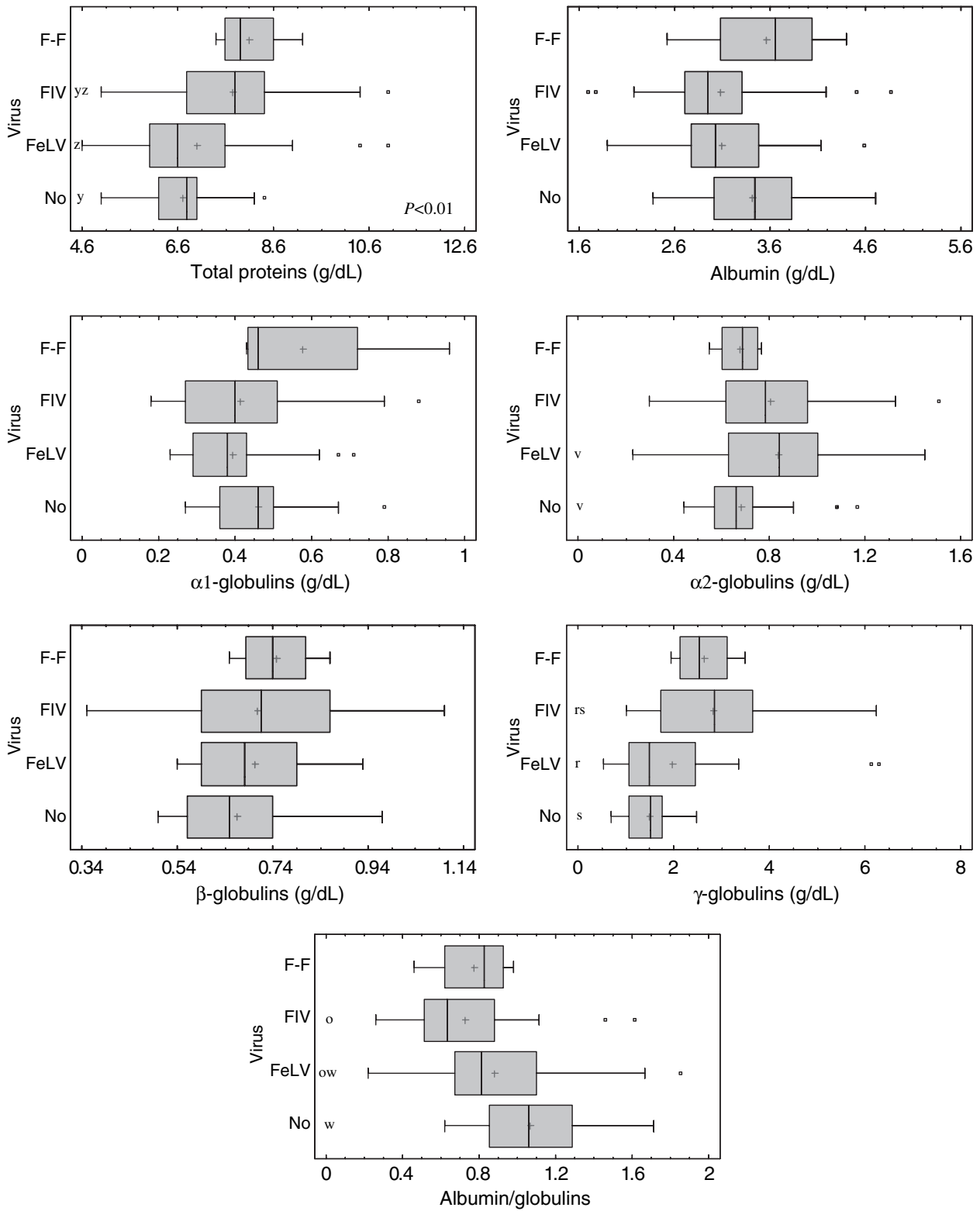


Fig. 1. Box and whiskers plot of the relationship between the different plasma protein fractions and the retroviral infection. The virus infection is indicated on the left, and the plasma fraction below each diagram. For each virus infection, the box encompasses the interquartile range, with the center line representing the median and the +, the sample mean. The whiskers represent the data range, and outliers are shown as dots. In each diagram, letters immediately to the right of the name of the virus designate statistically significant differences (determined by the Kruskal–Wallis test, at the P -value shown) between the groups which have been assigned the same letter.

animals included in the study had low gammaglobulin levels (86.7%), but four animals that had gammaglobulin levels higher than 3.0 g/dl raised the mean.

The mean CD4/CD8 ratio was low in both FIV+ (mean, 1.05), statistically different ($P < 0.05$) from FeLV+ (mean, 1.67) and non-infected (mean, 1.84) cats. The mean in F-F

Table 3. Values for the electrophoretic fractions in FeLV+, FIV+, F-F and non-infected cats

	FeLV	FIV	F-F	Non-infected
Number of animals	30 (33.71)	26 (29.21)	4 (4.49)	29 (32.58)
Protein (g/dl)	7.00 ± 1.45 Normal	7.73 ± 1.38 High	8.10 ± 0.77 Very high	6.71 ± 0.82
Albumin (g/dl)	3.10 ± 0.60 Normal	3.07 ± 0.75 Normal	3.55 ± 0.76 Normal	3.41 ± 0.40
α 1-globulins (g/dl)	0.39 ± 0.13 Discretely low	0.41 ± 0.17 Normal	0.57 ± 0.26 Very high	0.46 ± 0.13
α2-globulins (g/dl)	0.84 ± 0.31 Very high	0.81 ± 0.30 High	0.67 ± 0.10 Normal	0.68 ± 0.18
β -globulins (g/dl)	0.70 ± 0.11 Normal	0.71 ± 0.18 Normal	0.75 ± 0.09 Normal	0.66 ± 0.12
Gammaglobulins (g/dl)	1.98 ± 1.40 Normal	2.81 ± 1.21 Very high	2.63 ± 0.66 High	1.48 ± 0.48
Albumin/globulins	0.88 ± 0.36 Discretely low	0.72 ± 0.33 Very low	0.77 ± 0.22 Low	1.06 ± 0.26

Values highlighted in bold are significantly different between groups.

In each cell, the upper value is the mean \pm SD, and the description on the bottom is the qualitative comparison between the respective infected group and the non-infected group.

FeLV, feline leukaemia virus; FIV, feline immunodeficiency virus.

Values in parentheses are expressed in percentage.

Table 4. Significant differences in the plasma proteins using the Kruskal-Wallis test ($P < 0.05$) between the different group pairs included in the study

Non-infected vs FeLV+	Non-infected vs FIV+	FIV+ vs FeLV+
α 2-globulin A/G	Total protein γ -globulin A/G	Total protein γ -globulin

In each cell the electrophoretogram fraction which is significantly different between the two groups that converge in that cell is shown.

cats was 0.7, but the low number of animals in this group restricted statistical analysis. Even though no clear statistically significant differences were found, it seemed that animals with higher gammaglobulin levels tended to have lower CD4/CD8 ratios.

No correlation was seen between gammaglobulin levels and signs detectable by clinical examination, such as dermatitis or anorexia. However, negative correlations were detected between this parameter and haemoglobin content ($P < 0.01$), haematocrit ($P < 0.01$), mean corpuscular volume ($P < 0.0005$) and mean cell haemoglobin ($P < 0.01$). Also, high gammaglobulin levels corresponded to high absolute ($P < 0.01$) and relative ($P < 0.005$) neutrophil numbers (data not shown). No clear correlation was seen between the different serum fractions and the biochemical parameters tested (urea, creatinine, ALT, Na⁺, Cl⁻ or K⁺).

Double-infected animals had higher levels of α 1-globulin than either FIV+ or FeLV+ cats (Table 3), but no statistical conclusions may be drawn from this fact as a result of the low number of animals. No statistical differences were seen between non-infected cats and any other group. As regards α 2-globulin, statistically significant differences were also seen between FeLV+ and non-infected cats.

Discussion

This study has the deficiencies inherent to sampling under field conditions, in which the inclusion of animals is absolutely dependent on the owner, and the exact moment of infection is

unknown. On the other hand, when compared with specific pathogens free (SPF) cats, the use of conventional (non-laboratory) animals, most likely subjected to a variety of vaccination schedules, diets, diseases, stress, etc. of non-controlled conditions, represents an undeniable advantage. Even with this lack of background information, when compared with non-infected cats, statistically significant differences between cats infected by FIV or FeLV were seen in the levels of serum total proteins, gammaglobulins, α 2-globulins and albumin/globulin ratio (Tables 2 and 3).

As regards total serum proteins, FeLV+ cats had normal concentrations of total proteins, but FIV+ and F-F cats had marked hyperproteinemia (Table 3, Fig. 1), which was not caused by dehydration. Clinical signs were only observed in some of the infected cats, and non-infected cats had no relevant clinical signs. Protein levels were similar in healthy animals (infected and non-infected) (mean, 7.61 g/dl) and in cats with retrovirus-related clinical signs (mean, 7.55 g/dl).

The polyclonal hypergammaglobulinemia observed in FIV+ cats is consistent with results by other authors (Poli et al., 1992; Thomas et al., 1993; Bendinelli et al., 1995; Shelton et al., 1995), and it cannot be explained simply by the presence of secondary infections as they also occur in experimentally FIV-infected SPF cats not exposed to other pathogens (Hopper et al., 1989; Ackley et al., 1990; Poli et al., 1992; Bendinelli et al., 1995).

The CD4/CD8 ratio was very low in F-F cats, in the lower limit of normality in FIV+, and normal in FeLV+ animals. Animals with higher gammaglobulin levels tended to have lower CD4/CD8 ratios. As the CD4/CD8 ratio is the parameter considered as most important for diagnosing a situation of immunodeficiency, the serum electrophoretogram would be an inexpensive complementary method as the first approach for the practitioner, instead of the more costly flow cytometry to determine the CD4/CD8 ratio, although further studies need to be performed in this sense.

Hypergammaglobulinemia has also been reported in other lentiviral infections such as HIV infection (Shirai et al., 1992), and apparently reflects the response to the virus itself (Amadori et al., 1989). It has been stated that the hypergam-

maglobulinemia correlates with aberrant polyclonal hyperactivation of the B cells, although their total numbers in the peripheral blood remain normal (Ackley et al., 1990). In FIV infections, as in HIV ones, there is an increase in antibodies both against virus-specific and non-virus-related antigens (Flynn et al., 1994). Possibly, the high mutation rate of these viruses accounts, at least in part, for the hypergammaglobulinemia, as variants would be constantly emerging inducing the continuous formation of antibodies. In AIDS, most of the HIV-infected patients develop hypergammaglobulinemia during the first year post-infection, also associated with the inversion of the CD4/CD8 ratio (Shirai et al., 1992). Its development is difficult to explain. First, a low CD4/CD8 ratio implies low number of CD4+ T-helper cells to activate B cells; secondly, it has been shown in HIV+ patients that B cells undergo cell death through apoptosis and other mechanisms (De Milito, 2004). Thus, it seems paradoxical that gammaglobulins would increase if B cells are both dying and not being activated by T-helper cells.

As the study presented here was performed with conventional animals under field conditions, brought to the practice by their owners, it was difficult to determine in which stage of their disease samples were taken. It has been reported that hypergammaglobulinemia is generally observed in FIV-positive animals which have been infected for over 19 months (Ackley et al., 1990; Hofmann-Lehmann et al., 1997). According to some authors, the statistically significant differences in the gammaglobulins disappeared after month 56 (Hofmann-Lehmann et al., 1997), because of an increased variance of the gammaglobulin fraction in the FIV-positive cats. This may reflect the beginning of a decreased B-cell activation and, with it, an additional step towards immune suppression in some of the FIV-positive cats. As this was not observed in the cats of the present study, it could possibly mean that they had not reached this stage of infection.

No correlation was seen between gammaglobulin levels and clinical signs, such as dermatitis or anorexia, which is consistent with the observations by others (Ackley et al., 1990). However, gammaglobulin levels were seen to correlate negatively with haemoglobin content, haematocrit, mean corpuscular volume and mean cell haemoglobin, probably related to anaemia associated with the disease. Also, high gammaglobulin levels corresponded to high absolute and relative neutrophil numbers, which is also indicative of inflammation. However, other authors have reported that haematological parameters, such as haematocrit, total WBC, neutrophil and lymphocytes were not correlated with the gammaglobulin concentrations (Shelton et al., 1995). The discrepancies observed may be due to the different number of animals included in each study (89 in the present study, five in the one conducted by Shelton), or to the variability of the virulence of the infective strain (Hofmann-Lehmann et al., 1997). In any case, few studies have been undertaken in which the haematological parameters are compared with the serum fractions.

FeLV+ cats were found, on average, to be slightly hypergammaglobulinemic. It has been reported that amongst other observations, FeLV infection (specifically FeLV-induced immunodeficiency) is characterized by hypogammaglobulinemia (Hoover et al., 1987). In fact, most of the animals included in the study had low gammaglobulin levels (86.7%); however, four animals, non related to each other, that had gammaglobu-

lin levels higher than 3.0 g/dl raised the mean. Two of these animals were female kittens 4 and 5 months old, which died of acute respiratory signs shortly after being taken to the practitioner. The high gammaglobulin levels could be due, either to maternal antibodies or to a very severe infection in such young kittens (one of them had severe lymphocytosis of 91%). The other two (also females) had vesicular lesions in the oral mucosa, which could have been caused by secondary infections. The possibility of infection by FIP virus was investigated in these four cats, both serologically with sera 90 days apart (only in the last two cats), and clinically. Both parameters were negative, and FIP was not considered an explanation to the hypergammaglobulinemia.

Double-infected animals had higher levels of α 1-globulin than both FIV+ and FeLV+ cats (Table 3). However, these data have to be regarded cautiously, because of the small number of animals in the group. As regards α 2-globulin, statistically significant differences were also seen between FeLV+ cats and non-infected cats. Most α 1 and α 2 proteins are acute phase proteins, considered to be markers for acute inflammatory disease, infection or neoplastic and traumatic disorders (Duthie et al., 1997; Pocacqua et al., 2005). An increase in these fractions is an indicator of tissue damage and can be attributed to the fact that the pathogen induces an acute phase response in the host, as reported in FIP (Giordano et al., 2004) or in canine leishmaniosis (Miró and Fraile, 1999). One of these acute phase proteins, α 1-acid-glycoprotein or AGP, is considered as immunomodulator, and it has diagnostic value in FIP (Duthie et al., 1997; Kajikawa et al., 1999). It has been reported to be increased in FIV infections (Duthie et al., 1997), which would mean that FIV-positive cats would have elevated α -proteins in the serum, a fact we did not observe. In the present study, cats were not in the terminal stage of the disease (as were the cats in Duthie's study) which may account for this discrepancy. In addition, it further sustains that the altered electrophoretogram in these cats was not caused by FIP.

In conclusion, on the basis of our results, the electrophoretogram undergoes alterations with the retroviral infection, and the electrophoretic profiles become very distinctive depending on the feline retrovirus involved. The plasma electrophoretogram is useful for assessing the initial clinical status, and could be a valuable tool to determine the clinical course of the disease, during and after suitable therapy. High gammaproteins, as seen in FIV-infection, should alert about an increase in the humoral immune response, probably reflecting the uncontrolled replication of the virus and higher presence in the bloodstream. Acute phase proteins, which migrate in the α -globulins fractions, are indicative of microbial infection or following inflammatory stimulus, and also in neoplastic and traumatic disorders, and they are helpful as indicators in clinical diagnosis. After empirical treatment, such as with interferon (De Mari et al., 2004), it would be advisable to follow-up these parameters, to assess the results objectively, economically and easily. A time period study, analysing the changes in the electrophoretogram fractions, would improve our knowledge of the pathogenesis of these viruses.

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