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Short Paper

Antigenic detection of *Feline Panleukopenia virus* (FPV) in diarrhoeic companion cats in Ahvaz area

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Summary

The present study was carried out for the antigen detection of *Feline panleukopenia virus* (FPV) in diarrhoeic cats referred to the Veterinary Clinic of the School of Ahvaz University, in southwest Iran. Faecal samples were collected from 67 diarrhoeic household cats during 2005 to 2007. According to the age and clinical signs, the cats were divided into two groups; <6 months and >6 months, hemorrhagic and non hemorrhagic diarrhoea, respectively. Faecal samples were tested by immunochromatography assay test and 34% of cats were found positive to FPV antigen. The infection was more prevalent in cats less than 6 months (37%) compared with animals older than 6 months of age (31%). No significant differences were observed between different clinical signs, age and sex of the animals ($P>0.05$). The affected cats had no history of vaccination against Tri-cat, but in the healthy cat population, 18% were vaccinated. The difference between the two groups was significant ($P<0.05$).

Key words: *Feline Panleukopenia virus*, Immunochromatography, Cat, Diarrhoea, Ahvaz

Introduction

Feline panleukopenia is a severe disease of cats caused by *feline panleukopenia virus* (FPV), which belongs to the feline parvovirus group of the *Parvoviridae* family, together with canine parvovirus type 2 (CPV-2) and other parvovirus of carnivores (Parrish, 1994; Greene, 1998). Recent investigations demonstrate the prevalence of CPV infection in a wide range of cat populations (Ikeda *et al*., 2002). FPV-induced disease in cats has been known since the beginning of the 20th century, causing severe panleukopenia and enteritis with high morbidity and mortality. All susceptible cats can be exposed and infected within the first year of life. Unvaccinated kittens that acquire maternal immunity through colostrum are usually protected for up to 3 months of age. Lymphoid tissues, bone marrow, and intestinal mucosal crypts are most commonly invaded in adult animals. Also, CNS including the cerebrum, the cerebellum, the retina, and optic nerves, can be affected. *Feline panleukopenia virus* is most commonly transmitted by direct contact of susceptible animals with infected cats or their secretions. It is shed from all body secretions during the active stages of the disease but is most consistently recovered from the intestine and faeces (Inada *et al*., 1996; Greene, 1998).

Rapid diagnosis of FPV infection is especially important in order to isolate infected cats and prevent secondary infections of susceptible animals. Since clinical diagnosis is not definitive, several laboratory techniques have been developed to detect FPV in the infected cats such as polymerase chain reaction, hemaglutination, ELISA, immunoflurecence antibody test, virus isolation and monoclonal antibodies. Though these tests are more sensitive,

specific and more reproducible, they can be carried out only in specialized laboratories. On the other hand, the immuno-chromatography assay is the most rapid field diagnostic method used in clinical practice because the test procedure is simple and can be performed by veterinarians as well as by owners. Evaluation of the diagnostic kits (immunochromatography assay) showed an overall relative sensitivity and specifity of 95.8 and 99.7%, respectively (Esfandiari and Klingeborn, 2000). Furthermore, the comparative testing of 83 samples in Germany between the one-step test and an immune electron microscopy (IEM) agreed with 85.5%. The sensitivity and specifity were 83.9 and 88.9%, respectively (Esfandiari and Klingeborn, 2000). The aim of the present study was to investigate the presence of FPV antigens in diarrhoeic companion cats of different age groups in Ahvaz and the surrounding area, Khouzestan province, Iran.

Materials and Methods

Sample collection and preparation

Faecal samples were collected from 67 diarrhoeic domestic cats during 2005-2007 that referred to the Veterinary Clinic of the School in Ahvaz and the surrounding area in southwestern Iran. According to the age and clinical signs, cats were divided into two groups (<6 months and >6 months; hemorrhagic and non hemorrhagic diarrhoea). The age of cats ranged between 2 to 14 months. Blood samples (2 ml) were collected from the jugular vein of the cats to determine CBC. Ketamine (10 mg/kg) and acepromazine (0.15 mg/kg) were injected for sedative effect. The average weight of the studied cats was 1.4 kg. They were kept indoors and could not go outside. Duration of virus shedding was studied in some of the affected cats (12 cases); these cats were followed up daily until the samples became negative.

Immunochromatography assay

The test was carried out with a commercial rapid FPV Ag test kit (Manufactured by Anigen, Animal genetics, Inc., Korea, 2004), following the manufacturer's instructions. The kit is a chromatographic immunoassay for the qualitative detection of *Panleukopenia* antigen in feline faeces. The detection limit of this kit is about 104.5 TCID50/0.1 ml (Esfandiari and Klingeborn, 2000).

Test procedure

The samples were provided by a swab from the stool. They were then inputted and mixed into the assay diluents. They were left for a short time and finally four drops of supernatant were added into the sample hole. As the test began to work, a purple color was observed moving across the result window in the center of the test device. Interpretation of test results were at 5-10 minutes.

Interpretation of the test

A colour 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 colour bands (T and C) within the result window, no matter which band appears first, indicates a positive result. If the purple colour band is not visible within the result window after performing the test, the result is considered invalid (Esfandiari and Klingeborn, 2000).

Finally, treatment of the affected cats was directed at correcting the life-threatening dehydration that accompanied the diarrhoea with intravenous fluids (Ringer lactate or Nacl 0.9% + dextrose 5%), medicines that relax intestinal spasms and broad-spectrum antibiotics (Ceftizox 30 mg/kg q8h + Gentamicin 2 mg/kg q12h for 5 days) to prevent secondary bacterial infection. Low-dose oral oxazepam (2.5 mg total) was used a few minutes before feeding to stimulate the appetite of anorectic cats.

Statistical analysis

Potential association of the test results with age, sex, vaccination status and hemorrhagic diarrhoea were performed by

SPSS 10.0 for windows using Fisher's exact test and chi-square analysis. Differences were considered significant at $p<0.05$.

Results

Of 67 diarrhoeic cats, 23 were affected with FPV antigens (34.3%). The infection was more prevalent in cats less than 6 months (36.8%) and lower in animals older than 6 months of age (31.0%). No significant differences were observed between the different clinical signs, age and sex of the animals ($P>0.05$). Clinical examination of the cats showed depression, vomiting, hemorrhagic diarrhoea, profound dehydration, fever (40-41.6°C) and occasionally hypothermia during the terminal stages of the illness. On abdominal palpation, the intestinal loops had a thickened, ropelike consistency, and discomfort was noted in some cases. Forty seven cats manifested hemorrhagic and 20 non-hemorrhagic diarrhoea. Infection was higher in hemorrhagic diarrhoeic cats (40.4%) than those of non-hemorrhagic diarrhoeic cats (20%), but the difference was not significant ($P>0.05$). In our study, none of the affected cats had any history of vaccination against Tri-cat vaccine, but in the healthy cat populations, 8 cats (18.2%) were vaccinated. The difference between the groups (vaccinated and unvaccinated) was significant ($P<0.05$). Thirteen of the infected cats were male and 10 female (Table 1). Duration of virus shedding was 3, 4 and 5 days in 6, 4 and 2 cases that were under treatment respectively. Average shedding duration was 3.67 days post-infection in affected cats (12 cases). This is shown in Table 2.

Results indicated leukopenia (less than 5500 cells/µl), lymphopenia and neutropenia in most of the affected cats (87%). Seven of 23 of the affected cats died (30.4%) despite supportive treatment. Most of the dead cats were under 6 months old. Gross pathologic changes in the intestine of the infected cats were seen clearly. The intestinal tract was dilated, the bowel loops were firm and hyperermic with petechial and ecchymotic hemorrhages on the serosal surfaces. The faeces had a fetid odor when blood was present. Histologic abnormalities in the intestine included dilated crypts, with sloughing of epithelial cells and necrotic debris into the lumen. Shortening of villi had occured secondary to the necrosis of crypt cells. The most severe histologic lesions were found in the jejunum and ileum; the duodenum and colon were less affected. Focal damage was most prominent around lymphoid follicles in the submucosa of the small intestine.

Table 1: The status of *Feline panleukopenia* infections in diarrhoeic companion cats of different age in Ahvaz area, Iran by immunochromatography assay, 2005-2007

Sex	Age			
	<6 months		>6 months	
	Neg.	Pos.	Neg.	Pos.
Male	13	8	10	5
Female	11	6	10	4
Total	24	14	20	9

Table 2: Duration of virus shedding in 12 affected cats to FPV in Ahvaz area, Iran, 2005-2007

Cases	Duration of virus shedding/day: Days							
	1	2	3	4	5	6	7	8
1	+	+	+	–	–	–		
2	+	+	+	–	–	–		
3	+	+	+	+	–	–	–	
4	+	+	+	+	+	–	–	–
5	+	+	+	+	–	–	–	
6	+	+	+	–	–	–		
7	+	+	+	+	+	–	–	–
8	+	+	+	+	–	–	–	
9	+	+	+	–	–	–		
10	+	+	+	–	–	–		
11	+	+	+	+	–	–	–	
12	+	+	+	–	–	–		

Discussion

Our study revealed that 34.3% of diarrhoeic cats which were referred to the Veterinary Clinic of the School in Ahvaz area, southwest Iran, were affected with the FPV antigen. In the present survey, the ill cats had no history of vaccination against FPV, but in the healthy cat population, eight cats were vaccinated. It is obvious that vaccination can play a major role to protect animals from infectious diseases. Knowledge of the status of FPV in affected

cats in Ahvaz area is helpful for preventive measures. Stray cats can play a major role in transmitting the disease to other cats. Meanwhile, the virus is extremely stable and resistant to adverse environmental influences.

The prevalence of FPV infection varies in different countries. Blood samples were analyzed from 30 domestic cats from the Petén region of Guatemala to determine the seroprevalence of FPV. Fifty percent (15 of 30) of the cats sampled were seropositive for feline panleukopenia (Lickey *et al*., 2005). A serosurvey of feline panleukopenia (FPV) in cats from Ho Chi Minh City area in southern Vietnam was conducted and the results compared with their previous results in northern Vietnam. The seropositivity of FPV (44%) was similar to that in Hanoi area (Nakamura *et al*., 1999). The prevalence of antibodies to feline parvovirus (FPV) in 51 European sera was 2%. Samples were collected between 1996 and 1997 from cat populations in France, Switzerland, and Germany. From the low prevalence of FPV infections and from the fact that the European cats live solitarily, it was concluded that this viral infection does not spread readily within a population (Leutenegger *et al*., 1999). Serum samples from leopard cats in Taiwan and Vietnam were examined for the prevalence of antibodies against feline parvovirus. Nine of the 11 leopard cats were shown to have antibodies against feline parvovirus (Ikeda *et al*., 1999). Serological tests were also used to determine the prevalence of infections among 300 mainly adult feral cats in three different habitat types in south-eastern Australia. A high prevalence of specific antibody to feline panleukopaenia virus (79%) was observed (Berns *et al*., 2000).

Over a period from 1973 to 1979, a serologic survey of virus infections was conducted on feline sera collected in four universities located in different prefectures; Obihiro, Saitama, Kanagawa and Tokyo. A significant hemagglutination-inhibition (HI) antibody titer of 1:8 or higher to feline panleukopenia virus (FPV) was detected in 130 (58%) of the 226 sera used (Goto *et al*., 1981).

Feline panleukopenia is one of the most common causes of infectious diarrhoea in cats younger than 6 months. Kittens between 3 and 5 months of age are at high risk for FPV (Greene, 1998). Our study showed that the prevalence of infection was greater in cats less than 6 months of age, though the difference was not significant between age groups ($P>0.05$). Infection was not observed in cats above 1.5 year, presumably it may be due to resistance to the effects of *parvovirus*. Our results indicated that leukopenia may be an important sign of FPV, because most of the ill cats (87%) had WBC<5500 cells/µl.

Duration of virus shedding was 3 days (minimum) to 5 days (maximum) after the onset of diarrhoea in our study. Virus shedding was investigated only in cats that the onset of diarrhoea was less than 24 h from the time they were referred to hospital. We emphasize that isolation and treatment of the affected cats is very important, particularly in the first week of the disease, for the prevention of disease transmission to healthy cats.

High mortality in FPV may be due to secondary bacterial infections with enteric microflora (Greene, 1998). Gram-negative endotoxemia, with or without bacteremia and DIC are common complications of systemic FPV infection. The mortality rate was 30.4% in our study.

In conclusion, we emphasize that vaccination against *panleukopenia* and hygienic procedures are important measures for the prevention of FPV infections in the companion cat population, as FPV is a very stable virus (Greene, 1998). Further epidemiological and biological studies are needed for controlling parvovirus diseases in stray and domestic cats.

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