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Rapid One-Step Test for detection of Feline and Canine Parvoviruses in Cats

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Abstract

Feline parvovirus infection (FPV) is one of the serious diseases in Kittens that causes substantial morbidity and death. For the treatment of affected cats and the prevention of disease spread, early diagnosis of FPV infection is critical. To our knowledge, there have been no reports about the disease's situation in Egypt's Assiut province. As a result, the goal of this study was to find out how common FPV infection is among ill cats in this province. A total of 30 cats suspected of being infected with FPV were screened using an antigen rapid test to determine whether they were clinically suspicious. To determine the prevalence of FPV, each investigated cat's age, sex, breed, season, lifestyle (whether kept indoors or outdoors), and immunization were all documented. Overall, 26.7% of examined cats were affected. FPV infection was more common in young, unvaccinated cats who lived outdoor. Epizootiological monitoring of the prevalence rate based on cat breeds and sex revealed no statistically significant differences. In terms of season, spring had the highest infection rate (57.1%), followed by winter (33.3%), and autumn (7.69%). The rapid one-step test is a useful diagnostic tool for detecting FPV, which was found in the research area's cats.

KEYWORDS
Egypt, Assiut, Feline parvovirus, Survey, Rapid test.

INTRODUCTION

Feline panleukopenia (FP) is a devastating gastrointestinal disease that affects domestic and wild cats. It is caused by one of two closely related non-enveloped single-stranded DNA viruses, feline panleukopenia virus (FPLV) or canine parvovirus-2 (CPV-2) (Barrs, 2019). They are members of the genus protoparvovirus, subfamily parvovirinae, and family parvoviridae (Cotmore *et al*., 2014). In cats, FPV infection causes gastroenteritis, severe hematological abnormalities, primarily panleukopenia, and cerebellar hypoplasia specially in young kittens (Csiza *et al*., 1971).

Cats contract the infection mostly through the oral-fecal or intra-nasal routes, either through direct contact with infected secretions and excretions or by indirect contact with infected fomites. The virus replicates primarily in the oropharynx 12-18 hours after infection, enters the bloodstream within 2-7 days, and subsequently disseminates throughout the body, primarily in lymphoid tissue, bone marrow, and intestinal mucosa (Stuetzer and Hartmann, 2014).

Diseased cats shed high virus titers during the acute infection with FPV which can last up to two days, although the virus can survive in the environment for months (Poole, 1972). As a result of the virus's infectious nature and very lethal effects, rapid diagnosis is required to avoid further illness transmission and to decide the proper therapy for affected cats (Raj and Haryanto, 2020). Different laboratory confirmatory procedures, including PCR, ELISA, virus isolation and identification, are required because clinical signs are not decisive. Because these approaches are time-consuming and need specialist facilities, the employment of a rapid, simple diagnostic technique such as the one-step immuno-chromatographic assay, which is now available and can be used in the field by veterinarians or pet owners, is appropriate for quick disease diagnosis (Esfandiari and Klingeborn, 2000).

Although most rapid tests based on either enzyme linked immunosorbent assay (ELISA) or immuno-chromatographic technology are only licensed for canine parvovirus, due to the close structure and antigenic relationship between CPV-2 strains and FPV, they can also detect FPV with the same test kit (Abd-Eldaim *et al*., 2009). These tests had a relative sensitivity of less than 50% and a specificity of 100% (Kantere *et al*., 2015). Because false positives are less likely than false negatives in these fast assays, a positive test result in a cat presenting clinical signs suggests that the cat is infected with parvovirus (Neuerer *et al*., 2008).

According to the available literatures, only Awad *et al*. (2018)

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used direct ELISA and PCR to evaluate the prevalence of FPV in 165 sick cats in Egypt. As a result, the goal of this study was to determine the prevalence of FPV in the Assiut province of Egypt by detecting FPV antigen in cats with clinical indications of the disease. In addition, several epidemiological features of the condition are being investigated.

MATERIALS AND METHODS

Ethical statement

All procedures were carried out in conformity with the Assiut University institutional ethical committee's ethical norms. All cats were handled in accordance with Assiut University's animal research regulations, and samples were collected only with the owners' consent.

Study area, period, and population

A cross sectional study was conducted in Assiut Governorates, Egypt, from November 2020 to December 2021 on a total of 30 cats of all ages, sexes, and breeds who showed signs of parvovirus infection (lethargy, anorexia, vomiting, and/or bloody diarrhea).

Clinical examination

Thirty cats were referred to Smile pet clinic located in Assiut Governorates, Egypt. Firstly, comprehensive data and history from cat owners, including the primary complaint, food history, vaccination and deworming procedures, and the animal's lifestyle, which included access to the outdoors or confined indoors were collected. All the animals in the study were given a full clinical examination according to Gaskell and Bennett (1996). Physical examination included body temperature, visual mucus membranes, respiratory illness, digestive disturbances, and assessment of dehydration.

Screening of suspected cats for presence of FPV and CPV-2 antigen

There were two fast screening tests employed. The first is the IDEXX Laboratories' SNAP Canine Parvovirus Antigen Test Kit (SNAP Parvo, USA), which is a fast enzyme immunoassay for the detection of canine and feline parvovirus in feces. This test detects virus surface protein antigens (including intact virus particles) excreted in infected animals' feces. The second is the VDRG ®CPV Ag Rapid Kit Median, Korea, which is a lateral flow chromatographic immunoassay that can also detect parvovirus in feline feces.

Sampling and test procedures

For each diseased cat, stool samples were obtained and tested either by SNAP Parvo test or VDRG ®CPV Ag Rapid Kit according to the manufacturer's instructions. Firstly, we obtain a sampling swab to coat its tip with a thin coat of fecal material from the stool or rectum (fecal swabs), then the sample was mixed and stirred well with a dilution buffer to extract the virus from the stool sample thoroughly. Rapid Test Device was placed on a flat surface and 4-5 drops of the fluid were dispensed into the sample well of the test device. Finally, the result was verified within 5-10 minutes. The appearance of colored spot in the parvovirus sample spot meaning of positive result with SNAP test while with VDRG® test when both control and test lines were red meaning of positive result but when only control line was red meaning of negative result and only re-test when control line was not visible.

Statistical analysis

To measure the impact of each factor individually on the prevalence of the disease in cats (i.e., age, sex, breed, season, area, and vaccination status), Fisher's exact test in Epi Info™ 7.2.2.6 software was used. A probability value (P-Value) $P < 0.05$ was considered statistically significant.

RESULTS

Clinical findings

The thirty cats in this investigation displayed one or more of the usual gastroenteritis clinical symptoms (Fig. 1). A wide variety of clinical symptoms were recorded, which may vary from case to another. Eight cats were suffering from anorexia, lethargy, and abdominal pain. Hyperthermia was seen in 17 cats, and persistent vomiting was seen in ten of them. There were 23 cats with diarrhea, 9 of which had mucoid diarrhea and 14 of which had bloody feces. Dehydration was detected in 25 cats, including mild dehydration in 10 cases, moderate dehydration in 3 cases, and severe dehydration in 13 cases. Table 1 and Fig. 2 depict the clinical symptoms observed in cats of various ages, sexes, and breeds.

Results of rapid screening tests for presence of FPV and CPV-2 antigens

Eight (26.7%) of the 30 sick cats investigated tested positive for FPV antigens (Fig. 3 and Table 2), in the meantime, 22 cases (73.3%) were found to be negative. Infection was found to be more common in kittens aged 1 to 6 months (40%) than in other age groups. Also, according to our findings, male cats had a greater infection rate (30.8%) than female cats (23.5%), with no

Table 1. The reported clinical signs in examined cats in each age, sex, and breed.

Cases		Breed				Sex						Diarrhea			Dehydration	
Age	No.	Persian	Siamese	Egyptian Mau	Mixed	Male	Female	Anorexia	Lethargy	Fever	Vomiting	Mucoid	Bloody	Mild	Moderate	Severe
1-6 months	10	6	1	1	2	5	5	3	2	7	3	4	3	5	1	3
>6-12 months	10	6	1	-	3	5	5	1	2	5	3	4	4	1	2	4
>12 month	10	4	-	2	4	3	7	4	4	5	4	1	7	4	-	5
Total	30	16	2	3	9	13	17	8	8	17	10	9	14	10	3	12

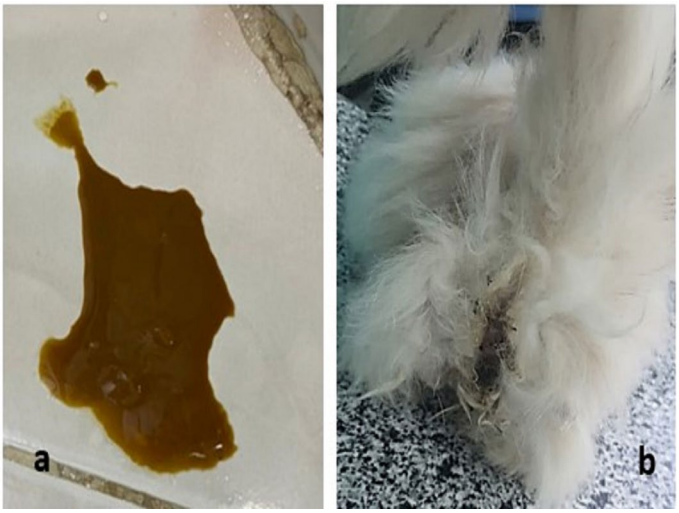

Fig. 1. a. bloody diarrhea b. Cat perineum soiled with watery mucoid diarrhea.

significant differences (P>0.05). Outdoor cats had a higher infection rate (33.33%) than indoor cats, notably during the spring (57.1%) and winter (33.33%) seasons. Siamese and Egyptian Mau cats were free of illness, but Persian and mixed breed cats were infected at about equal rates (31.5 and 33.33%, respectively). In our investigation, we found FPV in 8 (28.6%) of the 28 cats who had never been vaccinated, with a highly significant difference between the unvaccinated and vaccinated groups (P= 0.002343). Table 2 illustrates the results of the rapid test for FPV detection in relation to each studied cat's age, sex, breed, season, and vaccination status.

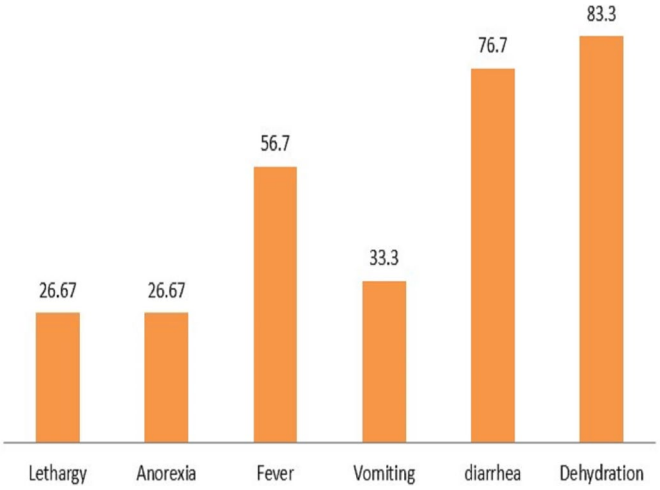

Fig. 2. The percentage of repeated clinical findings in the study population.

Table 2. Factors associated with parvovirus prevalence among the examined cats.

Factor	No. tested	Rapid test		Std Error	F-test	P-value
		Positive n (%)	Negative n (%)			
Age						
1-6 months	10	4 (40)	6 (60)			
> 6-12 months	10	1 (10)	9 (90)	0.347	0.241	0.628
>12 months	10	3 (30)	7 (70)			
Total	30	8 (26.7)	22 (73.3)			
Sex						
Male	13	4 (30.8)	9 (69.2)			
Female	17	4 (23.5)	13 (76.5)	0.211	0.186	0.670
Total	30	8 (26.7)	22 (73.3)			
Breed						
Persian	16	5 (31.25)	11 (68.75)			
Siamese	2	0	2 (100)			
Egyptian Mau	3	0	3 (100)	0.574	0.010	0.922
Mixed	9	3 (33.33)	6 (66.67)			
Total	30	8 (26.7)	22 (73.3)			
Season						
Spring	7	4 (57.1)	3 (42.9)			
Summer	1	0	1 (100)			
Autumn	13	1 (7.69)	12 (92.31)	0.46	1.584	0.219
Winter	9	3 (33.33)	6 (66.67)			
Total	30	8 (26.7)	22 (73.3)			
In/outdoor						
Indoor	21	5 (23.8)	16 (76.2)			
Outdoor	9	3 (33.33)	6 (66.67)	0.195	0.275	0.604
Total	30	8 (26.7)	22 (73.3)			
Vaccination						
Yes	2	0	2 (100)			
No	28	8 (28.6)	20 (71.4)	0.331	0.747	0.002
Total	30	8 (26.7)	22 (73.3)			

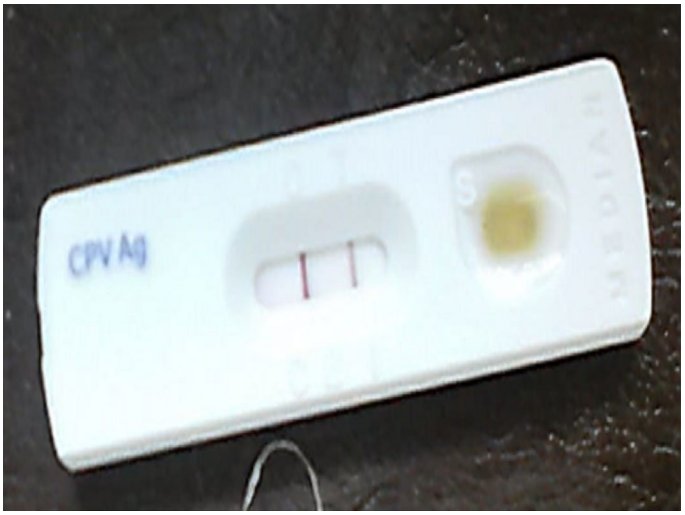

Fig. 3. Positive VDRG ®CPV Ag Rapid test.

DISCUSSION

Feline panleukopenia is still one of the most dangerous viral illnesses in cats, causing acute severe enteritis and significant mortality rates, especially in kittens who have not been immunized. The current study provides an excellent overview of the disease's clinical and epidemiological status among cats in the study area. In this study, the most noticeable clinical signs were fever, lethargy, anorexia, abdominal discomfort, vomiting, bloody or mucoid diarrhea, dehydration, and hypothermia, which occur in the late stages of the disease. These symptoms arose from severe gastroenteritis, which was caused by the virus replicating in the intestinal crypt epithelial cells, primarily in the jejunum and ileum, resulting in the destruction of the intestinal villi, increased intestinal permeability, malabsorption, and, as a result, the appearance of vomiting and diarrhea, which frequently contained blood and mucus, and so on dehydration occurred (Sykes, 2014). In addition, cats infected with FPV are more likely to develop secondary bacterial infections caused by intestinal bacteria, which can result in gram-negative endotoxemia and perhaps bacteremia (Mantione and Otto, 2005). These clinical signs have previously documented by Miranda *et al.* (2017).

Eight of the thirty infected cats tested positive for the FPV virus (26.7%). Various studies on the disease's prevalence have been conducted in various countries, with results that are more or less similar to the current findings. Islam *et al.* (2010) and Zenad and Radhy (2020) reported nearly similar prevalence rates in different areas. Using the ICG assay, they discovered that 13 out of 58 cats (22.41%) and 40 out of 180 cats (22.2%) were FPV infected, respectively.

In contrast, using the PCR approach, Awad *et al.* (2018) in Egypt and Raj and Haryanto (2020) in Indonesia assessed the illness prevalence to be 45% and 72.2%, respectively. In addition, seroprevalence was found in 51 European wild cat sera in different European nations (France, Switzerland, and Germany) (Leutenegger *et al.*, 1999). Variations in the number of samples analyzed and the diagnostic tests employed for sample analysis among various studies in different geographic locations could explain the observed variances in prevalence rates (Kim *et al.*, 2013 and Sayed-Ahmed *et al.*, 2020).

The majority of the cats evaluated in our study (28 of 30) had no history of vaccination, and 8 of these non-vaccinated cats tested positive (28.6%). Meanwhile, all of the vaccinated cats tested negative with a statistically high significant result (P=0.002343). This, predictably, explains the research area's substantially higher prevalence rate of FPV infection and emphasizes the necessity of immunization for disease prevention. This finding is consistent with prior research that found greater prevalence rates in unvaccinated cats (Horzinek, 2006 and Sykes, 2012). In addition, present work cats with access to the outdoors had a higher FPV infection rate (33.33%) than indoor cats (23.8%), with no statistically significant difference (P=0.603856). The virus's stable nature, which allows it to survive for at least a year in organically contaminated materials (Poole, 1972), and outdoor cats' exposure to high levels of environmental stress (Millán and Rodríguez, 2009), may boost the virus's infection rate. Household cat owners, on the other hand, are concerned about vaccinating their pets on a regular basis and avoiding contact with infected cats (Kim *et al.*, 2013).

The highest prevalence of FPV infection was found in cats aged 1 to 6 months (40%), followed by cats aged more than 12 months (30%), and cats aged > 6 months up to 12 months (10%), with no significant association between age and infection status (P=0.627533). These findings are consistent with those of (Bukar-Kolo *et al.*, 2018 and Mosallanejad *et al.*, 2009), who found a greater prevalence of FPV in kittens under 6 months of age. Because the virus prefers tissues with a high rate of mitotic activity in the s-phase of division, such as lymphoid tissues, bone marrow, and intestinal epithelium, which are permissive for viral replication, young kittens were more likely to acquire parvovirus illness (Csiza *et al.*, 1971 and Parker *et al.*, 2001). Inadequate immunization may also be to blame for the greater prevalence rate in older cats over 12 months (30%) in this study. The disease is most common in kittens under the age of one year, although it can also affect older, unvaccinated cats (Kruse *et al.*, 2010).

The findings of the rapid test for sex susceptibility of cats to FPV infection were 30.8% and 23.5% for positive male and female cats, respectively, with no significant difference between them. In contrast, (Bayati, 2016) found that the condition is somewhat more common in female cats than in male cats, but also with no significant differences between them.

Because the study was done on a small number of animals and the Persian breed was overrepresented in the study due to residents in this province preferring this type of cat, feline Parvovirus infection was only identified in two breeds of cats: Persian and mixed breeds. According to previous research, there was no significant difference in the prevalence of the disease by cat breed (Islam *et al.*, 2010; Bayati, 2016 and Awad *et al.*, 2018).

Spring (57.1%) had the highest infection rate, followed by winter (33.33%) and autumn (7.69%), with no cases documented in the summer (0%). FPV is a relatively stable virus that can survive for months at room temperature, primarily in feces and solid fomites. Heating and drying it in the summer reduce its survival and speeds up its inactivation (Truyen and Parrish, 1992). These findings are substantially identical to those published by El-Neshwy *et al.* (2019) in Egypt who found that the highest prevalence of CPV-2 infection in dogs occurred in the spring, followed by the winter. In the United States, however, outbreaks are more likely in July, August, and September due to the influx of kittens born in the spring and admitted to shelters, especially when mother immunity is decreasing (Reif, 1976).

CONCLUSION

The current study was the first to shed light on the clinical and epizootiological picture of FPV in Assiut Government, where it is widely spread and should be recognized quickly using FPV antigen fast tests to develop the best control methods.

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CONFLICT OF INTEREST

The authors declared that no conflict of interest exists.

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