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Research article

CLINICOPATHOLOGICAL FINDINGS IN CATS TESTED FOR FELINE IMMUNODEFICIENCY VIRUS (FIV) AND FELINE LEUKAEMIA VIRUS (FeLV)

Mara BATTILANI[1], Elisa KAEHLER[2], Alessandro TIROLO[1], Andrea BALBONI[1]*, Francesco DONDI[1]

[1]Department of Veterinary Medical Sciences, Alma Mater Studiorum - University of Bologna, Ozzano Emilia, Italy; [2]Ludwig-Maximilians-Universität, München, Germany

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This retrospective study aimed to evaluate the clinicopathological changes in a population of cats tested for feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV), in an Italian Veterinary University Hospital, in the period between January 2002 and May 2016. During the period of 14 years, 1834 cats were tested, and of these 241/1834 (13.1%) were positive for FIV antibodies and 92/1834 (5%) cats were positive for FeLV antigen. These data confirm the presence of a high prevalence of these viruses on Italian territory. To the authors' knowledge, this study describes findings that have never been evaluated before, such as iron status in retrovirus-infected cats and urinalysis in FeLV-positive cats. In this study, FIV-positive cats were more likely to have higher serum protein concentration and lower albumin-globulin ratio than other groups of cats. Lower urine specific gravity and higher urine protein to creatinine ratio were also detected for FIV-positive cats when compared with negative and healthy cats. FeLV-positive cats were more likely to have cytopenia, decreased haemoglobin, haematocrit and RBC compared with other groups of cats. The data obtained underline the importance of considering retroviral infections in the presence of a broad spectrum of risk factors and laboratory anomalies.

Keywords: clinicopathological findings; feline immunodeficiency virus; feline leukaemia virus; iron; UPC ratio; urinalysis

INTRODUCTION

Feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) are common pathogens occurring worldwide in domestic and wild felids [1,2]. Both pathogens belonging to the *Retroviridae* family, are associated with a variety of clinical signs and differences among them in the potential to cause disease. FIV is a *Lentivirus* that can trigger an acquired immunodeficiency syndrome, comparable to that caused by human immunodeficiency virus (HIV) in humans [3]. Infected cats are at high risk of

*Corresponding author: e-mail: a.balboni@unibo.it

developing secondary infections, neoplasia, neurological and chronic inflammatory diseases; despite this, most of the infected cats can remain clinically healthy for many years [4,5]. The symptomatic stage of FIV is frequently associated with haematological and biochemical abnormalities, in particular anaemia, leukopenia, hyperglobulinaemia, and azotaemia are reported [6,7]. FeLV is a *Gammaretrovirus* considered more pathogenic than FIV and has been known to be a cause of virus-related neoplasia, mainly lymphoma and leukaemia [3]. Furthermore, FeLV can cause bone marrow suppression frequently associated with haematological disorders such as anaemia, persistent, transient or cyclic neutropenia, thrombocytopenia, and combined cytopenias [8-10]. Guidelines for the management and prevention of feline retroviruses are available for decades and led to a decrease in prevalence, although the distribution of feline retroviruses infection varies considerably depending on the geographic region, the lifestyle of the feline population, and the diagnostic method chosen [11-14]. FeLV prevalence ranges between 2.3 - 7.5% in North America and 2% in Australia, while in Europe it is somewhat higher, 3.6 - 15.6% [15], although in some European countries FeLV was undetectable [14]. FIV prevalence levels are almost similar: 2.5 - 7.5% in North America, 15% in Australia, and 3.2 - 8.3% in Europe [14-17].

Given the importance and the diffusion of retroviruses infections in cats and since systematic studies about their impact on laboratory variables were primarily conducted under experimental conditions, we carried out a retrospective study aimed to describe clinicopathological abnormalities in naturally retrovirus-infected cats and to compare the results between infected and uninfected subjects. Additionally, the prevalence of the infections was estimated and correlated to signalment data.

MATERIALS AND METHODS

Study design, inclusion criteria, study groups and sampling

This was a retrospective study conducted at the Veterinary University Hospital (VUH) (Department of Veterinary Medical Sciences, University of Bologna), in Northern Italy. The electronic database of the VUH was reviewed and cats screened for FIV and FeLV infection between January 2002 and May 2016 were included in the study. Plasma, serum, or whole blood samples were tested for the presence of FIV antibodies (anti-p24 and anti-gp40) and FeLV antigen (p27) using a commercial point-of-care enzyme-linked immunosorbent assay (ELISA) based test (SNAP FIV/FeLV Combo Plus test, IDEXX, Westbrook, Maine, USA), following the manufacturer instruction. Cats were divided into four groups: FIV-positive, FeLV-positive, FIV and FeLV-positive, FIV and FeLV-negative. FIV and FeLV-positive cats were not included in the statistical analysis to avoid bias in the data interpretation. Medical records were also reviewed to collect signalment, history, clinical and clinicopathological data including complete blood count (CBC), serum chemistry, urinalysis, and urine protein to creatinine ratio (UPC). Another group, constituted of 21 cats selected among blood donors and faculty staff-owned cats and considered healthy on the basis of clinical

and laboratory data, was included in the study for comparison. All cats included in the healthy group were tested negative for FIV antibodies and FeLV antigen with the above-reported test.

Blood sampling was performed by venepuncture and samples were collected by vacuum system (Vacutest Kima, Arzegrande, PD, Italy). Samples of urine were collected by cystocentesis, spontaneous voiding or catheterization. FIV and FeLV tests and clinicopathological evaluation were carried out within 1 hour from the sampling and the samples were stored at –20°C after the examination.

Clinicopathological data

A complete blood count was performed using two automated blood cell counters available during the study period (CELL-DYN 3500 R, Abbott Diagnostics Division, Mountain View, CA, USA and ADVIA 2120, Siemens Healthcare Diagnostics, Erlangen, Germany). Automated haematology was completed by microscopic blood smear examination using May-Grünwald Giemsa staining. A serum chemistry profile including creatinine, urea, phosphate, total protein, albumin, albumin to globulin ratio (A:G), alanine transaminase, aspartate transaminase, alkaline phosphatase, γ-glutamyltransferase (GGT), total bilirubin, cholesterol, total calcium, sodium, potassium, chloride, glucose, serum amyloid A (SAA) as reported previously [18], total iron, total iron-binding capacity (TIBC) and TIBC-saturation was determined. Urinalysis included urine specific gravity (USG), dipstick (Com-bur10TestUX, Roche, Basel, Switzerland) and microscopic sediment examination, and the UPC. Urine samples with a visible red colour and/or >250 red blood cells (RBCs) in a high-power field (hpf) were excluded from the UPC analysis. Serum and urine chemical analysis were carried out using an automated analyser (AU480 Chemistry Analyzer, Beckman Coulter-Olympus, Brea, CA, USA).

Statistical analysis

All the collected data were captured in Microsoft Excel 2019 and analysed using statistical software (MedCalc Statistical Software version 18.5 bvba). Descriptive statistics was performed for all the evaluated variables and data are reported as mean ± standard deviation or median and (range), based on their distribution. Categorical data were analysed using the Chi-squared ($\chi 2$) test. Continuous data (age and clinicopathological results) were compared, using Kruskall Wallis ANOVA, among the study groups. A P value <0.05 was considered significant.

Ethics

Ethical review and approval were waived for this study because it was carried out using blood, serum and urine samples collected with the agreement of the cats' owners for clinical and diagnostic purposes independent of the study. No sampling activities were performed for the purposes of this study. All efforts were made to minimize the discomfort of the animals during sampling.

RESULTS

During the study period, 1834 cats were screened for retroviral infections and included in the study: 1049/1834 (57.2%) were male, of which 610/1049 (58.2%) were castrated, and 778/1834 (42.2%) were female, of which 488/788 (61.9%) were spayed. The median age was of 6 years (range <1 month to 21 years). Of the enrolled cats, 241/1834 (13.1%) showed antibodies against FIV and 92/1834 (5%) were positive for FeLV antigen. Among the tested positive cats, 16 (16/1834, 0.9%) were simultaneously positive for FIV and FeLV and were excluded from the statistical analysis. The 225/1834 (12.3%) cats tested positive only for FIV antibodies were included in the FIV-positive group and the 76/1834 (4.1%) cats tested positive only for FeLV antigen were included in the FeLV-positive one. The cats negative for both viruses were 1517/1834 (82.7%) and were included in the FIV and FeLV negative group. Signalment, clinical and clinicopathological findings are reported in Tables 1, 2, 3 and 4.

Table 1. Signalment of cats tested positive for FIV or FeLV infection, compared with cats tested negative.

	FIV-positive group	**FeLV-positive group**	**FIV and FeLV-negative group**	**P value[a]**
	(224/225) N (%)	**(76/76) N (%)**	**(1511/1517) N (%)**	
Sex				
Male	175 (78.1)	38 (50)	823 (54.5)	
Male intact	72 (32.1)	18 (23.7)	340 (22.5)	P=0.0406
Male neutered	103 (46)	20 (26.3)	483 (31.9)	P=0.0039
Female	49 (21.9)	38 (50)	688 (45.5)	
Female intact	19 (8.5)	10 (13.2)	261 (17.3)	P=0.0638
Female spayed	30 (13.4)	28 (36.8)	427 (28.2)	P<0.0001
	(204/225) N (%)	**(70/76) N (%)**	**(1227/1517) N (%)**	
Age groups (years)				
<1	0 (0)	4 (5.7)	61 (5)	P<0.0001
1.0-4.0	26 (12.8)	24 (34.3)	262 (21.3)	P=0.0002
4.0-8.0	51 (25)	15 (21.4)	365 (30)	P=0.0010
8.0-12.0	75 (36.7)	14 (20)	302 (24.5)	P=0.1050
>12	52 (25.5)	13 (18.6)	237 (19.2)	P<0.0001
	(204/225) Median (Range)	**(70/76) Median (Range)**	**(1227/1517) Median (Range)**	
Age median (years)	9 (<1–21)	6 (<1–17)	6 (<1–20)	P<0.0001

Data are given as: number of cats (% of number of cats/n). N, number of cats in each group for which the signalment data was available. [a] Significance P <0.05 ($\chi 2$ test).

Table 2. Complete blood cell count of cats tested positive for FIV or FeLV infection, compared with cats tested negative and healthy cats.

		FIV-positive group		FeLV-positive group		FIV and FeLV-negative group		Healthy cats group		
Variable	**RI**	**N**	**Median (Range)**	**N**	**Median (Range)**	**N**	**Median (Range)**	**N**	**Median (Range)**	**P value [d]**
Hb (gr%)	10-16	191	10.2 (3-16.6) [a b c]	73	9.2 (2.1-15.8) [a b c]	1249	11.8 (2-22.6) [b]	21	13.1 (11.2-16.2)	<0.001
Hct (%)	32-48	193	30.6 (8.9-48.1) [a b c]	73	26.5 (7-51.5) [a b c]	1256	34.7 (5.4-67.5) [b]	21	38.1 (33.3-48.7)	<0.001
RBC (/mm3) $X10^5$	70-110	192	72.15 (13-118.8) [a b c]	73	61.7 (13.7-116) [a b c]	1247	80.3 (4.95-139) [b]	21	87.3 (79.4-110.3)	<0.001
MCV (fL)	36-55	191	43.1 (32.6-73.4) [c]	73	44.5 (35.9-71.2) [a c]	1250	43.2 (26.2-87.5)	21	44.4 (36.8-73.7)	<0.05
MCHC (g%)	31-36	192	33.85 (12.2-40.4)	73	34.4 (27.7-40)	1250	34.1 (13.5-63.6)	21	33.6 (31.1-35.9)	
MCH (pg)	12.3-16.2	192	14.7 (10.7-29.6) [c]	73	15.2 (12.2-26.5) [a c]	1251	14.8 (6.4-22)	21	14.8 (12.5-18.4)	<0.05
RDW (%)	13-17	184	20.2 (12.3-40.1) [a b]	72	18.6 (14.1-39.3) [a b]	1228	17.3 (12.5-45.1) [b]	21	14.3 (13.4-15.7)	<0.001
Reticulocytes (/mm3)	0-80000	83	30650 (2230-515270)	43	29800 (3970-231900)	687	31650 (2292-308200)	21	49800 (15100-165800)	
PLT (/mm3) $X10^4$	15-50	192	13.95 (0.013-57.7) [a]	73	10.4 (0.4-101.7) [a]	1254	18.8 (0.01-178.7)	21	21.1 (4.5-56.3)	<0.001
MPV (fL)	8-26	115	15.6 (9.6-38.3)	48	18.6 (11.6-66.3)	875	15.8 (8.4-131)	21	16.6 (10-21.5)	
Leukocytes (/mm3)	4800-14930	192	11710 (240-44200)	73	11200 (400-65900)	1255	11205 (48-90200)	21	8530 (4980-18830)	
Neutrophils (/mm3)	1600-10000	185	7377 (30-38916) [b c]	70	5880 (28-47971) [a c]	1196	7600 (10-74866) [b]	21	4470 (1570-13520)	<0.001
Lymphocytes (/mm3)	900-5600	184	1810 (40-16940) [b]	71	1920.0 (70-65900)	1194	1910 (72-59010) [b]	21	3010 (1150-4610)	<0.05
Monocytes (/mm3)	0-650	172	345 (10-6360) [a b]	61	240.0 (10-4100) [b]	1114	270 (10-8708) [b]	21	140 (50-490)	<0.001
Eosinophils (/mm3)	60-1470	149	420 (30-12470) [c]	54	412.5 (12-4220) [a b c]	1047	450 (20-4620)	21	690 (60-1260)	<0.05
Basophils (/mm3)	0-60	50	20 (10-480)	22	10.0 (10-180)	521	20 (10-4090)	21	20 (0-40)	

RI, reference interval; Hb, haemoglobin concentration; HCT, haematocrit value;; MCH, mean corpuscular haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; N, number of cats for which the data were available; PLT, platelets; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell. [a] Significantly different from negative cats. [b] Significantly different from healthy cats. [c] Significantly different between FIV and FeLV positive cats. [d] Significance P <0.05.

Table 3. Serum chemistry of cats tested positive for FIV or FeLV infection, compared with cats tested negative and healthy cats

		FIV-positive group		FeLV-positive group		FIV and FeLV-negative group		Healthy cats group		
Variable	**RI**	**N**	**Median (Range)**	**N**	**Median (Range)**	**N**	**Median (Range)**	**N**	**Median (Range)**	**P value [d]**
Glucose (mg/dl)	63-148	181	110.0 (14.0-447.0) [c]	66	121.5 (55.0-356.0) [b c]	1099	114.0 (3.0-1497.0) [b]	21	101.0 (71.0 – 240)	<0.001
Urea (mg/dl)	30-65	180	60.2 (21.0-741.0) [b]	67	52.3 (24.0-455.6) [b]	1098	56.1 (6.9-946.8) [b]	21	46.7 (31.5 – 73.0)	<0.05
Creatinine (mg/dl)	0.8-1.8	178	1.5 (0.7-31.4)	69	1.3 (0.6-10.8)	1111	1.5 (0.3-25.8)	21	1.5 (0.8 – 2.1)	
Total bilirubin (mg/dl)	0-0.3	131	0.2 (0.0-3.7)	52	0.2 (0.1-13.2)	868	0.2 (0.0-23.1)	21	0.2 (0.2 – 0.3)	
ALT (U/l)	20-72	177	50.0 (13.0-1139.0) [a]	67	50.0 (16.0-857.0)	1102	58.0 (2.0-3826.0)	21	49.0 (33.0 – 92.0)	<0.05
AST (U/l)	9-40	175	33.0 (12.0-605.0) [a b]	65	38.0 (11.0-517.0) [b]	1096	36.0 (6.0-9585.0) [b]	21	26.0 (16.0 – 157.0)	<0.05
GGT (U/l)	0-4	171	0.1 (0.1-7.3) [a b c]	64	0.1 (0.1-3.2) [c]	857	0.1 (0.1-21.5)	13	0.1 (0.1 – 0.6)	<0.05
ALP (U/l)	20-140	168	39.0 (7.0-1101.0) [a b]	63	51.0 (10.0-324.0) [b]	1067	54.0 (5.0-4493.0) [b]	21	69.0 (22.0 – 163.0)	<0.001
CK (U/l)	91-326	42	129.5 (40.0-1339.0) [a]	14	147.0 (39.0-10256.0)	200	229.0 (2.0-26991.0)	0		<0.05
Ca (mg/dl)	8.5-10.5	130	9.4 (4.7-12.8) [a c]	51	9.6 (7.9-18.8) [c]	876	9.6 (2.0-16.7)	21	9.6 (8.8 – 10.2)	<0.05
P (mg/dl)	2.5-6.2	130	4.8 (2.2-32.2)	50	4.7 (2.2-8.7)	875	4.8 (1.7-32.5)	16	4.5 (3.0 – 6.1)	
Na (mEq/l)	145-155	176	149.0 (135.0-170.0) [b]	69	149.0 (137.0-165.0) [a b]	1095	150.0 (108.0-171.0) [b]	21	151.0 (147.0 – 155.0)	<0.05
K (mEq/l)	3.4-5.1	176	4.3 (3.0-7.2)	69	4.3 (2.7-7.0)	1096	4.3 (2.2-11.5)	21	4.2 (3.4 – 4.9)	
Cl (mEq/l)	110-123	72	114.0 (81.0-146.0) [b]	31	115.5 (95.0-144.0) [b]	599	115.0 (76.0-133.0) [b]	17	118.0 (113.0 – 122.0)	<0.05
Mg (mg/dl)	1.9-2.8	11	2.3 (1.8-5.2)	7	2.4 (2.2-4.3)	195	2.4 (1.4-8.6)	17	2.3 (2.1 – 2.5)	
Total protein (g/dl)	6.5-8.8	178	8.3 (5.1-13.1) [a b c]	67	7.8 (5.2-11.3) [c]	1105	7.6 (3.7-15.4)	21	7.7 (7.1 – 8.9)	<0.001
Albumin (g/dl)	2.6-4	178	2.8 (1.4-3.9) [a b c]	67	3.2 (1.9-4.0) [b c]	1098	3.2 (1.2-4.6) [b]	21	3.5 (2.9 – 4.0)	<0.001
Albumin/globulin	0.52-1.19	178	0.5 (0.2-1.0) [a b c]	67	0.7 (0.2-1.4) [b c]	1098	0.7 (0.1-1.5) [b]	21	0.8 (0.6 – 1.3)	<0.001
Total cholesterol (mg/dl)	59-230	127	149.0 (14.0-403.0)	49	137.0 (33.0-251.0)	866	148.0 (30.0-641.0)	13	125.0 (59.0 – 330.0)	
Triglycerides (mg/dl)	10-100	41	52.0 (13.0-2036.0)	13	78.0 (24.0-204.0)	198	58.0 (9.0-1982.0)	0		
SAA (μg/dl)	0-10	12	102.0 (2.0-260.0)	8	21.5 (1.0-218.0)	77	15.5 (1.0-245.0)	0		
Total iron (μg/dl)	68-215	47	62.0 (19.0-193.0) [c]	19	162.0 (37.0-360.0) [a c]	203	79.0 (13.0-424.0)	0		<0.001
TIBC (μg/dl)	205-390	47	242.0 (136.0-517.0) [a c]	19	269.0 (141.0-404.0) [c]	203	269.0 (121.0-479.0)	0		<0.05
TIBC Sat (%)	25-63	47	29.5 (6.0-86.0) [c]	19	61.0 (26.0-93.0) [a c]	203	30.5 (1.0-96.0)	0		<0.001

RI, reference interval; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; Ca, calcium; CK, creatine kinase; Cl, chlorine; GGT, γ-Glutamyl transferase; K, potassium; Mg, magnesium; N, number of cats for which the data was available; Na, sodium; P, phosphate; SAA, serum amyloid a protein; TIBC, total iron binding capacity; TIBC Sat %, total iron binding capacity saturation. [a] Significantly different from negative cats. [b] Significantly different from healthy cats. [c] Significantly different between FIV and FeLV positive cats. [d] Significance P <0.05.

Table 4. Complete urine analysis of cats tested positive for FIV or FeLV infection, compared with cats tested negative and healthy cats.

		FIV-positive group		FeLV-positive group		FIV and FeLV-negative group		Healthy cats group		
Variable	**RI**	**N**	**Median (Range)**	**N**	**Median (Range)**	**N**	**Median (Range)**	**N**	**Median (Range)**	**P value [d]**
pH [f]	5.5-7.5	69	6 (5-8)	29	6 (5-7.5)	480	6 (5-9)	21	6.5 (5.5-8)	
Specific gravity [g]	<1035	69	1024 (1010-1080) [b c]	29	1038 (1012-1080) [b c]	480	1028.5 (1002-1090) [b]	21	1068 (1040-1080)	<0.001
UPC [e]	<0.4 f	34	1.8 (0.1-8.7) [a b]	15	1.4 (0.06-6.8) [b]	83	1.1 (0.1-15.7) [b]	12	0.1 (0.04-0.4)	<0.001
Variable	**RI**	**N**	**Number of positive cats (%)**	**N**	**Number of positive cats (%)**	**N**	**Number of positive cats (%)**	**N**	**Number of positive cats (%)**	
Protein (mg/dl) [f]	negative	69	56 (81)	29	22 (76)	480	350 (73)	21	6 (28.6)	
Glucose (mg/dl) [f]	negative	69	6 (8.7)	29	3 (10.3)	480	64 (13.3)	21	0 (0)	
Bilirubin (mg/dl) [f]	negative	69	6 (8.7)	29	11 (38)	480	77 (16)	21	3 (14.3)	
Ketones (mg/dl) [f]	negative	69	1 (1.4)	29	2 (7)	480	18 (3.8)	21	2 (9.5)	

RI, reference interval; N, number of cats for which the data was available; UPC, urinary protein to creatinine ratio. [a] Significantly different from negative cats. [b] Significantly different from healthy cats. [c] Significantly different between FIV and FeLV-positive cats. [d] Significance P <0.05. [e] Parameter evaluated with the use of the automated chemistry analyzers (AU400 and AU480, Beckman Coulter, Brea, CA, USA). [f] Parameters evaluated using commercially available methods (Roche, Basel, CH). [h] Parameter measured with the use of the refractometer (American Optical Corporation, Buffalo, NY, USA).

DISCUSSION

This study was primarily aimed to describe clinicopathological abnormalities detected in a hospital population of retrovirally infected cats (FIV positive and FeLV positive) retrospectively enrolled in a 14-year period and to compare these findings with those obtained in negative and healthy cats. The values of FIV, FeLV, and FIV-FeLV infection prevalence obtained in this study (13.1%, 5% and 0.9%, respectively) are in line with previous surveys carried out in Italy from 2006 that reported variable prevalence values, ranging from 5.8% to 11.3% for FIV and from 3.8% to 6.1% for FeLV [14,19-22]. The prevalence of infection observed for feline retroviruses in European countries differed significantly among cats living in different regions. In Northern European countries FeLV is an unusual infection or has not been reported for many years, while in Southern Europe several studies reported higher prevalence values correlated to cats living at a much greater risk: stray colony free-ranging and shelter cats are very common in Southern Europe [14,16]. The frequency of positive FeLV cats detected in our study is comparable to findings of a recent epidemiological survey conducted throughout the European territory, which found a prevalence of 5.7% in Italy and Malta [14].

Several studies reported an increased risk of acquiring FIV infection for male intact cats and attributed this finding to the higher propensity of intact male cats to fight [12,14,23-25]. The present study confirms the association between the male gender and a higher frequency of infection, but without a considerable gap among intact and neutered cats, as suggested in other reports [26-29]. Furthermore, FeLV-positive cats were significantly younger than FIV-positive cats, as previously reported [12,30]. This finding was probably related to the decreased life expectancy of progressively FeLV-infected cats [31,32]. Conversely, the estimated death rate of FIV-infected cats was only 18% after five years from the hypothetical time of infection [33].

In the current study, FeLV-positive cats showed haemoglobin, haematocrit and RBCs values significantly lower than other groups. These results corroborate previous studies and are reasonably explained by the involvement of bone marrow affecting FeLV-positive cats [12,34]. Lower erythrocytes concentration can be also explained by increased destruction due to immune-mediated haemolytic anaemia or coinfection with haemoplasmas as previously reported [35,36]. Moreover, FeLV-positive cats had higher values of mean corpuscular volume (MCV) with respect to FIV-positive and negative cats, but not with respect to healthy cats, confirming the association between FeLV-positive status and a high frequency of macrocytosis or macrocytic anaemia [37]. FIV-positive cats showed significantly lower values of haemoglobin, haematocrit and RBC with respect to negative and healthy cats, but not to FeLV-positive cats. These findings contrast with those of a previous study in which FIV-positive cats did not show any significant differences in erythron with respect to control cats [12].

The present study had evaluated serum iron concentration in FIV- and FeLV-positive cats. In the FeLV-positive group, total iron concentration was significantly different

from the FIV-positive group, and 52% of FIV-positive cats had decreased serum iron levels with a median of 40 μg/dl. These findings confirm that a high percentage of cats affected by feline retrovirus displayed anaemia [38], and a probable explanation is an inflammatory response in chronic immune-compromise FIV-positive cats [39].

Another piece of evidence that emerged in FIV-positive cats is the increased value of SAA compared to FeLV-positive or negative cats. This major feline acute-phase protein increases early during inflammation, show higher sensitivity compared to WBC to detect inflammation and it can be used in cats with various diseases as a prognostic marker [18,40,41].

No significant differences in total leucocyte count were observed among groups, as reported in a previous study [30].

In our study, FIV and FeLV-positive cats had a significantly lower platelet count with respect to negative cats but not with healthy cats. Thrombocytopenia is considered a common finding in FeLV-infected cats and may be due to bone marrow suppression or neoplastic infiltration [42]. Conversely, in FIV-infected cats, bone marrow suppression is rarely reported and causes mainly neutropenia, not thrombocytopenia [12,43]. Our results should be cautiously evaluated due to the high frequency of platelet clumps in feline EDTA samples, therefore, in future studies, careful examination of blood smears is required to identify real thrombocytopenia [44,45].

The FIV-positive group had a significant increase in total protein and a decrease in serum albumin together with a higher frequency of hyperproteinaemia (34%), hypoalbuminemia (34%), and decreased albumin globulin ratio (46%), compared to the other groups. These data confirm the correlation between FIV infection and hyperglobulinaemia, which is frequently due to excessive natural immune response against the chronic persistent infection [46-48]. Hypergammaglobulinaemia reflects polyclonal B-cell stimulation and could be a direct consequence of FIV infection [38].

Urinalysis results showed that FIV-positive and FeLV-positive cats are more proteinuric than negative cats. Furthermore, FeLV-positive cats showed a significantly higher proteinuria, with a median UPC value of 1.4, than cats in the healthy group. Proteinuria was previously reported in FIV-infected cats [49-51] and an association between FIV- and FeLV-positive cats and the development of CKD has already been reported [52-54]. As urine analysis can provide essential information on the renal function of cats with retrovirosis, allowing to set up an appropriate therapy, the collection of urine samples and their analysis is indicated if infection is suspected. Furthermore, screening for FIV and FeLV should be considered when a kidney disease is suspected in cats.

The present study has some limitations. Anamnestic information and clinicopathological data were lacking for some patients, so prospective studies focused on the clinical course of cats with retroviruses are needed to better understand the impact of such infections in clinical practice. Another limit is linked to the use of rapid tests to detect FIV and FeLV infection, which in a situation of low prevalence could lead to false positives, inflating the prevalence values detected. Furthermore, antigenic tests are not

able to detect regressive FeLV infections, revealed only by molecular tests, affecting the estimated prevalence of FeLV.

In conclusion, our study provides an overview on the Italian epidemiological situation for FIV and FeLV infection in cats and describes our original findings such as iron status in retrovirus-infected cats and urinalysis in FeLV-positive cats. FIV-positive cats were more likely to have higher serum protein concentration and lower albumin-globulin ratio than other groups of cats. Lower urine specific gravity and higher UPC were also detected for FIV-positive cats when compared with negative and healthy cats. FeLV-positive cats were more likely to have cytopenia, decreased haemoglobin, haematocrit and RBC compared with other groups of cats. The obtained data underline the importance of considering, and screening for, retroviral infections in presence of a wide spectrum of risk factors and laboratory abnormalities.

Authors' contributions

MB and FD create concept and supervised the study. EK, AT, and AB participated in the methodology and participated in formal analysis. EK and AT applied software and data curation in the research process. MB, EK, and AT participated in the investigation, writing, and draft preparation. AB and FD participated in writing, reviewing, and editing the manuscript. FD participated in project administration. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

REFERENCES

1. Courchamp F, Suppo C, Fromont E, Bouloux C: Dynamics of two feline retroviruses (FIV and FeLV) within one population of cats. Proc Biol Sci 1997, 264(1383):785-794.
2. Levy JK, Scott HM, Lachtara JL, Crawford PC: Seroprevalence of feline leukaemia virus and feline immunodeficiency virus infection among cats in North America and risk factors for seropositivity. J Am Vet Med Assoc 2006, 228(3):371-376.
3. Hartmann K: Clinical aspects of feline immunodeficiency and feline leukaemia virus infection. Vet Immunol Immunopathol 2011, 143(3-4):190-201.

4. Abramo F, Bo S, Canese MG, Poli A: Regional distribution of lesions in the central nervous system of cats infected with feline immunodeficiency virus. AIDS Res Hum Retroviruses 1995, 11(10):1247-1253.
5. Gabor LJ, Love DN, Malik R, Canfield PJ: Feline immunodeficiency virus status of Australian cats with lymphosarcoma. Aust Vet J 2001, 79(8):540-545.
6. Shelton GH, Linenberger ML, Abkowitz JL: Haematologic abnormalities in cats seropositive for feline immunodeficiency virus. J Am Vet Med Assoc 1991, 199(10):1353-1357.
7. Thomas JB, Robinson WF, Chadwick BJ, Robertson ID, Beeston SA: Association of renal disease indicators with feline immunodeficiency infection. Journal (USA) 1993.
8. Hartmann K, Gerle K, Leutenegger C, Jarret O: Feline leukaemia virus-most important oncogene in cats. In 4th International Feline Retrovirus Research Symposium 1998.
9. Mauldin GE, Mooney SC, Meleo KA: Chemotherapy in 132 cats with lymphoma: 1988–1994. In 15th Annual Conference of the Veterinary Cancer Society, Tucson 1995, 190(2):174-178.
10. Stützer B, Müller F, Majzoub M, Lutz H, Greene CE, Hermanns W, Hartmann K: Role of latent feline leukaemia virus infection in nonregenerative cytopenias of cats. J Vet Intern Med 2010, 24(1):192-197.
11. Garigliany M, Jolly S, Dive M, Bayrou CL, Berthemin S, Robin P, Godenir R, Petry J, Dahout S, Cassart D, Thiry E, Desmecht D, Saegerman C: Risk factors and effect of selective removal on retroviral infections prevalence in Belgian stray cats. Vet Rec 2016, 178(2):45-45.
12. Gleich SE, Krieger S, Hartmann K: Prevalence of feline immunodeficiency virus and feline leukaemia virus among client-owned cats and risk factors for infection in Germany. J Feline Med Surg 2009, 11(12):985-992.
13. Stavisky J, Dean RS, Molloy MH; Prevalence of and risk factors for FIV and FeLV infection in two shelters in the United Kingdom (2011–2012). Vet Rec 2017, 181(17):451-451.
14. Studer N, Lutz H, Saegerman C, Gönczi E, Meli ML, Boo G, Hartmann K, Hosie MJ, Moestl K, Tasker S, Belák S, Lloret A, Boucraut-Baralon C, Egberink HF, Pennisi MG, Truyen U, Frymus T, Thiry E, Marsilio F, Addie D, Hochleithner M, Tkalec F, Vizi Z, Brunetti A, Georgiev B, Ludwig-Begall LF, Tschuor F, Mooney CT, Eliasson C, Orro J, Johansen H, Juuti K, Krampl I, Kovalenko K, Šengaut J, Sobral C, Borska P, Kovaříková S, Hofmann-Lehmann R: Pan-European study on the prevalence of the feline leukaemia virus infection–reported by the European Advisory Board on Cat Diseases (ABCD Europe). Viruses 2019, 11(11):993.
15. Szilasi A, Dénes L, Krikó E, Murray C, Mándoki M, Balka G: Prevalence of feline leukaemia virus and feline immunodeficiency virus in domestic cats in Ireland. Acta Vet Hung 2021, 68(4):413-420.
16. Little S, Levy J, Hartmann K, Hofmann-Lehmann R, Hosie MJ, Olah G, St. Denis K: 2020 American Association of Feline Practitioners' feline retrovirus testing and management guidelines. J Feline Med Surg 2020, 22:5–30.
17. Szilasi A, Dénes L, Krikó E, Heenemann K, Ertl R, Mándoki M, Vahlenkamp TW, Balka G: Prevalence of feline immunodeficiency virus and feline leukaemia virus in domestic cats in Hungary. JFMS Open Rep 2019, 5(2):2055116919892094.
18. Troìa R, Gruarin M, Foglia A, Agnoli C, Dondi F, Giunti M: Serum amyloid A in the diagnosis of feline sepsis. J Vet Diagn Invest 2017, 29(6):856 –859.
19. Bandecchi P, Dell'Omodarme M, Magi M, Palamidessi A, Prati MC: Feline leukaemia virus (FeLV) and feline immunodeficiency virus infections in cats in the Pisa district of Tuscany

and attempts to control FeLV infection in a colony of domestic cats by vaccination. Vet Rec 2006, 158(16):555-557.

20. Latrofa MS, Iatta R, Toniolo F, Furlanello T, Ravagnan S, Capelli G, Schunack B, Chomel B, Zatelli A, Mendoza-Roldan J, Dantas-Torres F, Otranto D: A molecular survey of vector-borne pathogens and haemoplasmas in owned cats across Italy. Parasit Vectors 2020, 13:1-8.
21. Spada E, Proverbio D, della Pepa A, Perego R, Baggiani L, DeGiorgi GB, Domenichini G, Ferro E, Cremonesi F: Seroprevalence of feline immunodeficiency virus, feline leukaemia virus and Toxoplasma gondii in stray cat colonies in northern Italy and correlation with clinical and laboratory data. J Feline Med Surg 2012, 14(6):369-377.
22. Spada E, Canzi I, Baggiani L, Perego R, Vitale F, Migliazzo A, Proverbio D: Prevalence of Leishmania infantum and co-infections in stray cats in northern Italy. Comp Immunol Microbiol Infect Dis 2016, 45:53-58.
23. Burling AN, Levy JK, Scott HM, Crandall MM, Tucker SJ, Wood EG, Foster JD: Seroprevalences of feline leukaemia virus and feline immunodeficiency virus infection in cats in the United States and Canada and risk factors for seropositivity. J Am Vet Med Assoc 2017, 251(2):187-194.
24. Gates MC, Vigeant S, Dale A: Prevalence and risk factors for cats testing positive for feline immunodeficiency virus and feline leukaemia virus infection in cats entering an animal shelter in New Zealand. N Z Vet J 2017, 65(6):285-291.
25. Goldkamp CE, Levy JK, Edinboro CH, Lachtara JL: Seroprevalences of feline leukaemia virus and feline immunodeficiency virus in cats with abscesses or bite wounds and rate of veterinarian compliance with current guidelines for retrovirus testing. J Am Vet Med Assoc 2008, 232(8):1152-1158.
26. Chhetri BK, Berke O, Pearl DL, Bienzle D: Comparison of risk factors for seropositivity to feline immunodeficiency virus and feline leukaemia virus among cats: a case-case study. BMC Vet Res 2015, 11(1):1-7.
27. Hitt ME, Spangler L, McCarville C: Prevalence of feline immunodeficiency virus in submissions of feline serum to a diagnostic laboratory in Atlantic Canada. Can Vet J 1992, 33(11):723.
28. Ravi M, Wobeser GA, Taylor SM, Jackson ML: Naturally acquired feline immunodeficiency virus (FIV) infection in cats from western Canada: prevalence, disease associations, and survival analysis. Can Vet J 2010, 51(3):271.
29. Yilmaz H, Ilgaz A, Harbour DA: Prevalence of FIV and FeLV infections in cats in Istanbul. J Feline Med Surg 2000, 2(1):69–70.
30. Gleich S, Hartmann K: Hematology and serum biochemistry of feline immunodeficiency virus-infected and feline leukaemia virus-infected cats. J Vet Intern Med 2009, 23(3):552-558.
31. Hartmann K: Feline Leukemia Virus Infection. In: *Infectious Diseases of the Dog and Cat*, 4th ed.; Greene, C.E. Ed. WB Saunders: Philadelphia; 2012, 108-136.
32. Hartmann K, Levy JK: Feline Leukemia Virus Infection. In: Ettinger SJ, Feldman EC, Côté E: *Veterinary Internal Medicine*. 8th ed. St. Louis: Elsevier, WB Saunders: Philadelphia; 2017, 978-983.
33. Beatty JA: Feline Immunodeficiency Virus Infection. In: Ettinger SJ, Feldman EC, Côté E: *Veterinary Internal Medicine*. 8th ed. St. Louis: Elsevier, WB Saunders: Philadelphia; 2017, 971-977.

34. Cotter SM: Management of healthy feline leukaemia virus-positive cats. J Am Vet Med Assoc 1991, 199(10):1470-1473.
35. Criado-Fornelio A, Martinez-Marcos A, Buling-Sarana A, Barba-Carretero JC: Presence of Mycoplasma haemofelis, Mycoplasma haemominutum and piroplasmids in cats from southern Europe: a molecular study. Vet Microbiol 2003, 93(4):307-317.
36. Macieira DB, de Menezes Rde C, Damico CB, Almosny NR, McLane HL, Daggy JK, Messick JB: Prevalence and risk factors for haemoplasmas in domestic cats naturally infected with feline immunodeficiency virus and/or feline leukaemia virus in Rio de Janeiro—Brazil. J Feline Med Surg 2008, 10(2):120-129.
37. Weiser MG, Kociba GJ: Erythrocyte macrocytosis in feline leukaemia virus associated anemia. Vet Pathol 1983, 20(6):687-697.
38. Hartmann K: Clinical aspects of feline retroviruses: a review. Viruses 2012, 4(11):2684-2710.
39. Tompkins MB, Tompkins WA: Lentivirus-induced immune dysregulation. Vet Immunol Immunopathol 2008, 123:45-55.
40. Paltrinieri S: The feline acute phase reaction. Vet J 2008, 177(1):26-35.
41. Tamamoto T, Ohno K, Ohmi A, Goto-Koshino Y, Tsujimoto H: Verification of measurement of the feline serum amyloid A (SAA) concentration by human SAA turbidimetric immunoassay and its clinical application. J Vet Med Sci 2008, 70(11), 1247-1252.
42. Shimoda T, Shiranaga N, Mashita T, Hasegawa A: A haematological study on thirteen cats with myelodysplastic syndrome. J Vet Med Sci 2000, 62(1):59-64.
43. Brown MR, Rogers KS: Neutropenia in dogs and cats: a retrospective study of 261 cases. J Am Anim Hosp Assoc 2001, 37(2):131-139.
44. Ellis J, Bell R, Barnes DC, Miller R: Prevalence and disease associations in feline thrombocytopenia: a retrospective study of 194 cases. J Small Anim Pract 2018, 59(9):531-538.
45. Norman EJ, Barron RC, Nash AS, Clampitt RB: Prevalence of low automated platelet counts in cats: comparison with prevalence of thrombocytopenia based on blood smear estimation. Vet Clin Pathol 2001, 30(3):137-140.
46. Shelton GH, Linenberger ML: Haematologic abnormalities associated with retroviral infections in the cat. In Seminars in Veterinary Medicine and Surgery (small animal) 1995, Vol. 10, No. 4:220-233.
47. Miro G, Domenech A, Escolar E, Collado VM, Tejerizo G, De Las Heras A, Gómez Lucía E: Plasma electrophoretogram in feline immunodeficiency virus (FIV) and/or feline leukaemia virus (FeLV) infections. J Vet Med A Physiol Pathol Clin Med 2007, 54(4):203-209.
48. Thomas JB, Robinson WF, Chadwick BJ: Leukogram and biochemical abnormalities in naturally occurring feline immunodeficiency virus infection. Journal (USA) 1993, 29:272–278.
49. Baxter KJ, Levy JK, Edinboro CH, Vaden SL, Tompkins MB: Renal disease in cats infected with feline immunodeficiency virus. J Vet Intern Med 2012, 26(2):238-243.
50. Poli A, Abramo F, Taccini E, Guidi G, Barsotti E, Bendinelli M, Malvaldi G: Renal involvement in feline immunodeficiency virus infection: a clinicopathological study. Nephron 1993,64(2):282-288.

51. Shelton GH, Linenberger ML, Persik MT, Abkowitz JL: Prospective haematologic and clinicopathologic study of asymptomatic cats with naturally acquired feline immunodeficiency virus infection. J Vet Intern Med 1995, 9(3):133-140.
52. White JD, Malik R, Norris JM, Malikides N: Association between naturally occurring chronic kidney disease and feline immunodeficiency virus infection status in cats. J Am Vet Med Assoc 2010, 236(4):424-429.
53. Barros VR, Bezerra JAB, Bochnakian MS, de Paula VV, Filgueira KD: Epidemiology of feline immunodeficiency virus and feline leukaemia virus in a veterinary teaching hospital. Revista Brasileira de Higiene e Sanidade Animal 2017, 11(2):151-160.
54. Piyarungsri K, Tangtrongsup S, Thitaram N, Lekklar P, Kittinuntasilp A: Prevalence and risk factors of feline lower urinary tract disease in Chiang Mai, Thailand. Sci Rep 2020, 10(1):1-8.

KLINIČKO-PATOLOŠKI NALAZI KOD MAČAKA TESTIRANIH NA VIRUS IMUNODEFICIJENCIJE (FIV) I VIRUS LEUKEMIJE MAČAKA (FeLV)

Mara BATTILANI, Elisa KAEHLER, Alessandro TIROLO, Andrea BALBONI, Francesco DONDI

Ova retrospektivna studija imala je za cilj da proceni kliničko-patološke promene u populaciji mačaka testiranih na virus imunodeficijencije (FIV) i virus leukemije (FeLV), u italijanskoj Veterinarskoj univerzitetskoj bolnici, u periodu od januara 2002. do maja 2016. godine. U periodu od 14 godina testirano je 1834 mačaka, od kojih je 241/1834 (13,1%) bilo pozitivno na FIV antitela i 92/1834 (5%) mačaka pozitivno na FeLV antigen. Ovi podaci potvrđuju prisustvo visoke prevalencije ovih virusa na teritoriji Italije. Prema saznanjima autora, ova studija opisuje nalaze koji nikada ranije nisu bili procenjeni, kao što je status gvožđa kod mačaka inficiranih retrovirusom i analiza urina kod FeLV pozitivnih mačaka. U ovoj studiji, FIV-pozitivne mačke su imale veću koncentraciju proteina u serumu i niži odnos albumin/globulin nego druge grupe mačaka. Niža specifična težina urina i veći odnos proteina i kreatinina u urinu takođe su otkriveni kod FIV-pozitivnih mačaka u poređenju sa negativnim i zdravim mačkama. FeLV-pozitivne mačke su češće imale citopeniju, smanjen hemoglobin, hematokrit i broj eritrocita u poređenju sa drugim grupama mačaka. Dobijeni podaci naglašavaju važnost razmatranja retrovirusnih infekcija u prisustvu širokog spektra faktora rizika i laboratorijskih anomalija.