

<u>Short Communication</u> Antibody detection of *feline infectious peritonitis virus* (FIPV) *in sera of companion cats in Ahvaz, south west of Iran*

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ABSTRACT

Feline infectious peritonitis virus (FIPV) is ubiquitous in domestic cats, especially in young cats and multicat environments. In the present study, a total of 248 companion cats of different ages were examined for serum antibody detection of FIPV by immunochromatography assay. The cats were selected from those referring to Veterinary Hospital of Ahvaz University, southwestern Iran from December 2006 to June 2009. Classification was made by age, sex, breed, region and season. The studied cats were divided based on age into three groups (<6 months, 6 months – 3 years and > 3 years) and based on area into five regions (north, east, west, south and central). The results were analyzed by using Chi-square analysis and Fischer's exact test. Seventeen of 248 serum samples (6.85%) had antibody against *feline infectious peritonitis virus*. Prevalence was significantly higher in young kittens less than 6 months (9.72%; 7 out of 72) and mean-age cats 6 months – 3 years (9.28%; 9 out of 97) compared with above 3 years (1.27%; 1 out of 79) (P<0.05). The most common clinical signs were loss weight and depressed appetite in the affected cats. Prevalence was higher in male cats (8.70%; 10 out of 115) than females (5.26%; 7 out of 133), the spring season (11.32%; 6 out of 53) and north region (10.53; 4 out of 38), but the difference was not significant between the prevalence of infection relative to host gender, season and region (P>0.05). It is necessary to control cat population in these area particular young cats to reduce risk of infection transmission between them.

Keywords: Feline infectious peritonitis virus, Cat, Prevalence, Immunochromatography assay, Ahvaz

INTRODUCTION*

Feline infectious peritonitis (FIP) is a viral disease affecting cats worldwide. Recent evidence suggests that the FIPV has evolved as a deletion mutation of *feline enteric coronavirus* (FECV). FECV is a large, enveloped RNA virus that is virtually nonpathogenic, whereas FIPV is almost invariably fatal (Greene 2006, Addie *et al* 2009). All FECV carriers have the potential to develop either enteritis or peritonitis, although only about 5% of infections develop into FIP. The epizootiology of FIP is closely linked to that of FECV (Foley *et al* 1998). FIP has been recognized in the African lion, Mountain lion, Leopard, Cheetah, Jaguar,

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Lynx, Caracal, African cat, European wild cat, Sand cat and Pallas cat (Watt et al 1993). Currently available diagnostic tests cannot differentiate FECV from FIPV with 100% accuracy (Suiter et al 1995). FIP is classified into serotype I and serotype II according to the amino acid sequence of its spike protein. A cause and effect relationship between FECV infection and FIP was first reported by Pedersen and co-workers (Pedersen 2009). Two basic forms of FIP have been characterized: granulomatous (dry, parenchymatous) or effusive (wet, non-parenchymatous). Cats with effusive FIP have ascites, thoracic effusion or both. Abdominal swelling with a fluid wave, mild pyrexia, weight loss, dyspnea, tachypnea, scrotal enlargement, muffled heart sounds and mucosal pallor or icterus may be noted (Greene 2006). Approximately half the cats with FIP are younger than 2 years, but cats of any age can be affected (Greene 2006). In general, antibodies against FECV are found in 80-90% of the animals living in catteries and in up to 50% of solitary cats (Foley et al 1997, Simons et al 2005). In Iran, large numbers of cats are found roaming residential streets. They can be an important potential source of transition of infection to other animals (Akhtar Danesh et al 2010). Diagnosis of FIP is extremely challenging. Difficulties in definitively diagnosing FIP arise from an absence of non-invasive confirmatory tests in cats with no effusion. Presence of effusion should first be ruled out because obtaining effusion and analysis is very useful and relatively non-invasive. Several laboratory methods have been developed to detect antigen or antibody in the serum of infected cats such as PCR, ELISA, LAT (Latex (Indirect Agglutination Test). IHA Hemagglutination Assay), VN (Virus Neutralization) IFAT (Indirect Fluorescent Antibody Tes) and immunochromatography assay (ICA). Though these more sensitive, specific and more tests are reproducible, but they are just expensive. ICA is one of the most common rapid field diagnostic methods used in clinical practice. Specificity and sensitivity for kits of FIP Ag Test (Biotech Co., Ltd, Shanghai, China) were found to be highly 98.8% and 100% respectively (Bonczynski *et al* 2002, Pratelli 2008). Although there are clinical cases of FIP in Iran, no study has been reported on the distribution of FIP virus in the Iran cat population. Thus the aim of this study was to investigate the antibody detection of FIPV in the serum samples of companion cats in Ahvaz area, southwestern Iran.

MATERIALS AND METHODS

Study area and sample population. The present survey was performed in Ahvaz area, southwestern Iran that is located at an elevation of 12 meters above sea level and the climate is warm-humid. In this study, a total of 248 companion cats of different ages were examined for serum antibody against FIPV by immunochromatography assay. The cats used in this study were referred cases to Veterinary hospital of Ahvaz University from December 2006 to June 2009. They were kept indoors without free access to outside sources in most cases. Classification was made by age, sex, breed, region and season. Information about companion cats was taken from their owners. The studied cats were examined and the clinical trials of signs were recorded. The studied cats were divided based on age into three groups (<6 months, 6 months-3 years and >3 years) and based on area into five regions (north, east, west, south and central). Seventy two of the studied cats had age less than 6 months, 97 were 6 months-3 years and 79 had age above 3 years. Number of female and male cats was 133 and 115 respectively. Most of the studied cats (223) were domestic short hair (DSH). Age was estimated by dental formulary and owner information's. Number of cats that were kept in the house was important in the present study.

Laboratory methods. Blood samples were collected from Jugular veins and allowed to clot and centrifuged for 5 min at $1800 \times g$. Serum was removed and stored at -20° C until assayed. Antibody against FIPV was detected with a commercial rapid test kit (FIP Ag Test, Biotech Co., Ltd, Shanghai, China) following the manufacturer's instructions. Sensitivity and specificity of these kits were 98.8% and 100% respectively (Esfandiari *et al* 2000). We added four drops of the serum sample into the holes using the dropper, drop by drop and slowly. As the test began to work, we saw purple color move across the result window in the center of the test device. Finally, test results were interpreted following the manufacturer's instructions (Catalog No. W81021).

Interpretation of the test. A color band will appear in the left section of the result window to show that the test is working properly. This band is the control band. The right section of the result window indicates the test results. If another color band appears in the right section of the result window, this band is the test band. The presence of only one band within the result window indicates a negative result. The presence of two color bands (T and C) within the result window, no matter which band appears first, indicates a positive result following the manufacturer's instructions (Catalog No. W81021) (Figures 1 and 2).

Statistical analysis. Cats were grouped by age, sex, breed, season and geographic area to determine whether these factors were associated with FIPV infection, using chi-square analysis and Fisher's exact test. Statistical comparisons were carried out using SPSS 16.0 statistical software. Differences were considered significant when P < 0.05.

RESULTS AND DISCUSSION

Seventeen of 248 serum samples (6.85%) had antibody against *feline infectious peritonitis virus*. Prevalence was significantly higher in young kittens less than 6 months (9.72%; 7 out of 72) and mean-age cats 6 months–3 years (9.28%; 9 out of 97) compared with above 3 years (1.27%; 1 out of 79) (P<0.05) (Table 1). Of course the difference was no significant between cats less than 6 months compared with 6 months – 3 years (P>0.05). Prevalence was higher in male cats (8.70%; 10 out of 115) than females (5.26%; 7 out of 133). The highest prevalence was in the spring season (11.32%; 6 out of 53) and north region (10.53; 4 out of 38), but the difference was not significant between the prevalence of infection relative to host gender, season and region (P>0.05).

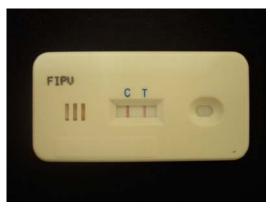


Figure 1. Positive sample of rapid FIP Ab test



Figure 2. Negative sample of rapid FIP Ab test

Clinical signs were none-specific. Most of the cats were apparently healthy and had referred for other reasons mostly for vaccination. The most common clinical signs were loss weight and depressed appetite. Eight out of 17 cases (47.06%) which had anorexia and depressed appetite and nine out of 187 cases (4.81%) which had no above signs were seropositive. The difference was significant between two groups (P<0.05). Three, two and five cases of the referred cats had icterus, ascite and iritis respectively. All of them were negative for FIP. The difference was not significant for above signs (P>0.05). Twelve out of 83 (14.46%) cats were FIP positive from multi-cat households, while 5 out of 165 (3.03%) were positive among single cat households. The difference was significant between cats that lived as group and single

(P<0.05). Prevalence in other seasons (winter, summer and autumn) were (4.54; 3 out of 66, 7,04; 5 out of 71 and 5.17; 3 out of 58) respectively. Prevalence in other regions (east – west - south and central) were (6.25; 3 out of 48, 8.20; 5 out of 61, 5.36; 3 out of 56 and 4.44; 2 out of 45) respectively also (Table 2). Two hundred twenty three out of 248 (89.92%) of companion cats were DSH breed. Sixteen of 248 (6.45%) of the studied cats were Persian and nine of 248 (3.63%) were domestic long hair (DLH).

 Table 1. Prevalence of Feline infectious peritonitis virus infection in companion cats of different age and sex in Ahvaz district, Iran by ICA, 2006-2009.

Age	< 6 months		<u>6 months</u>	– <u>3 years</u>	> 3 years	
Sex	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Male	34	4	40	5	31	1
Female	31	3	48	4	47	0
Total = 248	65	7	88	9	78	1

Table 2. Prevalence of *Feline infectious peritonitis virus* infection in companion cats of different age and region in Ahvaz district, Iran by ICA. 2006-2009.

Age	< 6 months		<u>6 months – 3 ears</u>		> 3 years	
Region	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
North	11	2	9	2	14	0
East	17	1	16	1	12	1
West	12	1	22	4	22	0
South	12	1	23	2	18	0
Central	13	2	18	0	12	0
Total	65	7	88	9	78	1

The present study that is the first report on prevalence of *feline infectious peritonitis* in companion cats in Ahvaz district revealed that 6.85% of referred cats were affected with FIP virus. These results indicate that virus is present in cats of this area. Due to close contact of cats together they can be an important potential source of transmission of infection. The prevalence of FIP has not been studied in different areas in Iran. Only in a research, Shirani *et al* (2006) reported occurrence of infectious peritonitis in a cat with severe ascite in

Tehran. Studies indicate that the incidence of FIP can vary greatly between countries. It was found the seroprevalence of *feline enteric coronavirus* to be 34% among pedigree cats in the Sydney (Bell et al 2006b). In another investigation, the overall prevalence of antibodies against FCoV was 31% in Swedish cats (Holst et al 2006). In the Republic of South Africa and Namibia, 195 of 342 animals had evidence of infection (Kennedy et al 2003). Sera collected in California revealed an occurrence of 20% (Pedersen 1976). While in Austria type I virus was found in 62% of the studied cats (Posch et al 2001). A total of 58% of the Czech cats were seropositive against FECV I (Moestl et al 2002). FECVs I and II distribution was also studied in southern Italy and a seroprevalence of 72% and 82%, respectively, was detected (Pratelli et al 2008). Feline sera from Turkey were assayed for coronavirus antibody on 100 sera. The VN yielded 79 negative and 21 positive sera but the ELISA confirmed only 74 as negative (Pratelli et al 2009).

Seroprevalence is higher in cats that are kept in groups of at least five than in cats kept in smaller groups (Pedersen 2009). The prevalence of feline enteric coronavirus antibodies in the sampled population based on the gold standard was 62% among multi-cat environments, and 4% among single cat households in Turkey (Pratelli et al 2009). In the present study, 14.46% were FIP positive in multi-cat environments, while 3.03% were positive in single cat households. The difference was significant between cats that lived as group and single. There was no significant difference between the sex distribution of the male and female kittens (Cave et al 2002). A higher seroprevalence was seen in male companion cats (8.70%) than females (5.26%) in our study. It can be explained by the territorial habits associated with them, of course the difference was not significant. It doesn't seem sex to be a determining factor as other studies have concluded.

A report of 42 confirmed FIP cases from Australia has shown FIP to be over-represented in certain pure breeds such as Burmese and Australian Mist (Norris *et al* 2005). This is probably a reflection of the dynamics of FECV-infection in cats (Addie et al 1995). Reports from the US and Europe indicate an increased risk for purebreds, young cats, and intact males (Rohrbach et al 2001). Most of the companion cats were DSH breed (89.92%; 223 out of 248) in our survey. Among domestic cats, young cats aged 3 months to 3 years and geriatric cats older than 13 years are most frequently affected (Greene 2006). In one study, FIP was the most common single cause of disease in cats younger than 2 years of age (Marioni-Henry et al 2004). Cave et al (2002) studied the causes of death among 274 sheltered and privately owned kittens. Deaths from FIP (8.4%) were more frequent in fall and winter and on the basis of cattery records, the number of deaths varied yearly. In our study, Prevalence was higher in the season of spring (11.32%), but the difference was not significant. In our survey, prevalence was significantly higher in kittens and juvenile cats less than 6 months and 6 months - 3 years compared with adult cats above 3 years and this was similar to that described by Heeney et al 1990, Cave et al 2002, Marioni-Henry et al 2004 and Bell et al 2006a. Our survey showed that signs of FIP were often vague. Signs such as icterus, ascite and iritis were not seen, so clinical signs are not specific. The most common clinical signs were loss weight and depressed appetite in the affected cats. Breeders of uninfected cats must be careful if they want to avoid introducing the virus into the group. It has been estimated that in multi-cat households where FECV has been introduced, 80-90% of all the cats will be infected (Greene 2006, Pedersen 2009). At present, only one FIP vaccine is available, which is considered as being non-core. Kittens may profit from vaccination when they have not been exposed to FECV, particularly if they enter a FECV-endemic environment (Addie et al 2009). Minimizing exposure is the best method for prevention of infection. Our study showed that available tests such as ICA are more valuable for clinicians who have already narrowed down the diagnosis to FIP.

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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. and Horzinek, M.C. (2009). Feline infectious peritonitis. Guidelines on prevention and management. *Journal of Feline Medicine* and Surgery 11, 594-604.
- Addie, D.D., Toth, S., Murray, G.D. and Jarrett, O. (1995). Risk of feline infectious peritonitis in cats naturally infected with feline coronavirus. *American Journal of Veterinary Research* 56, 429-434.
- Akhtardanesh, B., Ziaali, N., Sharifi, H. and Rezaei, Sh. (2010). Feline immunodeficiency virus, feline leukemia virus and Toxoplasma gondii in stray and household cats in Kerman–Iran: Seroprevalence and correlation with clinical and laboratory findings. Research in Veterinary Science 89, 306-310.
- Bell, E.T., Malik, R. and Norris, J.M. (2006a). The relationship between the feline coronavirus antibody titre and the age, breed, gender and health status of Australian cats. *Australian Veterinary Journal* 84, 2-7.
- Bell, E.T., Toribio, J.A., White, J.D., Malik, R. and Norris, J.M. (2006b). Seroprevalence study of feline coronavirus in companion and feral cats in Sydney, Australia. *Australian Veterinary Journal* 84, 74-81.
- Bonczynski, J.J., Ludwig, L.L., Barton, L.J., Loar, A. and Peterson, M.E. (2002). Comparison of peritoneal fluid and peripheral blood pH, bicarbonate, glucose, and lactate concentration as a diagnostic tool for septic peritonitis in dogs and cats. *The Veterinary Record* 151, 497-501.
- Cave, T.A., Thompson, H., Reid, S.W., Hodgson, D.R. and Addie, D.D. (2002). Kitten mortality in the United Kingdom: a retrospective analysis of 274 histopathological examinations (1986 to 2000). *The Veterinary Record* 151, 497-501.
- Foley, J.E., Lapointe, J.M., Koblik, P., Poland, A. and Pedersen, N.C. (1998). Diagnostic features of clinical neurologic feline infectious peritonitis. *Journal of Veterinary Internal Medicine* 12, 415-423.
- Foley, J.E., Poland, A., Carlson, J. and Pedersen, N.C. (1997). Risk factors for feline infectious peritonitis among cats in multiple-cat environments with endemic feline

enteric coronavirus. *Journal of the American Veterinary Medical Association* 210, 1313-1318.

- Greene, C.E. (2006). Infectious diseases of the dog and cat, 3rd ed. WB Saunders, Philadelphia., Pp 754-775.
- Heeney, J.L., Evermann, J.F., McKeirnan, A.J., Marker-Kraus, L., Roelke, M.E., Bush, M., Wildt, D.E., Meltzer, D.G., Colly, L. and Lukas, J. (1990). Prevalence and implications of feline coronavirus infections of captive and free-ranging cheetahs (Acinonyx jubatus). *Journal of Virology* 64, 1964-1972.
- Holst, B.S., Englund, L., Palacios, S., Renstrum, L. and Berndtsson, L.T. (2006). Prevalence of antibodies against feline coronavirus and Chlamydophila felis in Swedish cats. *Journal of Feline Medicine and Surgery* 8, 207-211.
- Kennedy, M., Kania, S., Stylianides, E., Bertschinger, H., Keet, D. and Van-Vuuren, M. (2003). Detection of feline coronavirus infection in southern African nondomestic felids. *Journal of Wildlife diseases* 39, 529-535.
- Marioni-Henry, K., Vite, C.H., Newton, A.L. and Van-Winkle, T.J. (2004). Prevalence of diseases of the spinal cord of cats. *Journal of Veterinary Internal Medicine* 18, 851-858.
- Moestl, K., Knotek, Z., Toman, M. and Faldyna, M. (2002). Seroprevalence of feline coronaviruses in two central European countries: Czech Republic and Austria. 2nd International Feline Coronavirus/Feline Infectious Peritonitis Symposium. Glasgow, Scotland, August 2002.
- Norris, J.M., Bosward, K.L., White, J.D., Baral, R.M., Catt, M.J. and Malik, R. (2005).Clinicopathological findings associated with feline infectious peritonitis in Sydney, Australia: 42 cases (1990e2002). *Australian Veterinary Journal* 83, 666-673.
- Pedersen, N.C. (1976). Serology studies of naturally occurring feline infectious peritonitis. *American Journal of Veterinary Research* 37, 1449-1453.
- Pedersen, N.C. (2009). A review of feline infectious peritonitis

virus infection: 1963-2008. Journal of Feline Medicine and Surgery 11, 225-258.

- Posch, A., Posh, U., Kuebber-Heiss, A., Stur, I., Seiser, M. and Moestl, K. (2001). Feline coronaviruses: differentiation of the types I and II by RT-PCR and their occurrence in Austrian cat populations. *Wien Tiera rztl Monatsschr* 88, 1-9.
- Pratelli, A. (2008). Comparison of serologic techniques for the detection of antibodies against feline coronaviruses. *Journal of Veterinary Diagnostic Investigation* 20, 45-50.
- Pratelli, A., Yesilbag, K., Siniscalchi, M., Yalc, E. and Yilmaz, Z. (2009). Prevalence of feline coronavirus antibodies in cats in Bursa province, Turkey, by an enzyme-linked immunosorbent assay). *Journal of Feline Medicine and Surgery* 11, 881-888.
- Rohrbach, B.W., Legendre, A.M., Baldwin, C.A., Lein, D.H., Reed, W.M. and Wilson, R.B. (2001). Epidemiology of feline infectious peritonitis among cats examined at veterinary medical teaching hospitals. *Journal of the American Veterinary Medical Association* 218, 1111.
- Shirani, D., Akhtar Danesh, B., Ghaffari, M., Dezfulian, O., Veshkini, A. (2006) Clinical and pathological findings in a suspected cat to feline infectious peritonitis. *Journal of Veterinary Medical University of Tehran* 61, 311-315.
- Simons, F.A., Vennema, H. and Rofina, J.E. (2005). A mRNA PCR for the diagnosis of feline infectious peritonitis. *Journal of Virology Methods* 124, 111.
- Suiter, B.T., Pfeiffer, N.E., Jones, E.V., Reed, A.P., Klepfer, S.R., Miller, T.J. and Srikumaran, S. (1995). Serological recognition of feline infectious peritonitis virus spike gene regions expressed as synthetic peptides and E. coli fusion protein. *Archive Virology* 140, 687-702.
- Watt, N.J., MacIntyre, N.J. and McOrist, S. (1993). An extended outbreak of infectious peritonitis in a closed colony of European wildcats (Felis sylvestris). *Journal of Comparative Pathology* 108, 73.

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