Cross-sectional and histopathological studies of Feline Coronavirus infections in stray cats in Kuwait

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FCoV,
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Summary
Feline Coronavirus (FCoV) is a worldwide viral infection of felids. The disease is usually asymptomatic, but it can cause mild diarrhoea; however, few numbers of cases may develop a severe systemic disease known as feline infectious peritonitis (FIP). This study aims to determine the prevalence of FCoV shedding in the faeces of stray cats in Kuwait and detect antibodies against FCoV in their serum. Histopathological analyses and RT-PCR were used to prove cases of FIP. A total of 178 cats were examined for the presence of FCoV in their faeces using a rapid immunochromatography (IC) test. Anti-FCoV Antibody (Anti-FCoV Ab) was detected in their serum using ELISA. Eleven samples were tested using RT-PCR to confirm positive cases. The prevalence of FCoV faecal antigen in stray cats was 32.6%. The overall detection rate of Anti-FCoV Ab in stray cats was 44.9%. Nine cats tested positive using the RT-PCR test. Six out of those nine were confirmed to be FIP positive through gross and histopathological examination. The characteristic uveitis and discoloration of the irises were seen. The present study is the first report confirming FCoV infection in stray cats in Kuwait. Postmortem and histopathological lesions in cases of FIP were recorded.

Introduction

Stray and feral cats are increasing in number in Kuwait. The majority of these were lost or abandoned by their owners, and they gather where there are resources–food (often garbage, small mammals, reptiles, and native birds), water, and shelter–forming small colonies. Stray cats play a valuable role in any ecosystems by controlling the rodent population; thus, removing them is a major risk. Nevertheless, they raise public health concerns due to their large population numbers and ability to harbour zoonotic pathogens (Hildreth *et al.* 2010, El-Azazy *et al.* 2015).Coronavirus infection is prevalent in cats as most feline coronavirus (FCoV) strains are found in their gastrointestinal tract (Tasker 2018). Anti-FCoV Ab could be detected in serum samples of infected cats minimum 9 days post-infection (Desmarets *et al.* 2016); however, in other cases, the seroconversion may take up to 28 days (Meli *et al*. 2004). The majority of cats infected with coronavirus are only mildly symptomatic, while up to 10% develop FIP similarly as in SARS-CoV2 infection, the majority of infections are asymptomatic, and only a tiny percent develops COVID (Tekes and Thiel 2016, Tasker 2018, Felten and Hartmann 2019, Kennedy 2020, Paltrinieri *et al*. 2021). Once a cat is infected with FCoV, FIP could develop. Therefore, the only way to prevent FIP in cats is to avoid FCoV infection by detecting chronic shedders. In a study that lasted up

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to five years, Addie and Jarrett (2001) reported three patterns of FCoV shedding in naturally infected cats.

Pattern I: cats continuously shed the virus (13.2%; 18/136); these cats were persistent and possibly long-life carriers, and none of them developed FIP. Long-term carriers were defined as cats that gave positive FCoV RT-PCR results on faecal samples for at least eight consecutive months. Pattern II: cats stopped shedding the virus but were still susceptible to reinfection (41.2%; 56/136). These cats gave negative FCoV RT-PCR negative results for five months or became seronegative. Pattern III: continually reinfected cats (32.4%; 44/136).

Chronic carrier cats maintained an antibody response, gave positive FCoV RT-PCR results in blood and faeces and usually shed the virus for life. Whereas cats ceased viral shedding, became seronegative. Although there was a link between FCoV shedding and antibody titres, the cats could still be seropositive after they stopped shedding the virus for some time (Addie and Jarrett 2001, Gonon *et al*. 1999).

Ultimately, neither the presence of FCoV in a cat's faeces nor the elevation of Ab titres against FCoV in a cat's blood is used to diagnose FIP. A decisive FIP diagnosis can be established only by the histopathological examination of a biopsy or postmortem (PM) material (Benetka *et al.* 2004).

Simultaneously no studies on FCoV and FIP have been reported in Kuwait. This study is the first to determine the prevalence of FCoV shedding in the faeces of stray cats in Kuwait and detect Anti-FCoV Ab in their serum. Histopathological studies and RTPCR test were employed to prove the cases of FIP.

Materials and methods

Study design

A cross-sectional study was conducted during the period extending from June 2011 to May 2012 to study parasitic, viral, bacteriological, and pathological lesions in stray cats in Kuwait (El-Azazy *et al*. 2015, El-Azazy *et al*. 2016). Special traps were set at night in different localities in Kuwait to collect random samples of stray cats. The following day, the cats were transferred to the laboratory for investigation. Trapped cats were checked for the presence of microchips to avoid including owned cats in the study. Descriptive data, including the date of trapping, localities, and the age and gender of the cats, were recorded. The trapped cats appeared to be domestic shorthair / longhair cats rather than purebred cats. Based on dental development, the maturation of the genital structure, and the size of the body, the cats were divided into two age groups: young (≤6 months of age and <1.4 kg) and adults (1.5–6 kg). In General, the climate in Kuwait is characterized by its hot and dry season (April-November) and cold, wet season (December-March).

Sample collection

The cats were carefully transferred to a squeezing box at the pathology laboratory and injected intramuscularly with Rompun 2% (1.5ml/ 10kg). Then, they were examined, and every evidence of clinical signs was recorded. Blood samples were then collected from each cat and placed in tubes with anticoagulants and without anticoagulants to separate the serum. The serum was stored at -20°C for serological examination. Subsequently, the cats were euthanized using T61 (active ingredients are Embutramide, Mebezonium iodide, Tetracaine hydrochloride, and Dimethylformamide). T61 was injected directly and slowly into the heart, and the dose was calculated according to the cat's weight (0.3ml/kg), then the cat necropsied. Detailed PM and histopathological examinations were conducted. Faecal samples were collected from the rectum during PM examination.

Rapid immunomigration test

A total of 178 faecal samples from stray cats were subjected to qualitative detection of FCoV Ag through a rapid immunochromatography (IC). Rapid immunochromatography, also called immunomigration, is one of the most efficient and feasible methods for detecting antibody–antigen interactions. An antibody specific to a certain antigen is fixed to colloidal gold molecules, which are dried as a stripe on a chromatography paper (nitrocellulose) strip contained in a plastic holder in the most frequent iteration of this approach. The Anigen Rapid FCoV Ag Test Kit (Bionote, Inc., Gyeonggi-do, Republic of Korea) was used as described by the manufacturer. The manufacturer claims a diagnostic performance of the IC when tested by the manufacturer compared to the RT-PCR method; a relative sensitivity of 95.12% (95%CI: 83.47–99.40%), relative specificity of 95.87% (95%CI: 90.62–98.65%), and accuracy of 95.68% (95%CI: 91.30–98.25%).

RT-PCR test

Eleven cats were examined for RNA using Norgen's FIP RT-PCR Kit (Norgen Biotech Corp., Ontario, Canada). The kit enabled FCoV RNA binding from the blood samples through spin-column chromatography based on Norgen's proprietary resin and was used as prescribed by the manufacturer.

Before RNA detection in effusion samples, viral RNA was extracted using a QIAamp Viral RNA Mini Kit (Qiagen Inc., Maryland, USA), as per the manufacturer's instructions.

The criteria for selecting cats for PCR examination were positive results of IC for FCoV Ag in faeces and/or the presence of PM lesions, diarrhoea, and emaciation. Blood samples from 10 cats and blood and ascitic fluid from one cat were collected.

Serological examination

One hundred and seventy-eight stray cats were examined for detecting IgG antibodies against FCoV in serum using a quantitative enzyme immunoassay (EIA) (MyBioSource, Inc., San Diego, USA). EIA, also known as ELISA (Enzyme-Linked ImmunoSorbent Assay), is a test that identifies and quantifies antibodies in serum samples as a reaction to specific antigens. The optical densities were read at 405 nm using a microplate reader (BioTek Instrument, Inc. Winooski, USA).

The results were validated and interpreted as described by the manufacturer. Samples were considered positive if the difference (Δ) between the sample absorbance at 405 nm on the positive viral antigen well and the absorbance at 405 nm on the negative control antigen well was greater than or equal to 0.300 (relative level of antibodies).

Histopathological examination

All cats (178) were examined antemortem and postmortem (PM). Clinical signs and PM lesions were recorded. Samples were collected from the affected organs and fixed in 10% formalin.

A total of 124 cats were histopathologically examined (80 livers, 64 kidneys, 18 spleens, 40 lungs, 36 intestines, 11 stomachs, 5 brains, 15 mesenteric lymph nodes, 8 pancreas). Histopathological change was evaluated using the paraffin method. Sections were cut, stained with haematoxylin and eosin dyes (H&E). Then, the lesions were observed under a light microscope.

Statistical analysis

A univariate analysis (chi square test, χ^2) was used to distinguish the risk factors associated with FCoV shedding in the faeces of the examined stray cats in Kuwait and the presence of Anti-FCoV Ab in their blood. If χ^2 suggested that the studied variables may have been associated with FCoV ($P < 0.05$), multivariate logistic regression was applied to detect confounding. Analysis of variance (ANOVA) was used to assess the effect of FCoV infection on body weight of stray cats in different age -groups and sexes. Statistix 10© Analytical Software was used to do statistical analyses and calculate P value and odds ratio (OR).

Furthermore, to generate box and whisker plots of EIA relative level of antibodies in different age groups and box and whisker plots of body weight in positive and negative IC-FCoV stray cats concerning different age groups and sexes.

Results

Prevalence of FCoV Ag and Anti-FCoV Ab in stray cats in Kuwait

The prevalence of FCoV faecal shedding in stray cats was 32.6% (58/178). The rates of FCoV faecal excretion were higher in tom and young than queen and adult cats (42.3%, 45.9%, 25%, 25.6%, respectively).

The rate of detection of FCoV in the faeces of stray cats was higher in dry than wet season (37.1%, 26%, respectively), as shown in Table I. Anti-FCoV Abs were detected in 47.4% tomcats, 43%queens, 44.3% young cats, and 45.3% adult cats.

The overall detection rate of Anti-FCoV Ab in stray cats was 44.9% (80/178), with more reported in the dry season (50.4%) (Table I). Statistical analysis showed that only the age group was significantly associated with FCoV shedding in the faeces of stray cats (logistic regression; P= 0.0379).

The number of kittens shedding FCoV was 2.04 times greater than the number of adult cats shedding FCoV (OR = 2.04; Table I). In contrast, the factors under study were non-significantly associated with the presence of Anti-FCoV Ab in the blood of the examined stray cats (χ^2; $P > 0.05$; Table I).

In the 80 positive EIA cats, the median of EIA relative level of antibodies results was higher in the young than the adults (Figure 1).

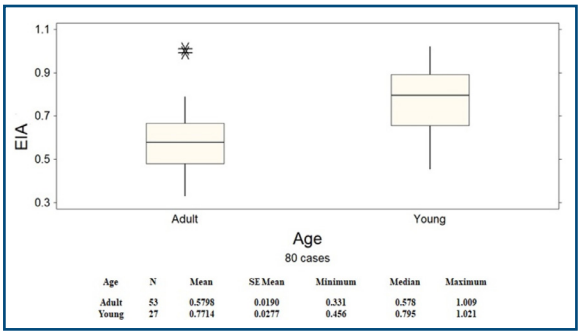

Figure 1. *Box and whisker plots of EIA relative level of antibodies in different age groups showed the median of EIA relative level of antibodies results was higher in the young than the adults.*

Table I. *Statistical comparison of prevalence of FCoV positive antigen detection in faeces and antibody detection in serum in different age groups, sexes and seasons of the examined stray cats.*

		Total	Antigen Detection by IC			Antibody Detection by EIA		
			No Positive	%	P value (OR)* Multivariate**	No Positive	%	P value Univariate***
Age	Adult	117	30	25.6	0.038 (2.04 {1.04-4.02})	53	45.3	0.89
	Young	61	28	45.9		27	44.3	
Sex	Male	78	33	42.3	0.051 (1.93 {1.00-3.73})	37	47.4	0.56
	Female	100	25	25.0		43	43.0	
Season	Wet	73	19	26.0	0.21 (1.55 {0.78 -3.05})	27	37.0	0.08
	Dry	105	39	37.1		53	50.4	
	Total	178	58	32.6		80	44.9	

OR (odds ratio {95% C.I. lower and upper limit}); **multivariate analysis logistic regression, * univariate analysis χ^2 test*

FCoV faecal shedding was higher in tom and young than queen and adult cats. The rate of detection of FCoV in the faeces of stray cats was higher in dry than wet season. Statistical analysis showed that only the age group was significantly associated with FCoV shedding in the faeces of stray cats (logistic regression; P= 0.0379). The number of kittens shedding FCoV was 2.04 times greater than the number of adult cats shedding (OR = 2.04). There was no statistical significant association between FCoV Ab detected by EIA and the three factors under study (age, sex, and season).

Effect of FCoV infection on body weight of stray cats

There was a significant body weight loss in stray cats that were positive for FCoV in their faeces using IC test than those negative regarding age groups and sexes (P= 0.0253). Figure 2 illustrated box whisker plots of body weight in positive and negative FCoV stray cats in different age groups and sexes.

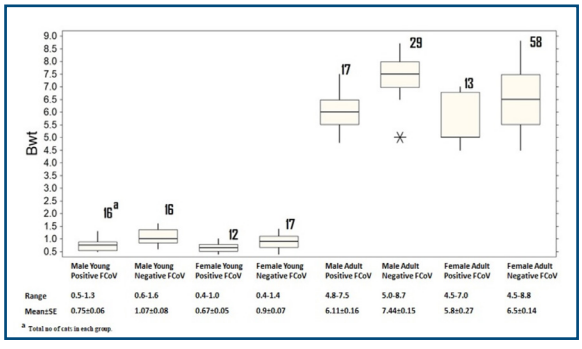

Figure 2. *Box and whisker plots of body weight in positive and negative FCoV stray cats in different age groups and sexes illustrated that infected cats weighed less than did FCoV - negative cats when compared in the same age groups and sexes.*

RT-PCR results in the examined stray cats

Nine out of 11 cats tested positive in the RT-PCR test (Figure 3). Comparing the results of RT-PCR with IC results, EIA relative level of antibodies and PM lesions in the 11 examined cats revealed that two positive cats using IC had no PM lesions; one of them

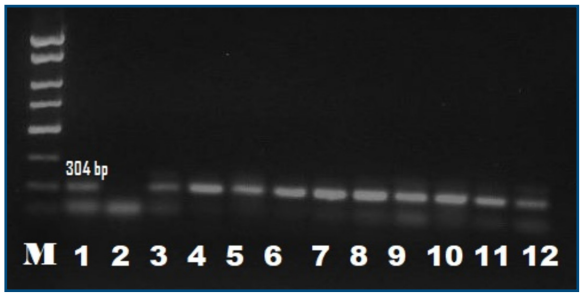

Figure 3. *RT-PCR product gel electrophoresis showed Marker M; Lane 1: positive control; Lane 2: negative control; Lane 3 to 10: positive end point RT-PCR reaction from blood samples of 8 cats; Lane 11 and 12: positive end point RT-PCR reaction of 2 samples from one cat (ascetic fluid and blood).*

was positive according to EIA and RT-PCR (Table II). Simultaneously, two out of nine cats with different pathognomonic lesions were negative according to IC and positive according to EIA and RT-PCR (Table II). One cat with PM lesions was positive according to IC and negative according to EIA and RT-PCR.

Gross and histopathological lesions

All 178 cats were subjected to antemortem and PM examinations. One hundred twenty-four cats (28 kittens and 96 adults) were subjected to histopathological examination by collecting samples from organs showing lesions. In the present study, FIP cases were diagnosed by histopathology examination. Six cats showed characteristic histopathology and PM lesions and had FCoV-RNA detected by RT-PCR in their blood samples (Table II).

Table II. *Comparison of the results for RT-PCR, IC, EIA titer, and gross lesions.*

Cat No	Gross Lesions	IC	EIA	RT-PCR	Diagnosis
1	Congested mesenteric blood vessels	Positive	0.991	+ve	FCoV enteritis +ve
2	Enteritis	Positive	0.801	+ve	FCoV enteritis +ve
3	No PM lesion, diarrhoea	Positive	0.236*	-ve	-
4	No PM lesion, Diarrhoea, emaciation	Positive	0.900	+ve	FCoV enteritis +ve
5	Visceral congestion, enlarged spleen and liver, fibrinous flakes on the serosal surface of the visceral organs, particularly spleen	Positive	0.781	+ve	FIP +ve
6	Severe congestion of intestine,yellowish-white discolouration of liver, fibrinous flakes on the serosal surface of the visceral organs, particularly spleen	Negative	0.896	+ve	FIP +ve
7	Accumulation of sparse quantity of fluids in the peritoneal cavity; ascites peritonitis, congested and enlarged visceral organs and opaque mesentery	Positive	0.991	+ve**	FIP +ve
8	Eye lesion, changes in the colour of the irises and cornea reddish yellow with yellowish white thick discharge from the eyes, congestion and enlargement of visceral organs (liver and kidney); FPV Antigen +ve by IC test	Positive	1.021	+ve	FIP +ve
9	Eye lesion, changes in the colour of the irises and cornea reddish yellow with yellowish white thick discharge from the eyes, Visceral congestion and enlarged mesenteric lymph nodes	Negative	1.009	+ve	FIP +ve
10	Mild congestion in spleen and lung	Positive	0.224*	-ve	-
11	Enlarged liver with distinct lobule demark, pale central area with peripheral congestion, mild congestion of portal veins	Positive	0.789	+ve	FIP +ve

**Negative EIA test; **Blood and ascites effusion samples were examined by PCR and both were positive; +ve: Positive.*

The prevalence of FIP in total examined stray cats was 3.4% (6/178). In particular, the affected cats had severe inflammation of the mesentery/peritoneum, and one of them showed accumulation of a sparse quantity of fluids in the peritoneal cavity; ascites (Figures 4A, 4B).

Out of the six cats, two showed eye lesion, changes in the colour of the irises and cornea reddish yellow with yellowish white thick discharge from the eyes (Figure 5).

In general, the lesions observed were visceral congestion, peritonitis, enteritis, lymphadenitis/sinus histiocytosis of the mesenteric lymph nodes, pneumonia, nephritis, periportal mononuclear infiltration in the liver, and meningitis.

Microscopically, the lesions appeared as thickening of the mesenteric wall with localised perivascular accumulation of mixed inflammatory cells (macrophages, neutrophils, lymphocytes, plasma cells) and fibrin.

Two cats showed acute mesenteric lymphadenitis and sinus histiocytosis. In one case, there were thrombi in the portal vessels (Figure 6). Neurologic FIP caused by meningitis was also reported in two other cats (Figure 7).

Mild to moderate enteritis was diagnosed in three other cats, and they tested positive for FCoV by RT-PCR. These findings suggested these cats show enteric form of FCoV (Figures 8A, 8B).

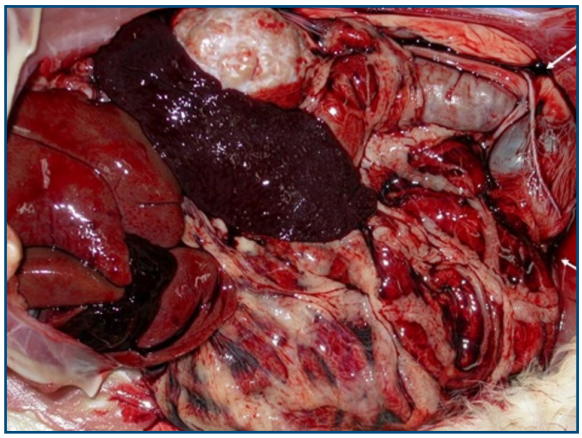

Figure 4A. *Severely congested mesentery with accumulation of sparse quantity of fluids in the peritoneal cavity (arrows).*

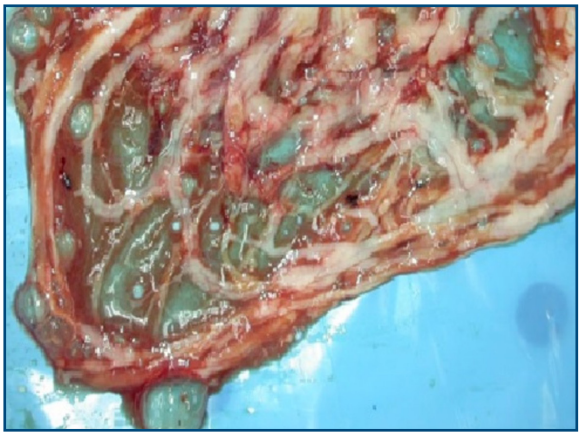

Figure 4B. *Part of the mesentery from same cat (Figure 4a) showing congested and opaque mesentery with accumulation of sparse quantity of fluids on the membrane.*

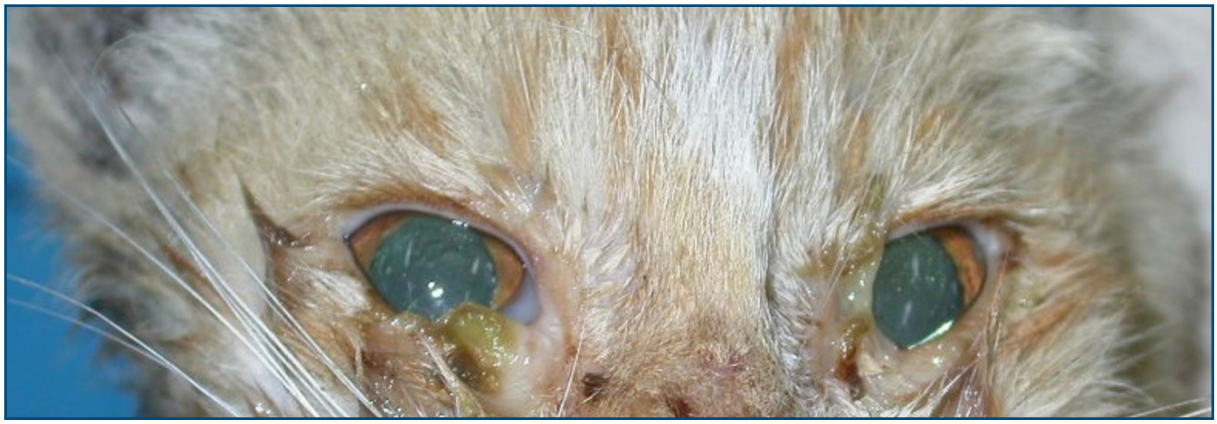

Figure 5. *Eye lesion, discolouration of the iris, cornea reddish yellow with yellowish white thick discharge from the eyes.*

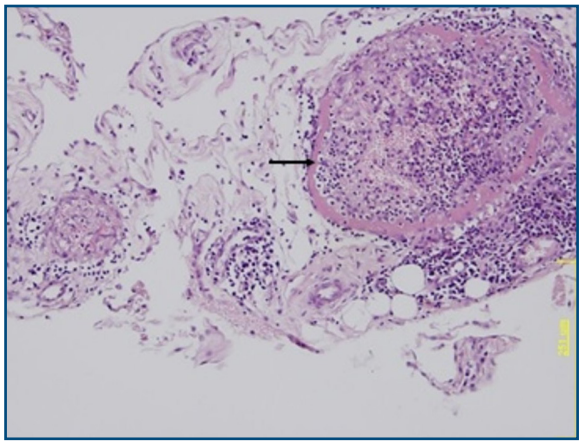

Figure 6. *Mesentery wall thickened with mononuclear infiltration and thrombi in blood vessel (arrow) - H&E stain - 200x.*

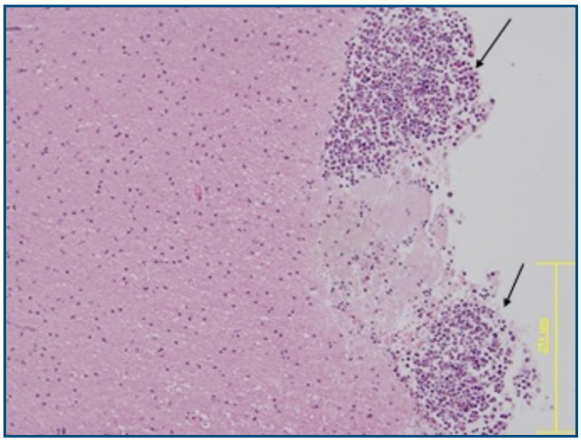

Figure 7. *Brain - foci of mononuclear infiltration in the meninges (arrows) - H& E - 200x.*

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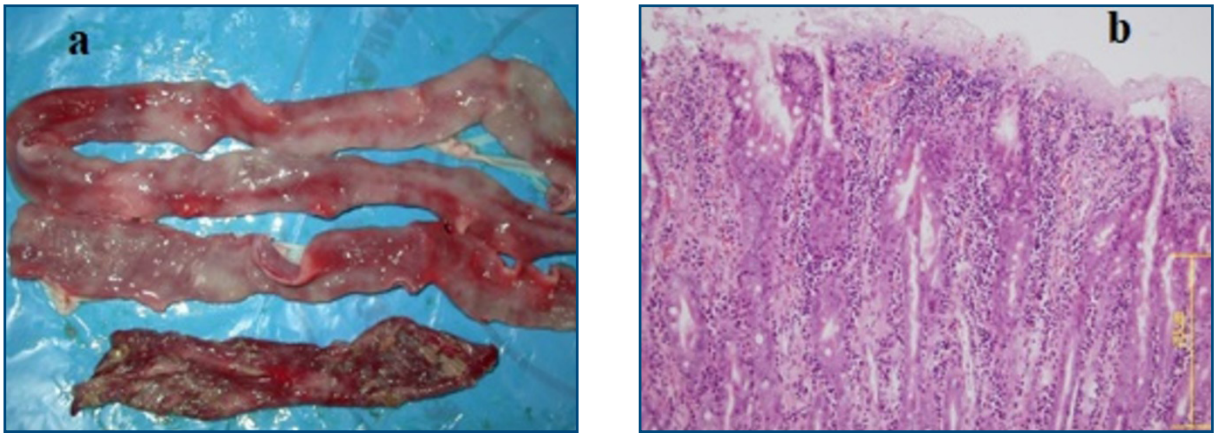

Figure 8. *Intestine – jejunum – showing congested mucosa (a). Histopathology (b) moderate mononuclear infiltration in the mucosa with loss of villous epithelium - H & E - 200x.*

Discussion

The overall prevalence of FCoV shedding in the examined stray cats was 32.6%. Previous studies revealed different FCoV shedding ratios due to differences in terms of the cat population examined and the diagnostic test used.

In the USA, 100 cats were tested at an open-admission municipal animal shelter in Florida; the FCoV excretion rate was 58.0% in cats with diarrhoea and 36.0% in cats with normal faeces using RT-PCR test and electron microscopy examinations (Sabshin *et al.* 2012). In Canada, 46.5% of the examined healthy cats from shelters and private households tested positive for FCoV in their faeces by RT- PCR (McKay *et al.* 2020). In German catteries, 137 of 179 cats (76.5%) tested positive for FCoV RNA in at least one of the four samples collected from each cat (Klein-Richers *et al.*, 2020).

In the present study, the proportion of cats shedding FCoV was lower than in other studies; this may be due to other studies examining cats in multi-cat households, catteries, and shelters (Pedersen *et al*. 2004, Pedersen *et al*. 2009, Klein-Richers *et al.* 2020). Gathering cats in catteries makes this highly infectious virus easier to spread through faecal-oral route transmission due to FCoV replication in shared litter boxes (Addie and Jarrett 2001, Hartmann 2005). In this study, the examined cats were stray and living outdoors, which may decrease the infection rate. Furthermore, coronaviruses cannot survive outdoors as they are inactivated by sunlight (Horzinek and Osterhaus 1979, Addie *et al*. 2012).

The present study showed that a higher percentage of young cats (below six months of age) shed FCoV virus in their faeces compared to older cats. Age was the most significant risk factor; the prevalence of kittens disseminating FCoV was 2.04 times higher than adult cats. This result is in line with previous investigations (Hartmann 2005, Pedersen *et al.* 2008, Klein-Richers *et al.* 2020). Kittens typically get infected when maternal Ab diminution occurs between six and ten weeks old, enabling the virus to propagate effectively (Hartmann 2005, Pedersen *et al.* 2008). Although Harpold *et al.* (1999) suggested that FCoV shedding in kittens begins as early as at the age of four weeks, other researchers could not report virus shedding in kittens aged less than nine weeks (Hartmann 2005, Pedersen *et al.* 2008).

In the present study, more tomcats shed FCoV than queens, and more shedders were reported in the dry season. These results may be due to confounding as most examined tomcats were young and caught during the summer. Klein-Richers *et al.* (2020) studied the risk factors associated with FCoV shedding, such as age, sex, breed, number of cats in a household, and litter box-cleaning frequency per day. They concluded that young age was the most critical risk factor for FCoV shedding, while the other factors were not significantly associated with it.

The prevalence of stray cats seropositive to Anti-FCoV Ab was 44.9% (80/178) in this study. Other studies reported different seropositivity prevalence percentages in the examined cats. The overall prevalence of Anti-FCoV Ab was 31% in Swedish cats (Holst *et al.* 2006). In German catteries, antibodies against FCoV were detected in 64 of the 82 examined cats (78%) (Felten *et al.* 2020). Mosallanejad *et al.* (2012) studied the FCoV-seropositive companion cats in Ahvaz district, Iran. They found that 6.85% (17/248) of the examined cats had Anti-FCoV Ab in their sera. There was no statistical association between age, sex, and season and the presence of Anti-FCoV Ab in the sera of the examined stray cats. However, the percentage was higher in males and the dry season. These findings were consistent with a previous study, which reported no significant association between seropositivity prevalence and the host's gender and delete the season (Mosallanejad *et al.* 2012).

Many researchers investigated a possible correlation between anti-FCoV Ab level and faecal virus shedding. However, some researchers suggested that serum Ab titre is not significantly associated with the shedding of FCoV. In addition, it cannot be a good indicator of FCoV shedding in the faeces as kittens may shed FCoV in their faeces before they seroconvert (Harpold *et al.* 1999).
Other researchers demonstrated a correlation between the Ab level and FCoV shedding, along with shedding frequency and shedding intensity (Addie and Jarrett 2001, Holst *et al.* 2006, Felten *et al.* 2020).
Thus, Ab titre evaluation can help manage FCoV infection in catteries or multi-cat households but cannot substitute for the faecal examination. Compared to using only one test, carrying out quantitative RT-PCR and Ab titre together can yield a more accurate view of a cat's status. The median of EIA relative level of Ab in the positive group was higher in the young than in the adult.
The variation in Ab levels is linked to a complex interaction between the virus and the host. This interaction may differ from cat to cat for different reasons, including age, the host's genetic background, and the virus's infecting dosage, strain, and virulence. This interaction may explain the observed differences in natural or experimental infection (Gonon *et al*. 1999).

FCoV – infected cats weighed less than did FCoV - negative cats when compared in the same age groups and sexes, this could be due to weight loss or failure to gain weight. Primary FCoV infection can cause a severe acute or chronic course of diarrhoea with weight loss that is resistant to therapy (Kipar *et al.* 1998). Addie and Jarrett (1992) reported that the uneven size of the cats, which may not become evident until kittens become slightly older, was a significant sign pointing to FCoV as the cause of delete the diarrhoea. However, many other causes of diarrhoea in cats must be ruled out before a diagnosis of FCoV diarrhoea can be made.

In the present study, six cats showed different characteristic signs and lesions of FIP depending on the FIP form. On PM examination, one cat was considered to have wet effusive form characterised by accumulation of a sparse quantity of fluids in the peritoneal cavity: ascites. The wet effusive form always manifests as abdominal, pleural, or pericardial effusion (Tasker, 2018). The other five cats were diagnosed as having a dry non-effusive form; two of these cats showed ocular signs (cornea reddish yellow with yellowish white thick discharge from the eyes and iris discolouration). Ocular involvement in FIP is a characteristic lesion in non- effusive FIP and can include uveitis, keratitis, and changes in the colour of the iris (Pedersen 2014, Tasker 2018). Meningitis was also reported in two other cats. Neurologic FIP is mainly reported in dry form and microscopically shows lymphoplasmacytic infiltration into the meninges of the spinal cord and brainstem (Crawford *et al.* 2017, Tasker, 2018). Histopathology of the examined tissues revealed focal accumulation of inflammatory cells and fibrin with necrotic proliferative lesions in different organs such as liver, kidney, spleen, and mesenteric lymph nodes (Crawford *et al.* 2017).
Histopathological findings of FIP are utilised to confirm cases and are considered the "gold standard" for evaluating diagnostic tests (Sharif *et al.* 2010).

PM examination and histopathology revealed that six cats showed signs and lesions of FIP. Two of them tested negative for FCoV Ag in their faeces; however, they tested positive for FCoV-RNA by RT-PCR in their blood samples and seropositive by EIA. FIP prevalence in the total examined stray cats was 3.4% (6/178).

In the present study, the pyogranulomatous foci on the surface of visceral organs, reported in the literature, were not observed; this may be because the animals in this study were random stray cats. They were euthanised early, i.e., before the disease could develop into the typical lesions.
Three of the examined cats tested positive as FCoV shedders in the IC test and they were RT-PCR positive for FCoV RNA in their blood samples. Additionally, those three cats suffered from diarrhoea, emaciation, enteritis, and mild congestion in the viscera, which suggested a mild enteric form FCoV infection.

In the present study, blood samples collected from 10 cats and ascitic fluid and blood from one cat were examined through RT-PCR. Many studies reported using the molecular diagnosis of FCoV RNA in blood and effusion samples collected from FIP suspected positive cats (Simons *et al*. 2005, Soma *et al*. 2013, Felten *et al*. 2017).
Effusion samples (ascites, pleural, or pericardial effusions), if available, are the best sample used for the RT-PCR test (Felten *et al*. 2017). However, the molecular methods can not differentiate between enteric and virulent FCoVs, as there is no specific genetic marker to detect the virulent strain (Soma et al. 2013, Fish et al. 2018).
Furthermore, enteric FCoV could cause transient fever and low replication in the blood, and the detection of FCoV RNA in blood samples collected from cats without signs of FIP appears to be rare (Fish *et al*. 2018).
Hence, the blood samples could be used in FIP diagnosis through RT-PCR, significantly when correlated with the gold standard pathognomonic lesions.

The present study is the first report on the detection of FCoV in stray cats in Kuwait. Moreover, PM and histopathological lesions in FIP and enteric FCoV.

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Statement of animal rights

This study was approved by the Animal Ethics Committee of Kuwait Foundation for the Advancement of Sciences (KFAS-Award Number 01-1202-2010) and the Kuwaiti Public Authority for Agriculture Resources and Fisheries.

All methods were carried out following relevant institutional, national, and international guidelines and regulations. This study conformed to appropriate ethical standards and the journal's policies .

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