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Research Article

Prevalence and Pathology of Feline Panleukopenia in Domestic Cats

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Abstract

Prevalence and pathology of feline panleukopenia was conducted in cats of Mumbai region from January 2020 to January 2021 and prevalence was found to be 70% (35/50). Age wise study revealed higher incidence of FPL in the age group of 0- 6 months (56%), followed by 6-12 months (8%) and lowest in one year and above group (6%). Non- descript cats/kittens (85.72%) were found to be affected at a higher percentage than pure breed (Persian) cats (14.28%). The higher percentage of female cats (65.71%) were affected by FPL than males (34.29%) cats. Necropsy examination of cat died due to FPL showed catarrhal exudation with hyperaemia, haemorrhages in stomach and intestinal mucosa, enlarged mesenteric lymph nodes and slightly pale bone marrow. Lung and liver were congested. Microscopically, section of small intestine showed coagulative necrosis, dilatation of crypt containing mucus and necrotic debris and denudation of villi and proliferative changes in submucosa. Infiltration of inflammatory cells like polymorphs, lymphocytes and basophilic intranuclear inclusions in intestine and stomach as well as bacterial colonies in stomach were observed. Lymph nodes and spleen showed lymphoid cell depletion whereas lung showed acute interstitial pneumonia. For diagnosis of FPL, SNAP test could able to detect 58% (29/50) of sample while PCR detected 70% (35/50) and PCR appeared to be more sensitive than SNAP test.

Keywords: Feline Panleukopenia; Cats; Pathology; Prevalence; PCR and SNAP test

Introduction

Feline panleukopenia (FP) is highly contagious viral disease of cat and other felids such as wild cat, cheetah and tiger. The FP is caused by a Feline panleukopenia virus (FPLV) of parvoviridae family and is a single-stranded non enveloped virus. The virus is highly stable, resistant to chemical and physical reagents, and not affected seriously by heat at 60 ^{0}C for an hour. Thus, the environment remains contaminated for longer period after diseased cat excrete the virus in the environment through all excretions and secretions. The FPV is considered to be one of the most leading cause of death in kittens (>50%), cats and other members of feline family like big cats. Kittens below 6 months are most severely affected by the virus. Parvovirus specially affects rapidly growing and dividing cells, such as those in the intestines, bone marrow, lymphoid organs and the developing foetus [1,2].

Feline panleukopenia virus is antigenically closely related to canine parvovirus type 2. In Germany, CPV was detected in only approximately 10% of feline samples, but in Southeast Asia, reports showed that, about 80% of diseased cats were infected with CPV. In 1978, canine parvovirus type 2 (CPV2) emerged in domestic dogs and spread and genetic drift soon changed its antigenicity. The serotype CPV2a appeared in 1979 and CPV2b in 1984 [3].

These antigenic types have different host ranges; CPV2a and CPV2b are found in both cats and dogs, although CPV2 has been

found only in dogs. Both feline parvovirus and canine parvovirus can cause the FPL although CPV infection in cats are not common. Feline panleukopenia is the oldest known viral disease of cats [4].

The present study was designed to study the prevalence of feline panleukopenia in domestic cats from Mumbai region by using SNAP test (Lateral flow assay) and PCR, respectively. The study also highlighted the gross and microscopic features of the disease.

Material and Method

Clinical samples

A total of 50 stool samples were collected from cats and kittens suspected for Feline panleukopenia from different pet clinics and animal hospitals of Mumbai region. The samples were collected from cats that had history anorexia, fever (104-105 ^{0}F), intractable vomiting and diarrhoea. Faecal samples were collected aseptically in sterile container and swab and transported to lab on ice. The details information about age, gender and breeds were collected. According to their age, the dogs were divided into 3 groups (0-6 months, 6-12 months and above 12 months). Feline panleukopenia cases were confirmed by SNAP test and reconfirmed by conventional PCR. A detailed necropsy was conducted on cats died due to FP infection and gross observations were recorded. Different organs like stomach, intestine and spleen were collected in 10% formalin to study histopathological changes.

Diagnosis of FPV by lateral flow assay

All the fifty faecal samples collected from the affected cats and kittens were subjected for diagnosis of FPV by lateral flow assay by using feline panleukopenia antigen detection kit (petX Biotech).

Diagnosis of FPV by polymerase chain reaction

Isolation of viral DNA from stool

All the fifty faecal samples were subjected to viral DNA isolation by using a stool DNA isolation mini kit (Favorgen Biotech corp; HCN code-38220090)). Stool DNA isolation kits of were procured from Virion Enterprises, Kamothe, Navi Mumbai. All the solutions and buffers were procured from SRL laboratory Inc., Mumbai and prepared using deionized double glass distilled water.

Processing of stool sample

The freezing (-20 ^{0}C) and thawing at (room temperature-RT) of the stool sample were carried out three times for a period of 2 to 4 h in each step. The viral DNA was extracted from stool as per the protocol provided with the stool DNA isolation kit.

Determination of purity and yield of the deoxyribonucleic acid (DNA) samples

The purity and concentration of the extracted DNA were estimated by UV spectrophotometry (Nanodrop, M/s Thermo Scientific). The OD was measured for 1 µL sample at 260/280 nm and finally, DNA concentration was adjusted to 100 ng /µL for PCR studies.

Polymerase chain reaction

Extracted DNA from suspected representative stool samples was used as template DNA. The vaccine virus (FPV) DNA was extracted and used as positive control.

PCR mixture (single sample)

Primers used

Forward primer FM-F (5-GCTTTAGATGATACTCATGT-3 and Reverse primer FM-R (5-GTAGCTTCAGTAATATAGTC-3) were used to amplify 698 bp of the single-copy conserved 5 ends of the VP genes [5].

Component/Item	Volume/sample (µL)
Reaction mixture	12.5
Master mix	6.25
Forward primer	0.5
Reverse primer	0.5
Nuclease free water	4.25
Template DNA	01
Total	12.5

Table a

The details of PCR conditions are illustrated in table 1.

	Initial denaturation	Denaturation	Annealing	Extension	Final extension
Temp.	95°C	95°C	49°C	72C	72°C
Time	3min.	1min.	2 min.	45 sec.	10 min.
		35 cycles			

Table 1: Feline parvovirus PCR conditions.

PCR product visualisation

The products obtained by PCR for FPLV were subjected to agarose gel (1%) electrophoresis along with positive control and 100 bp DNA molecular weight marker for each sample. The product obtained was read with reference to the positive control and 100 bp DNA molecular weight marker. The specificity of PCR products was confirmed by the appearance of the desired band of specific molecular weight (698-700 bp) under UV illuminator and photographed for permanent record.

Results

Prevalence of feline panleukopenia

In the present study, total of fifty samples were collected from FPV non-vaccinated cats/kittens showing clinical signs vomiting, diarrhoea, dehydration, fever, anorexia and depression. The samples were screened for FPL using SNAP stool test kit (Lateral flow assay) out of which 29 samples (58%) were found positive by Lateral flow assay**.** All these fifty samples were further subjected to polymerase chain reaction (PCR) in which 35 (70%) samples were found positive and recorded a prevalence of 70%.

Age wise prevalence of feline panleukopenia

The prevalence of feline panleukopenia in cat in relation to different age groups are presented in table 2**.** In the present study, the age of the cats affected with feline panleukopenia ranged from one month to seven years. Out of total clinical cases (positive by PCR), highest incidence of feline panleukopenia was observed in the age group of 0- 6 month (80%), followed by 6-12 month (11.43%) and lowest in an age group of more than one year (8.57%).

Age (Months)	No cases positive by PCR	Percent positive cases
0-6	28	80
6-12	04	11.43
>12	03	8.57
Total	35	100

Table 2: Distribution of feline panleukopenia cases in cat based on Age (n = 50).

Breed wise prevalence of feline panleukopenia

Breed wise distribution of clinical cases of feline pan leukopenia in cats are presented in table 3. In the present study, the highest prevalence of feline panleukopenia was observed in non- descript cats/kittens (85.72%) followed by pure breed (Persian) cats (14.28%). Prevalence of feline panleukopenia in non-descript cat is more as compared to pure breed cats (14.28%) and could be due to a greater number of non-descript cats (85.71%) being presented and screened for FPL.

Breed	No. of cases tested for FPL	No cases positive by PCR	Percentage positive cases
Non-descript (ND)	50	30	85.72
Pure breed (Persian)		05	14.28
Total	50	35	100

Table 3: Distribution of feline panleukopenia cases in cat based on breed.

Gender wise prevalence of feline panleukopenia

Gender wise distributions of clinical cases of feline pan leukopenia in cats was carried out in 35 cats/kittens. Out of 35 cases, 23 cases were recorded in females (65.71%) and 12 cases in males (34.29%). The female cat has higher incidence of infection as compared to male counterpart.

Gross pathology

Grossly, the carcass of the kitten died due to FPL appeared severely dehydrated and emaciated with pale mucus membrane and rough body coat. The principle gross lesions were observed in stomach, intestine and lymphoid organs. The gastric mucosa appeared hyperemic and showed catarrhal exudation with focal ulcerations (Figure 1). Jejunum and ileum were severely affected than the duodenum and large intestine. The intestinal mucosa showed severe catarrhal enteritis with yellowish to brown tinged exudate (Figure 2). Upon removal of exudate from mucosa, the underneath mucosa was hyperemic and showed petechial hemorrhages with focal ulcerations.

Lymph nodes (LN) of the mesentery were enlarged and edematous. The bone marrows of the long bones appeared slightly pink rather than deep red. The lungs were moderately congested and liver showed enlargement with congestion. The other organs like heart and kidney did not show any appreciable gross lesions.

Histopathology

Microscopically, the principal lesions were found in gastrointestinal tract and lymphoid organs. Section of stomach showed gastri-

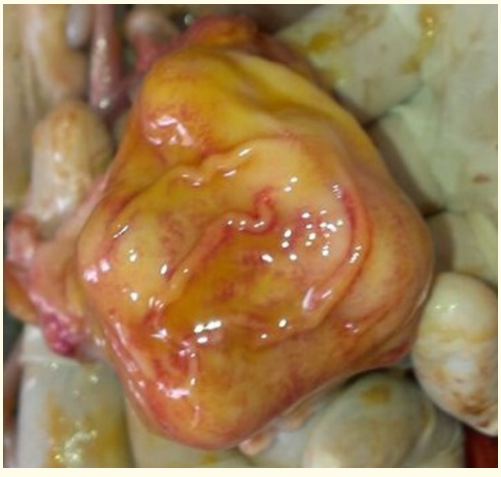

Figure 1: Stomach of FPL affected cat showing catarrhal exudate with hyperemia on mucosa.

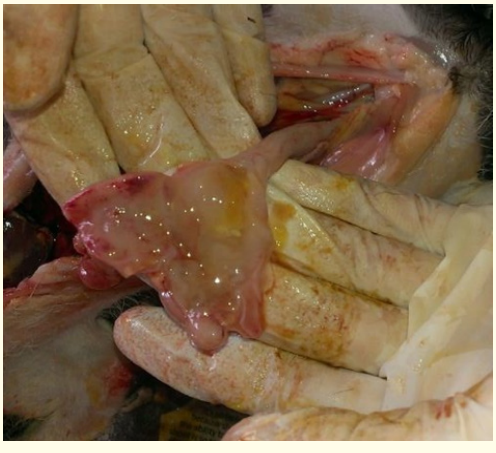

Figure 2: Intestine of FPL affected cat showing catarrhal exudate in lumen.

tis with infiltration of inflammatory cells like polymorph and lymphocytes with numerous bacterial colonies (Figure 3). The mucosa of small intestine showed coagulative necrosis and denudation and loss of villi due to which the remaining epithelium undergoes proliferative changes (Figure 4). The crypts were dilated and contain mucus and necrotic debris (Figure 5). The mucosa and sub mucosa of small intestine showed sparse infiltration of polymorphs and lymphocytes with basophilic intranuclear inclusions bodies (Figure 6). Section of the lymph nodes and spleen showed necrosis (hyperchromasia, pyknosis and karyorrhexis) of lymphocytes, histolytic proliferation and lymphoid cell depletion in follicular and paracortical region in LN (Figure 7 and 8) and Malpighian corpuscles of the spleen. Section of lung showed moderate diffuse acute interstitial pneumonia characterized by exudation and infiltration of neutrophils and predominant population of lymphocytes in interstitium. Heart did not show any inclusion bodies.

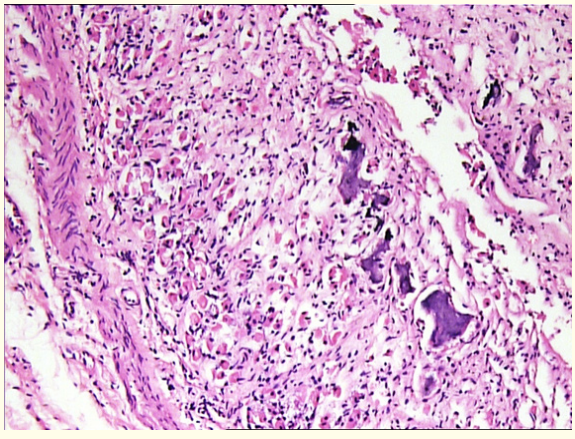

Figure 3: FPL-Photomicrograph of stomach showing severe gastritis with infiltration of inflammatory cell like polymorphs and lymphocytes with numerous bacterial colonies. (H and E x 200X).

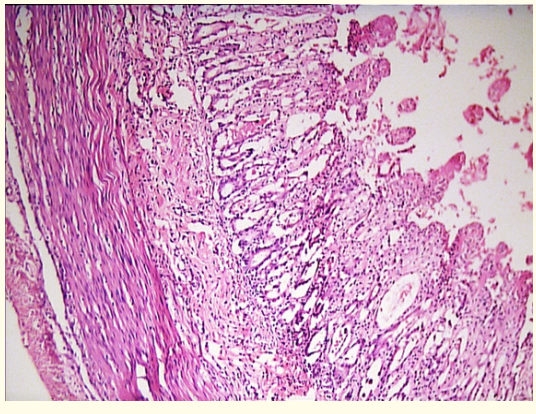

Figure 4: FPL-Photomicrograph of intestine showing coagulative necrosis, loss of villi and proliferative changes in submucosa (H and E x 100X).

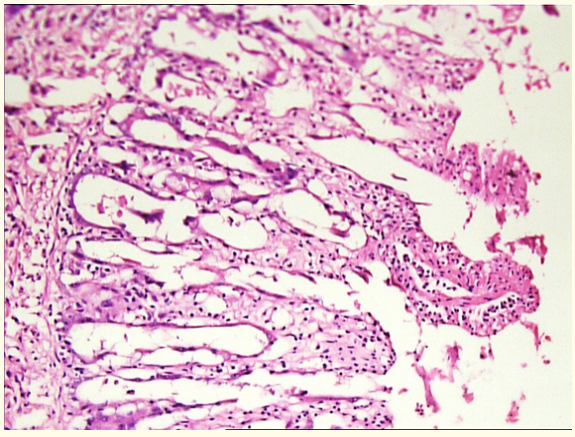

Figure 5: FPL-Photomicrograph of intestine showing dilated crypts containing mucus and necrotic debris (H and E x 200X).

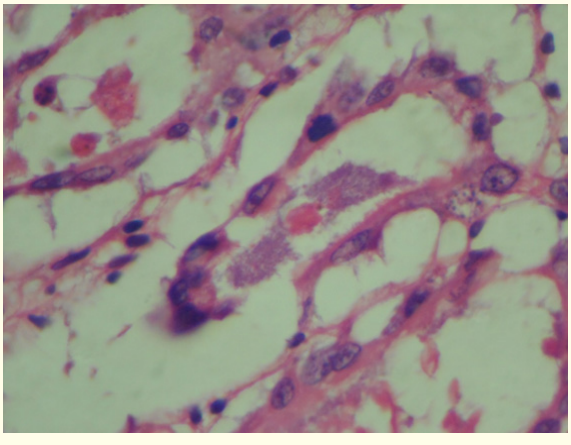

Figure 6: FPL-Photomicrograph of intestine showing intranuclear basophilic inclusion (Arrow) (H and E x 400X).

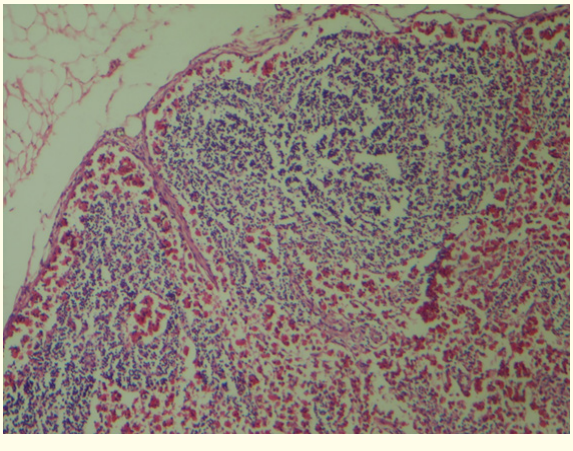

Figure 7: FPL-Photomicrograph of lymph node showing lymphoid depletion in follicular and paracortical region (H and E x 100X).

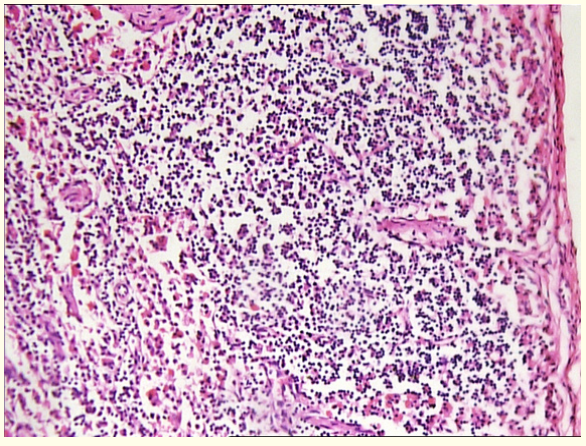

Figure 8: FPL-Photomicrograph of lymph node showing severe lymphoid necrosis indicated by hyperchromasia, pyknosis and karyorrhexis (H and E x 200X).

Diagnosis of FPV

During the study period (January 2020 to January 2021), total of fifty cat's samples were screened by SNAP (PetX) and PCR. Out of 50 faecal samples, 29 samples were positive (58%) by SNAP test (lateral flow assay) (Plate-19). And 35 samples (faeces) for feline pan leukopenia specific primers of VP genes by conventional PCR, and showed a specific band of 698 bp (Figure 9). All the sample positive by SNAP test were also positive by PCR test.

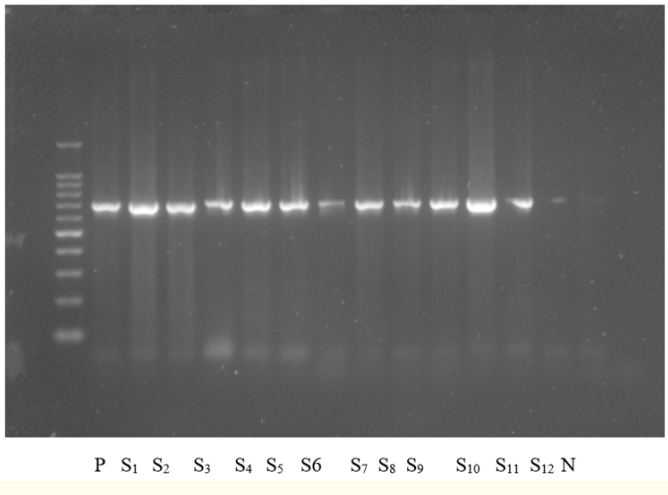

Figure 9: Detection of feline panleukopenia by PCR (698 bp amplicon): P - Positive control; L-DNA Ladder; S_1 to S_{12}-Cats samples; N - Negative control

Discussion

Prevalence of feline panleukopenia

The cats/kittens affected with FPV showed vomition, diarrhoea, dehydration, fever, anorexia and depression. Out of 50 faecal samples, 29 samples (58%) were found positive for FPV by Lateral flow assay and 35 (70%) samples by PCR. These findings are in agreement with those of Bayati., *et al*. [6] who reported 51. 1% prevalence of FPV by PCR and 38% by SNAP test in Iraq. Similarly, Raheena., *et al*. [7] reported 77.77% and 37.03% prevalence of FPL by PCR and IC strips, respectively in five districts of Kerala, and Bakde [9] reported 85% prevalence of feline panleukopenia by PCR method in different districts of Kerala, India**.** On contrary to the present study, Abd-Eldaim., *et al.* [10] reported 55 (100%) samples positive by SNAP Parvo and 54 (98.18%) out of 55 samples positives by conventional PCR method. Whereas, lower prevalence of FPL was recorded by Islam., *et al.* [11] who tested 58 cats by SNAP test kit out of which 13 (22.41%) were found positive for FPL at different villages at Tangail district in Bangladesh, and Bukar-Kolo., *et al.* [12] recorded 4% prevalence of FPL in pet cats and 9.5% in stray cats, respectively in Maiduguri, Northeastern Nigeria. Kim., *et al*. [13] tested blood sample for FPL in Seoul, Korea by using PCR and reported a prevalence of 2% (4 cats) out of which 3% (3 cats) were stray cats and 1% (1 cat) were household cats.

In present investigation, prevalence of FPL was higher in Mumbai region (India) and could be due to ubiquitous nature of virus, persistence of virus in the environment for longer periods leading to frequent exposures in native cat population and the lack of vaccination in cat population as also reported by Bakde [9] in Kerala, India.

Variation in the prevalence data observed by various authors could be due to epidemiological factors viz. prevalence of virus, geographical location, weather conditions, seasonal variations and type of sample collected (feces/blood). Kim., *et al*. [13] reported very low prevalence (2%) of FPL in Seoul, Korea and could be due the fact that blood samples were being used for detection of virus by PCR and all the cats were selected for the study irrespective of their health status (Health/sick).

Age wise prevalence of feline panleukopenia

In the present study, highest incidence of feline panleukopenia was observed in the age group of 0- 6 month (80%), followed by 6-12 month (11.43%) and lowest in an age group of more than one year (8.57%). These observations are in accordance with observation recorded earlier by various researchers [4,8,11,12,14-18].

The present study indicated that virus can affect kittens or cats of all age groups although in varied proportion. Similarly, earlier studies on FPL revealed that virus affects cats or kittens of all age groups but kittens below 6 months are more prone to infection than older cats. Moreover, stray cats were commonly affected by FPV than home cats as they were exposed to the various viruses in nature than the home cats [11,14].

Breed wise prevalence of feline panleukopenia

In the present study, the highest prevalence of feline panleukopenia was observed in non- descript cats/kittens (85.72%) followed by pure breed (Persian) cats (14.28%). Prevalence of feline panleukopenia in non-descript cat is more as compared to pure breed cats (14.28%) and could be due to a greater number of non-descript cats (85.71%) being presented and screened for FPL. The literature on the prevalence of FPL in relation to breed appears to be scanty.

Contrary to this, Awad., *et al.* [8] reported that FPL infection did not show any significant difference between Siam and Persian breeds. They observed that there was no correlation on occurrence of FPL in different breed of cat.

Gender wise prevalence of feline panleukopenia

In the present study, out of 35 cases, 23 cases were recorded in females (65.71%) and 12 cases in males (34.29%). The female cat has higher incidence of infection as compared to male counterpart. These findings are similar with Islam., *et al.* [11] who reported 26.92% prevalence of FPL in female than those of male (18.75%) cats. Also, Awad., *et al.* [8] reported higher prevalence of FPV in female (40.5%) than male (39.5%) cats. On the contrary, Citarová and Mojzisova [18] reported higher incidence of FPL in male cats than female cats. However, Mosallanejad., *et al.* [14], Kim., *et al.* [13] and Zenad and Radhy [17] did not observe any significant correlation of gender on prevalence of FPL.

Gross pathology

The kitten died due to FPL on gross examination showed dehydration, emaciation, anaemia and rough body coat. The stomach, intestine and lymphoid organs were severely affected. Stomach showed gastritis with catarrhal exudate and haemorrhages. The

intestinal mucosa showed severe catarrhal enteritis with yellowish to brown tinged exudate with hyperemia and haemorrhages on underneath mucosa. Mesenteric LN were enlarged and edematous, while lung and liver appeared to be congested.

The gross lesions observed in the present study corroborate the observation described earlier [14,19-20]. Jones., *et al*. [19] were of the opinion that in initial stages of disease, the lymph nodes appear edematous and hyperemic on gross examination. Microscopically, proliferation of histiocytes seen in lymphoid organ was soon followed by necrosis of lymphocytes in follicular and paracortical region in LN, Malpighian corpuscles of the spleen and cortex of the thymus and Payer's patches leading to atrophy of lymphoid organ in terminal stages of disease. Similar to the present study, Mosallanejad., *et al*. [14] also reported severe lesion in Jejunum and ileum with mild lesion in duodenum and colon. The pale color of bone marrow could possibly due to destruction of hematopoietic tissue as virus has affinity for dividing cells of bone marrow *viz*. stem cell population leading to their destruction as reported by various authors [14,19-20].

Histopathology

The section of stomach and intestine showed microscopic lesion characteristic of FP and characterized by severe inflammatory changes with infiltration of inflammatory cells and intranuclear inclusion bodies. Section of the lymph nodes and spleen showed lymphoid depletion and indicated immunosuppression. Lung and liver showed mild to moderate inflammatory changes. The microscopic lesions observed in the present study support the observation reported by Carison., *et al.* [21] and Jones., *et al.* [19]. Jones., *et al.* [19]. Carlson., *et al.* [22] were of the opinion that the severe clinical signs observed in FP are result of severe intestinal destruction. Jones., *et al*. [19] was of the opinion that the principal microscopic lesions in FPL were observed in gastrointestinal tract where the virus multiplies in the dividing cells in the crypt. Similar to the present study, presence of intranuclear basophilic inclusion bodies in section of small intestine due to FP has been reported [21]. The diffuse acute interstitial pneumonia observed in the present study could possibly be due to secondary bacterial infection which was supported by observing bacterial colonies in section of stomach and in accordance with observation reported by Lane., *et al.* [20] who observed moderate diffuse acute interstitial pneumonia with moderate multifocal acute pulmonary oedema and multifocal acute moderate necro hemorrhagic bacterial gastritis in addition to lesion specific to FPL. They suggested that the addition lesions could be due to secondary bacterial enteritis and septicemia. The destruction of lymphocytes in various lymphoid organs could have resulted into immunosuppression and multiplication of bacteria present in intestine as observed in histological section of stomach during the study. Similarly, Boes and Durham, [23] were of the opinion that immunosuppression in FPL occurs directly through lymphocytolysis and indirectly through depletion of lymphocyte precursors. Diagnosis of feline panleukopenia.

Diagnosis of feline panleukopenia by SNAP test and PCR

Diagnosis of feline panleukopenia by SNAP test

Out of 50 faecal samples, collected from affected cat/kitten, 29 samples (58%) were positive by SNAP test (lateral flow assay). Findings in the present study are in accordance with Bayati., *et al.* [6] who reported 32 (38%) FPL affected cats by rapid test kit and 43 (51. 1%) by PCR. However, Abd-Eldaim., *et al.* [10] reported that most of the cases which were positive by SNAP test were also positive by PCR method. They found total of Fifty-five samples were positive by SNAP Parvo and 54 out of 55 were positive by conventional PCR in FPL affected cat's samples. On contrary to this Awad., *et al.* [8] reported that those cases which were negative by ELISA method were positive by PCR method.

Diagnosis of feline panleukopenia by PCR test

In the present study, total of 70% (35/50) samples were found positive for feline pan leukopenia by PCR. It has been reported that sensitivity of PCR technique in detecting FPL cases is better than sensitivity of other techniques *viz*. SNAP test, ELISA and IC strips [6-8,12]. Abd-Eldaim., *et al.* [10] observed that most of the cases diagnosed by PCR technique were positive than other diagnostic methods. It may be due to better sensitivity of PCR in diagnosing FPL cases than the sensitivity of other diagnostic techniques like ELISA/SNAP test). They opined that many samples which were positive by ELISA/SNAP test were negative by PCR and could be due to recent recovery from the feline panleukopenia infection or could be due to recent vaccination history.

Conclusion

In conclusion, present study put on record the occurrence of feline panleukopenia first time in Mumbai region along with detail incidence in male and female cat/kitten population along with gross, microscopic and molecular techniques and its comparison with SNAP test.

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