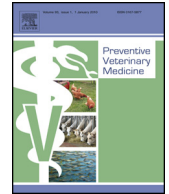




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A retrospective clinical and epidemiological study on feline coronavirus (FCoV) in cats in Istanbul, Turkey



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ARTICLE INFO

Article history:

Received 16 June 2014

Received in revised form 6 January 2015

Accepted 24 January 2015

Keywords:

Antibody

Cat

Feline coronavirus

Clinical

Epidemiology

Istanbul

Turkey

ABSTRACT

The presence of antibodies to feline coronavirus (FCoV) and feline immunodeficiency virus (FIV), together with feline leukemia virus (FeLV) antigen was investigated in 169 ill household and stray cats attending a veterinary surgery in Istanbul in 2009–14. The estimated FCoV and FIV seroprevalence (95% confidence intervals) were 37% (30–45%) and 11% (6–16%), respectively and FeLV prevalence was 1% (0–3%). FCoV seroprevalence increased until 2 years of age, was highest in 2014 and among household cats living with other cats and with outdoor access, and was lower in FIV seropositive compared to seronegative cats. Symptoms typically associated with wet feline infectious peritonitis (FIP) including ascites, abdominal distention or pleural effusion, coupled in many cases with non-antibiotic responsive fever, were observed in 19% (32/169) of cats, and 75% (24/32) of these cats were FCoV seropositive. FCoV seropositivity was also associated with a high white blood cell count, high plasma globulin, low plasma albumin and low blood urea nitrogen. The percentage of FCoV seropositive and seronegative cats that died in spite of supportive veterinary treatment was 33% (21/63) and 12% (13/106), respectively. These results indicate that FCoV is widespread and has a severe clinical impact in cats from Istanbul. Moreover, the incidence of FCoV infections could be rising, and in the absence of effective vaccination cat owners need to be made aware of ways to minimize the spread of this virus.

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1. Introduction

Feline coronaviruses (FCoVs) are enveloped, positive-sense, single-stranded RNA viruses classified as "subgroup 1a" in the family *Coronaviridae* within the order *Nidovirales* (Vijaykrishna et al., 2007). FCoV consists of two biotypes designated as feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV), which are both divided into two serotypes, I and II. Serotype I is of feline origin

and difficult to grow in cell culture. Serotype II appears to have arisen from the recombination of FCoV serotype I with canine coronavirus and grows rapidly in cell culture causing a lytic cytopathic effect (Benetka et al., 2004; Hartmann, 2005; Pedersen, 2009). It is thought that the FIPV biotype may arise from FECVs in individual cats by internal mutation, often in immune suppressed cats (Poland et al., 1996; Vennema, 1999). An alternative hypothesis is that FECVs and FIPVs form distinct viral populations with infection by FIPV causing FIP (Brown et al., 2009).

FCoVs are transmitted by the fecal–oral route and the virus can persist on fomites for 3–7 weeks where they pose a risk of transmission (Hartmann, 2005; Pedersen, 2009;

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Kipar et al., 2010). FCoV primarily infect enterocytes and spread from the intestine by monocyte-associated viremia (Gunn-Moore et al., 1998; Kipar et al., 2005). They have also been shown to replicate in monocytes/macrophages of healthy cats (Can-Sahna et al., 2007; Dye et al., 2008). Vertical transmission has not been demonstrated (Foley et al., 1997). Persistently infected, asymptomatic carriers spread FCoV since most of these cats shed the virus for a period of months or years, either continuously or transiently (Foley et al., 1997; Cave et al., 2004; Dye et al., 2008; Kipar et al., 2010; Sabshin et al., 2012).

The symptoms of FCoV infection are highly variable. Most FCoV-infected cats look healthy with the exception of a mild enteritis (Pedersen, 2009). Up to 12% of FCoV infected cats develop feline infectious peritonitis (FIP), which is a fatal form of the infection (Addie et al., 2009). Development of FIP is strongly associated with stress, immunity, multi-cat households and mainly occurs in young cats between 3 and 16 months of age (Cave et al., 2004; Hartmann, 2005; Bell et al., 2006; Addie et al., 2009; Vogel et al., 2010). Clinically, two forms of FIP are well documented: a 'wet' or effusive form (polyserositis and vasculitis) and a 'dry' or non-effusive form (pyogranulomatous lesions in organs) (Kipar et al., 2005). Ascites is the most prominent manifestation of 'wet form' FIP while lethargy, anorexia, weight loss and fever refractory to antibiotics are common and non-specific signs of FIP (Kipar et al., 2005; Addie et al., 2009).

Diagnosis of FIP is complicated and the cat's clinical history together with results from several analyses including serology, PCR and postmortem analyses are often required before a definite diagnosis can be reached (Shelly et al., 1988; Hartmann et al., 2003; Addie et al., 2004, 2009; Pratelli, 2008; Sharif et al., 2010; Taylor et al., 2010). Hematological and biochemical changes in FIP cases are not very specific, but ascites, increase in serum protein level, increase in bilirubin, decrease in hematocrit and decrease in A:G ratio are prominent (Addie et al., 2009). Serological tests may fail to detect recent infections and cross-reactions occur between FIPV and low pathogenic FECV strains (Hartmann, 2005; Sharif et al., 2010). Molecular detection systems like standard and real time reverse transcription polymerase chain reaction (PCR) have certain advantages as they are rapid and sensitive, particularly when using abdominal or pleural fluid or tissue biopsy or aspirates (Pedersen, 2009; Sharif et al., 2010). A recent PCR test that is commercially available (FIP Virus RealPCR™ Test, IDEXX) allows differentiating FIPV and low pathogenic FECV biotypes, and according to the manufacturers, the test was 99.4% accurate in samples from 88% infected cats with a positive PCR result. PCR results should be evaluated together with clinical findings and postmortem samples should be analyzed by molecular methods (Sparkes et al., 1994; Hartmann et al., 2003; Pratelli, 2008; Addie et al., 2009; Pedersen, 2009; Sharif et al., 2010).

Worldwide the prevalence of FCoV infections may be up to 90% in multi-cat environments and 10–60% in household cats (Herrewegh et al., 1997; Pedersen et al., 2004; Bell et al., 2006; Addie et al., 2009; Sharif et al., 2009; Taharaguchi et al., 2012). Detection of FCoV antibodies in the early stage of infection can be useful to minimize the

spread of FCoVs in a breeding cattery, multi-cat household and FCoV-free household (Cave et al., 2004; Dye et al., 2008; Drechsler et al., 2011). Therefore, it is important to monitor cats living in multi-cat environments in order to reduce and control FCoV infection.

The aim of this study was to investigate FCoV seroprevalence and its relationship with the animal's signalment, habitat, hematological and biochemical parameters and symptoms in cats from Istanbul.

2. Materials and methods

2.1. Study population and sampling

During 5 years, from January 2009 to April 2014, a total of 169 cats with symptoms compatible with feline viral infections were included in the study population. They included individuals with fever, depression, dullness and/or weight loss. They were examined by two different veterinarians working at a private Veterinary Clinic in Istanbul. The animals' gender, breed, age and habitat whether household, shelter or street (stray cats) was recorded. Other data from household cats included if they were adopted or home raised from birth, they cohabitated with other cats and had outdoor access.

Cats were clinically examined to detect fever, skin lesions, behavioral changes (insidious onset, depression) and symptoms related to organ systems were recorded; specifically, cardiorespiratory (dyspnea, abnormal heart and lung sounds), gastrointestinal (anorexia, weight loss, stomatitis, enteritis, abdominal distension, vomiting, ascites), urinary, circulatory (lymphadenopathy, anemia, icterus), ocular lesions (keratic precipitates, uveitis, hyphema, iridocyclitis, chorioretinitis) and central nervous system (epileptic seizures, ataxia) symptoms.

Blood samples were taken from the cephalic vein by the veterinarians examining the cats for hematological and biochemical analyses and to detect antibodies against FCoV and feline immunodeficiency virus (FIV) together with feline leukemia virus (FeLV) antigen as described below. All analysis except FCoV IFAT antibodies were carried out at the veterinary clinic within an hour of taking the blood sample. IFAT tests and protein electrophoresis were carried out at an external private veterinary laboratory.

Disease progression of the study cats was evaluated during repeat visits to the clinic and mortality was considered to be associated to the current infection when the cat did not respond to standard treatments which included fluid and antibiotic therapy.

2.2. Analysis of serum samples for antibodies to FCoV and FIV and for FeLV antigen

All serum samples ($n=169$) were analyzed by rapid tests for the presence of antibodies to FCoV (Bionote, Anigen, FCoV) and FIV (Bionote, Anigen FIV Ab), and FeLV antigen (Bionote, FeLV Ag) following kits' instructions. According to the manufacturers, the sensitivity (Se) and specificity (Sp) of the FCoV test compared to the reference immunofluorescence antibody test (IFA) were 96.0% and 97.9%, respectively, Se and SP of the FeLV test versus virus

Table 1a
Percentage of FCoV seropositive cats according to examination year, gender, breed, and age.

Variables	Level	N	No. seropositive	% seropositive	95% CI		p value
					Lower	Upper	
Study year	2009	13	9	31	6	56	<0.0001
	2010	20	15	25	6	44	
	2011	19	17	11	0	24	
	2012	35	24	31	16	47	
	2013	52	36	31	18	43	
	2014	30	5	83	70	97	
Gender	Female	70	42	40	29	51	0.6499
	Male	99	64	35	26	45	
Breed	Cross breed	142	88	38	30	46	0.8062
	Pure breed	27	18	33	16	51	
Age (years)	0.1–0.4	25	20	20	4	36	0.2031
	0.6–0.8	13	8	38	12	65	
	1	38	22	42	26	58	
	2	24	12	50	30	70	
	3	24	12	50	30	70	
	4–5	16	13	19	0	38	
	6–9	14	10	29	5	52	
	10–15	15	9	40	15	65	

isolation were 94.7% and 99.7%, respectively, and that of the FIV tests versus Western Blot were 96.8% and 99.6%, respectively. Sera found to be positive for antibodies to FCoV by the rapid test were analyzed by IFA in an external private laboratory to confirm the result. Serum giving fluorescence at a dilution above 1:20 was considered positive for antibodies against this virus.

2.3. Hematological and biochemical analyses

All blood samples were analyzed for a complete blood hemogram–histogram (18 parameters) using a Veterinary Specific Mindray blood analyzing kit and the Hemogram Instrument (Mindray). Samples were also analyzed for comprehensive blood biochemistry (14 parameters); 60 samples were analyzed using the Vet-Scan (Abaxis) kit and the remaining samples were analyzed using Reflotron (Roche) kit. Serum protein electrophoresis was performed for serum samples positive for antibodies to FCoV. Ascitic fluid from 14 cats was analyzed for albumin/globulin (A:G) ratio. Hematological and biochemical tests included total white blood cell count (WBC), lymphocyte and red blood cell counts, hematocrit, hemoglobin, total protein, albumin (alb.), globulin (glob.), alkaline phosphatase (ALP), alanine transaminase (ALT), amylase, total bilirubin, blood urea nitrogen (BUN), creatinine, glucose, calcium, phosphorus, sodium and potassium.

2.4. Statistical analyses

Data were analyzed using R (<http://cran.r-project.org/>) software. Approximately unbiased estimates of prevalence were calculated assuming known values of test Se and Sp using the Rogan–Gladen statistic (Greiner and Gardner, 2000). Yates-corrected chi squared test, or when appropriate Fisher's exact test (Kirwood and Sterne, 2003), was used to compare the proportion of FCoV seropositive cats

according to cat demographic and habitat explanatory variables and the proportion of signs in FCoV seropositive and seronegative cats. Biochemical and hematological results were categorized as being within (normal), above (high) or below (low) reference values (Villiers and Blackwood, 2005).

The independent relationship between FCoV serological status, and a cat's demographic, habitat and FIV serological status was further investigated using logistic regression analysis (Kleinbaum and Klein, 2010). FCoV was the binary outcome variable (seropositive or seronegative). The explanatory variables included those associated at $p < 0.05$ with FCoV serological status in the univariable analysis; they were FIV serological status, age, examination year and the variable reflecting living place, outdoor access and contact with other cats (Tables 1a and 1b). All variables were included in the model as categorical variables as shown in Tables 1a and 1b, except age, which included seven levels after combining data from 4 to 15 year old cats into a single level. Model parameters were estimated using the maximum likelihood estimation method and significance was taken for alpha less than 5% for a double sided test.

3. Results

3.1. Estimated FCoV and FIV seroprevalence and FeLV prevalence

Antibodies to FCoV and FIV were detected in 63/169 and 19/169 cats. Two out of 169 cats were positive for FeLV antigen. The estimated FCoV and FIV seroprevalence and FeLV prevalence (95% CI) adjusted for tests Se and Sp, were 37% (30–45%), 11% (6–16%) and 1% (0–3%), respectively. All cats testing FCoV antibody positive to the rapid test were also IFAT antibody positive.

Table 1b
Percentage of FCoV seropositive cats according to origin, habitat and FIV status.

Variables	Level	N	No. seropositive	% seropositive	95% CI		p value
					Lower	Upper	
Home	Household	122	73	40	31	49	0.2835
	Street	47	33	30	17	43	
Origin	Home raised	50	23	54	40	68	<0.0001
	Pet shop	16	6	63	39	86	
	Shelter adopted	3	0	100	100	100	
	Street adopted	100	77	26	18	34	
Outdoor access	No	75	53	29	19	40	0.0805
	Yes	94	53	44	34	54	
Cohabiting with other cats	No	37	12	14	2	25	0.0014
	Yes	132	94	44	35	52	
Home; outdoor access; cohabiting with other cats	Street, yes; yes	47	33	30	17	43	0.0002
	House; no; no	37	32	14	2	25	
	House; no; yes	37	21	43	27	59	
	House; yes; yes	47	20	57	43	72	

3.2. Relationship between FCoV serological status, year, demographic and habitat variables

FCoV seroprevalence varied significantly by study year, origin and habitat variables ($p < 0.05$) (Tables 1a and 1b). Furthermore, FCoV seroprevalence was 5% (1/19) and 41% (62/150) among FIV seropositive and seronegative cats, respectively ($p < 0.05$). Logistic regression analysis confirmed the independent relationship of FCoV serological status with examination year, age, FIV status, habitat and contact with other cats (Table 2).

3.3. Clinical symptoms, prognosis and relationship with FCoV serological status

Clinical examination revealed depression or dullness, fever and low body weight in 81% (137/169), 76% (128/169) and 70% (119/169) of study cats, respectively, and 57% (96/169) of cats had all three signs. The percentage of cats with ascites, abdominal distension and pleural effusion was 10% (17/169), 14% (24/169) and 5% (8/169), respectively. All three symptoms were present in only one cat, no cats had pleural effusion and ascites or abdominal enlargement alone; in contrast, ascites and abdominal enlargement without pleural effusion were observed in 9% (15/169) of cats and 19% (32/169) of cats presented one of these three conditions. The cat with all three symptoms was FCoV seronegative instead; FCoV seroprevalence was 93% (14/15) in cats with ascites and abdominal enlargement and 75% (24/32) in cats with at least one of the three symptoms, and 94% (30/32) of these were dull or depressed, had fever and/or low body weight.

The percentage of some clinical signs differed according to the cat's FCoV serological status (Table 3a). Other symptoms found included dyspnea (20/169), stomatitis (13/169), ocular signs (12/169), urinary tract signs (12/169) and epilepsy (2/169) (not shown in table format). The prevalence of these symptoms was not significantly different between FCoV seropositive and seronegative cats.

Thirty-three percent of FCoV seropositive cats (21/63) and 12% (13/106) of seronegative cats died from the

condition for which they were admitted in spite of receiving treatment ($p < 0.05$).

3.4. Hematological and biochemical parameters and relationship with FCoV serological status

Results of the hematology and clinical chemistry are shown in Table 3b. Abnormalities were particularly frequent in cats with ascites and pleural effusion and 72% (23/32) and 76% (24/29) of cats with these signs had high WBC and low A:G ratio, respectively (not tabulated).

4. Discussion

This study shows that FCoV infections are widespread in cats from Istanbul and this is in agreement with other studies elsewhere (Sparkes et al., 1992; Pedersen et al., 2004; Pesteanu-Somogyi et al., 2006; Sharif et al., 2009; Taharaguchi et al., 2012; Paris et al., 2014). Moreover, FCoV seroprevalence increased in 2014 compared to previous years and this may suggest that FCoV infections are an increasing health problem in cats in Istanbul. High FCoV seroprevalence (up to 84%) has been reported in many countries (Sparkes et al., 1992; Holst et al., 2006; Pratelli, 2008; Pratelli et al., 2009; Sabshin et al., 2012; Taharaguchi et al., 2012). In contrast, FCoV seroprevalence was comparatively low in chronically ill (19.3%) and even lower in healthy cats (10.1%) in Korea (Dong-Jun et al., 2011). Prevalence may vary depending on the inclusion criteria used (normal versus ill cats) and estimates may be affected by selection bias, analytical errors and imperfect diagnostic tests.

Several risk factors have been reported to be associated with FCoV infection and with FIP development, including age, breed, gender, multi-cat environment and stress (Bell et al., 2006; Pesteanu-Somogyi et al., 2006; Addie et al., 2009; Sharif et al., 2009; Worthing et al., 2012). In the present study, FCoV serological status was significantly associated with year, age, FIP serological status and habitat variables. The risk of infection would be expected to rise during the first months or years of life due to

Table 2

Estimates from the logistic-regression model of FCoV serological status conversion. A study of cats from Istanbul in 2009–14.

Variable	Level	OR	95% CI		p value
			Lower	High	
Examination year	2009	1	–	–	–
	2010	0.54	0.08	3.54	0.5228
	2011	0.14	0.01	1.35	0.0896
	2012	0.57	0.11	3.01	0.5088
	2013	0.72	0.15	3.55	0.6916
	2014	7.57	1.25	45.83	0.0275
Age (years)	0.1–0.4	1	–	–	–
	0.6–0.8	1.55	0.22	10.86	0.6587
	1	1.41	0.30	6.68	0.6641
	2	3.98	0.83	19.12	0.0844
	3	7.57	1.34	42.86	0.0222
	4–15	1.32	0.28	6.23	0.7258
Home; outdoor access	House; no; no	1	–	–	–
Cohabiting with cats	House; no; yes	6.09	1.48	25.03	0.0122
	House; yes; yes	12.87	3.15	52.57	0.0004
	Street; yes; yes	3.12	0.77	12.63	0.1111
FIV status	Seronegative	1	–	–	–
	Seropositive	0.05	0.01	0.50	0.0106

Table 3a

Percentage of cats with clinical signs according to their FCoV serological status. A study of cats from Istanbul in 2009–14.

Variable	FCoV						p value
	Seropositives			Seronegatives			
	N	No. affected	% (95 CI)	N	No. affected	% (95 CI)	
Fever	63	41	65 (53–77)	106	87	82 (75–89)	0.0211
Depression or dullness	63	49	78 (68–88)	106	88	83 (76–90)	0.5235
Weight loss	63	37	59 (47–71)	106	82	77 (69–85)	0.0144
Vomiting	63	7	11 (3–19)	106	35	33 (24–42)	0.0027
Abdominal distention	63	17	27 (16–38)	106	7	7 (2–11)	0.0006
Ascites	63	15	24 (13–34)	106	2	2 (0–4)	<0.0001
Diarrhea	63	9	14 (6–23)	106	4	4 (0–7)	<0.0001
Pleural effusion	63	6	10 (2–17)	106	2	2 (0–4)	0.0532

increasing cat-to-cat contact. However, it is possible that infection prevalence among 0.1–0.4 year-olds may have been underestimated as several weeks would be needed for anti-FCoV antibodies to develop following infection. Other studies have reported greater FCoV prevalence in cats 3–11 months of age (Bell et al., 2006; Pedersen, 2009; Taharaguchi et al., 2012). Instead, a study in Australia and Malaysia found no association between age and FCoV infection in cats (Bell et al., 2006; Sharif et al., 2009).

Household cats living alone had the lowest risk of being FCoV seropositive, as reported in other studies (Addie et al., 2009; Drechsler et al., 2011). In contrast, this study found that household cats that cohabitated with other cats had a high risk of being FCoV seropositive, as has been previously shown (Foley et al., 1997; Herrewegh et al., 1997; Pedersen et al., 2004; Pesteau-Somogyi et al., 2006; Sharif et al., 2009; Sabshin et al., 2012). Moreover, in the present study, FCoV seroprevalence was lower in stray cats (30%) compared to cats living at home (57%). It is possible that stray cats have poorer health and increased risk of dying from FCoV infections compared to household cats. Alternatively, stray cats could be exposed to less FCoV compared to household cats, who commonly share the same litter box and eat from the same food bowl as other cats in the

household. Furthermore, immunological differences could exist between stray and household cats, with the latter being naturally selected for a protective Th-1 mediated rather than a Th-2 antibody mediated response. This, however, has not been investigated and remains speculative.

Interestingly, FIV seroprevalence was negatively associated with FCoV infection in this study. The reason for this is unclear. It could be because household cats are vaccinated for FIV in Istanbul. It is also possible that coinfecting cats are at greater risk of dying as a result of FIV immunosuppression compared to cats that are only FCoV seropositive.

In the present study, no difference in FCoV seroprevalence was found between females and males. Similar results have been found by others (Cave et al., 2004; Bell et al., 2006; Holst et al., 2006; Sharif et al., 2009; Taharaguchi et al., 2012). Instead, in Australia and the USA, male cats were found to be more frequently infected with FCoV (Pesteau-Somogyi et al., 2006; Worthing et al., 2012). There is no known biological reason supporting gender-associated susceptibility and resistance to FCoV, and differences between studies could be related to males and females having different lifestyles and FCoV exposure.

Breed was not associated with FCoV seropositivity in the present study. In contrast, higher FCoV seroprevalence has

Table 3b

Percentage of cats with altered hematological and biochemical parameters according to their FCoV serological status. A study of cats from Istanbul in 2009–14.

Variable	FCoV						p value
	Seropositives			Seronegatives			
	N	No. affected	% (95 CI)	N	No. affected	% (95 CI)	
Hematology							
High WBC	63	41	65 (53–77)	106	41	39 (29–48)	0.0016
Low WBC	63	3	5 (0–10)	106	18	17 (10–24)	0.0369
High lymphocytic count	63	18	29 (17–40)	106	22	21 (13–28)	0.3326
Low lymphocytic count	63	4	6 (0–12)	106	9	8 (3–14)	0.7690
High red blood cell count	63	21	33 (22–45)	106	25	24 (16–32)	0.2309
Low red blood cell count	63	20	32 (20–43)	106	21	20 (12–27)	0.1177
High hemoglobin	63	53	84 (75–93)	106	96	91 (85–96)	0.3140
Low hemoglobin	63	0	0 (0–0)	106	0	0 (0–0)	1.0000
High hematocrit	63	9	14 (6–23)	106	6	6 (1–10)	0.1038
Low hematocrit	63	29	46 (34–58)	106	36	34 (25–43)	0.1627
Proteins							
High albumin	63	0	0 (0–0)	63	1	2 (0–5)	1.0000
Low albumin	63	28	44 (32–57)	63	5	8 (1–15)	<0.0001
High globulin	63	47	65 (53–77)	63	7	11 (3–19)	<0.0001
Low globulin	63	0	0 (0–0)	63	0	0 (0–0)	1.0000
High alb./glob. ratio	63	2	3 (0–8)	63	2	3 (0–8)	1.0000
Low alb./glob. ratio	63	43	68 (58–80)	63	9	14 (6–23)	<0.0001
High total protein	63	27	43 (31–55)	63	9	14 (6–23)	0.0008
Low total protein	63	0	0 (0–0)	63	0	0 (0–0)	1.0000
Kidney							
High BUN	63	0	0 (0–0)	63	4	6 (0–12)	0.1190
Low BUN	63	6	10 (2–17)	63	0	0 (0–0)	0.0276
High urea	63	15	24 (13–34)	76	50	66 (55–76)	0.0001
Low urea	63	0	0 (0–0)	76	0	0 (0–0)	0.2702
High creatinine	63	4	6 (0–12)	74	7	9 (3–16)	0.7246
Low creatinine	63	0	0 (0–0)	74	0	0 (0–0)	0.3473
Liver							
High ALT	63	12	19 (9–29)	106	35	33 (24–42)	0.0747
Low ALT	63	0	0 (0–0)	106	2	2 (0–4)	0.5296
High ALP	63	5	8 (1–15)	68	2	3 (0–7)	0.2603
Low ALP	63	4	6 (0–12)	68	6	9 (2–16)	0.7460
High bilirubin	63	0	0 (0–0)	65	2	3 (1–7)	0.4962
Low bilirubin	63	0	0 (0–0)	65	0	0 (0–0)	0.8597

been reported in purebred cats compared to non-pedigree cats in several studies (Bell et al., 2006; Pesteanu-Somogyi et al., 2006; Holst et al., 2006; Taharaguchi et al., 2012). In Japan, seroprevalence was higher among pedigree cats including American curl, Maine coon, Norwegian forest cat, Ragdoll and Scottish fold compared to American shorthair, Himalayan, Oriental, Persian, and Siamese (Taharaguchi et al., 2012). In Australia, Siamese, Persian, Domestic Shorthairs and Bengal cats had significantly lower prevalence than British Shorthairs, Cornish Rex and Burmese cats (Bell et al., 2006). Australian studies reported FCoV to be prevalent among British Shorthair, Devon Rex and Abyssinian breeds (Worthing et al., 2012). In Malaysia, FCoV seroprevalence was higher in Persian (96%) than in mix-breed cats (70%) (Sharif et al., 2009). In the present study there were too few cats of specific breeds to allow robust statistical comparisons. Moreover, pure and mixed breed cats did not differ significantly in terms of street access and contact with other cats.

Critical evaluation is necessary for a cat to be diagnosed with FIP. Diagnosis is based on evaluation of history, symptoms, hematological and biochemical parameters, diagnostic tests, radiology and tissue biopsy results (Shelly et al., 1988; Sparkes et al., 1994; Hartmann et al., 2003;

Addie et al., 2009; Pedersen, 2009; Sharif et al., 2010; Tsai et al., 2011). It has been reported that up to 12% of FCoV infected cats develop FIP (Hartmann, 2005; Addie et al., 2009; Pedersen, 2009). Although in the present study no definitive diagnosis of FIP was attempted, 19% of cats had typical signs of wet FIP including abdominal distension, ascites and pleural effusion, and increased γ -globulin and decreased albumin-to-globulin (A:G) ratio. Differences in the percentage of FCoV cats developing FIP between studies could be associated with the cat populations examined; cats investigated in this study were clinically ill and suspected of having a viral infection.

5. Conclusions

This study indicates that FCoV infection in cats from Istanbul is high and possibly increasing. Preventive actions are necessary in multi-cat environments (shelters, catteries and pet shops) and single household cats with outdoor access. Cats presenting with general malaise, including fever not responding to antibiotics, depression, ascites, abdominal distension, diarrhea, pleural effusion, postsurgical complications and a low A:G ratio should be suspected of suffering from FIP.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Addie, D., Belak, S., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Hartmann, K., Hosie, M.J., Lloret, A., Lutz, H., Marsilio, F., Pennisi, M.G., Radford, A.D., Thiry, E., Truyen, U., Horzinek, M.C., 2009. Feline infectious peritonitis. ABCD guidelines on prevention and management. *J. Feline Med. Surg.* 11 (7), 594–604.
- Addie, D.D., McLachlan, S.A., Golder, M., Ramsey, I., Jarrett, O., 2004. Evaluation of an in-practice test for feline coronavirus antibodies. *J. Feline Med. Surg.* 6 (2), 63–67.
- Bell, E.T., Malik, R., Norris, J.M., 2006. The relationship between the feline coronavirus antibody titre and the age, breed, gender and health status of Australian cats. *Aust. Vet. J.* 84, 2–7.
- Benetka, V., Kubber-Heiss, A., Kolodziejek, J., Nowotny, N., Hofmann-Parisot, M., Mostl, K., 2004. Prevalence of feline coronavirus types I and II in cats with histopathologically verified feline infectious peritonitis. *Vet. Microbiol.* 99, 31–42.
- Brown, M.A., Troyer, J.L., Pecon-Slatery, J., Roelke, M.E., O'Brien, S.J., 2009. Genetics and pathogenesis of feline infectious peritonitis virus. *Emerg. Infect. Dis.* 15, 1445–1452.
- Can-Sahna, K., Ataseven, V.S., Pinar, D., Oguzoglu, T.C., 2007. The detection of feline coronaviruses in blood samples from cats by mRNA RT-PCR. *J. Feline Med. Surg.* 9, 369–372.
- Cave, T.A., Golder, M.C., Simpson, J., Addie, D.D., 2004. Risk factors for feline coronavirus seropositivity in cats relinquished to a UK rescue charity. *J. Feline Med. Surg.* 6 (2), 53–58.
- Dong-Jun, A., Hye-Young, J., WooSeog, J., Jee-Yong, P., Myoung-Heon, L., Bong-Kyun, P., 2011. Prevalence of Korean cats with natural feline coronavirus infections. *Virology* 41, 8, 455.
- Drechsler, Y., Alcaraz, A., Bossong, F.J., Collisson, E.W., Diniz, P.P., 2011. Feline coronavirus in multicat environments. *Vet. Clin. N. Am. Small Anim. Clin.* 41 (6), 1133–1169. <http://dx.doi.org/10.1016/j.cvsm.2011.08.004>.
- Dye, C., Helps, C.R., Siddell, S.G., 2008. Evaluation of real-time RT-PCR for the quantification of FCoV shedding in the faeces of domestic cats. *J. Feline Med. Surg.* 10 (2), 167–174.
- Foley, J.E., Poland, A., Carlson, J., Pedersen, N.C., 1997. Patterns of feline coronavirus infection and fecal shedding from cats in multiple-cat environments. *J. Am. Vet. Med. Assoc.* 210, 1307–1312.
- Greiner, M., Gardner, I.A., 2000. Application of diagnostic tests in veterinary epidemiologic studies. *Prev. Vet. Med.* 45 (1–2), 43–59.
- Gunn-Moore, D.A., Gruffydd-Jones, T.J., Harbour, D.A., 1998. Detection of feline coronaviruses by culture and reverse transcriptase polymerase chain reaction of blood samples from healthy cats and cats with clinical feline infectious peritonitis. *Vet. Microbiol.* 62, 193–205.
- Hartmann, K., 2005. Feline infectious peritonitis. *Vet. Clin. North Am. Small Anim. Pract* 35 (1), 39–79.
- Hartmann, K., Binder, C., Hirschberger, J., Cole, D., Reinacher, M., Schroo, S., Frost, J., Egberink, H., Lutz, H., Hermanns, W., 2003. Comparison of different tests to diagnose feline infectious peritonitis. *J. Vet. Intern. Med.* 17 (6), 781–790.
- Herrewegh, A.A., Mahler, M., Hedrich, H.J., Haagmans, B.L., Egberink, H.F., Horzinek, M.C., Rottier, P.J., de Groot, R.J., 1997. Persistence and evolution of feline coronavirus in a closed cat-breeding colony. *Virology* 234 (2), 349–363.
- Holst, B.S., Englund, L., Palacios, S., Renstrom, L., Berndtsson, L.T., 2006. Prevalence of antibodies against feline coronavirus and *Chlamydomydia felis* in Swedish cats. *J. Feline Med. Surg.* 8, 207–211.
- Kipar, A., May, H., Menger, S., Weber, M., Leukert, W., Reinacher, M., 2005. Morphological features and development of granulomatous vasculitis in feline infectious peritonitis. *Vet. Pathol.* 42, 321–330.
- Kipar, A., Meli, M.L., Baptiste, K.E., Bowker, L.J., Lutz, H., 2010. Sites of feline coronavirus persistence in healthy cats. *J. Gen. Virol.* 7 (91), 1698–1707.
- Kirwood, B.R., Sterne, J.A.C., 2003. *Essential Medical Statistics*, second ed. Blackwell Publishing, Oxford, UK.
- Kleinbaum, D.G., Klein, M., 2010. *Logistic Regression. A Self-Learning Test. Statistics for Biology and Health*, third ed. Springer, London.
- Paris, J.K., Wills, S., Balzer, H.J., Shaw, D.J., Gunn-Moore, D.A., 2014. Enteropathogen co-infection in UK cats with diarrhoea. *BMC Vet. Res.* 12, 10–13.
- Pedersen, N.C., Sato, R., Foley, J.E., Poland, A.M., 2004. Common virus infections in cats, before and after being placed in shelters, with emphasis on feline enteric coronavirus. *J. Feline Med. Surg.* 6 (2), 83–88.
- Pedersen, N.C., 2009. A review of feline infectious peritonitis virus infection: 1963–2008. *J. Feline Med. Surg.* 11 (4).
- Pesteanu-Somogyi, L.D., Radzai, C., Pressler, B.M., 2006. Prevalence of feline infectious peritonitis in specific cat breeds. *J. Feline Med. Surg.* 8 (1), 1–5.
- Poland, A.M., Vennema, H., Foley, J.E., Pedersen, N.C., 1996. Two related strains of feline infectious peritonitis virus isolated from immunocompromised cats infected with a feline enteric coronavirus. *J. Clin. Microbiol.* 34 (12), 3180–3184.
- Pratelli, A., Yesilbag, K., Siniscalchi, M., Yalcin, E., Yilmaz, Z., 2009. Prevalence of feline coronavirus antibodies in cats in Bursa province, Turkey, by an enzyme-linked immunosorbent assay. *J. Feline Med. Surg.* 11 (10), 881–884.
- Pratelli, A., 2008. Comparison of serologic techniques for the detection of antibodies against feline coronaviruses. *J. Vet. Diagn. Invest.* 20 (1), 45–50.
- Sabshin, S.J., Levy, J.K., Tupler, T., Tucker, S.J., Greiner, E.C., Leutenegger, C.M., 2012. Enteropathogens identified in cats entering a Florida animal shelter with normal feces or diarrhea. *J. Am. Vet. Med. Assoc.* 241, 331–337.
- Sharif, S., Arshad, S.S., Hair-Bejo, M., Omar, A.R., Zeenathul, N.A., Hafidz, M.A., 2009. Prevalence of feline coronavirus in two cat populations in Malaysia. *J. Feline Med. Surg.* 12, 1031–1034.
- Sharif, S., Arshad, S.S., Hair-Bejo, M., Omar, A.R., Zeenathul, N.A., Alazawy, A., 2010. Diagnostic methods for feline coronavirus: a review. *Vet. Med. Int.* 7, 28.
- Shelly, S.M., Scarlett-Kranz, J., Blue, J.T., 1988. Protein electrophoresis on effusions from cats as a diagnostic test for feline infectious peritonitis. *J. Am. Anim. Hosp. Assoc.* 24, 495–500.
- Sparkes, A.H., Gruffydd-Jones, T.J., Harbour, D.A., 1994. An appraisal of the value of laboratory tests in the diagnosis of feline infectious peritonitis. *J. Am. Anim. Hosp. Assoc.* 30, 345.
- Sparkes, A.H., Gruffydd-Jones, T.J., Harbour, D.A., 1992. Feline coronavirus antibodies in UK cats. *Vet. Rec.* 131, 223–224.
- Taharaguchi, S., Soma, T., Hara, M., 2012. Prevalence of feline coronavirus antibodies in Japanese domestic cats during the past decade. *J. Vet. Med. Sci.* 74, 1355–1358.
- Taylor, S.S., Tappin, S.W., Dodkin, S.J., Papasouliotis, K., Casamian-Sorrosal, D., Tasker, S., 2010. Serum protein electrophoresis in 155 cats. *J. Feline Med. Surg.* 12, 643–653.
- Tsai, H.Y., Chueh, L.L., Lin, C.N., Su, B.L., 2011. Clinicopathological findings and disease staging of feline infectious peritonitis: 51 cases from 2003 to 2009 in Taiwan. *J. Feline Med. Surg.* 13, 74–80.
- Vennema, H., 1999. Genetic shift and drift during feline coronavirus evolution. *Vet. Microbiol.* 69, 139–141.
- Vijaykrishna, D., Smith, G.J., Zhang, J.X., Peiris, J.S., Chen, H., Guan, Y., 2007. Evolutionary insights into the ecology of coronaviruses. *J. Virol.* 81, 4012–4020.
- Villiers, E., Blackwood, L. (Eds.), 2005. *BSAVA Manual of Canine and Feline Clinical Pathology*. British Small Anim. Vet. Assoc., Gloucester, UK.
- Vogel, L., Van der Lubben, M., Telintelo, E.G., Bekker, C.P., Geerts, T., Schuijff, L.S., Grinwis, G.C., Egberink, H.F., Rottier, P.J., 2010. Pathogenic characteristics of persistent feline enteric coronavirus infection in cats. *Vet. Res.* 41, 71.
- Worthing, K., Wigney, D., Dhand, N., Fawcett, Q.A., McDonagh, P., Malik, K.R., Norris, J., 2012. Risk factors for feline infectious peritonitis in Australian cats. *J. Feline Med. Surg.* 14, 405–412.