

FELINE PANLEUKOPENIA VIRUS INFECTION IN A CAPTIVE-BRED BENGAL TIGER (*Panther tigris tigris*) AND A LEOPARD (*Panthera pardus*)

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INTRODUCTION

Feline panleukopenia (FPL) (also known as feline distemper) is a contagious and fatal disease of both domesticated and wild felids. The disease is caused by Feline panleukopenia virus (FPLV) of the family parvoviridae. This virus is closely related to the canine parvovirus (CPV), mink enteritis virus (MEV) and raccoon parvovirus and only show <2 % difference in their genome (Steinel *et al.*, 2001; Taylor *et al.*, 1999). *In vitro* culture and *in vivo* inoculation of virus of the family parvoviridae have confirmed overlapping host ranges, and their classification is mostly based on the host from which the virus has been isolated (Shackelton *et al.*, 2005; Truyen and Parrish, 1992). The FPLV among wild felids are less commonly reported due to difficulties in observing clinical signs, although, many evidences confirm that FPLV can affect almost all species of wild felids (Lane *et al.*, 2016; Olmsted *et al.*, 1992).

The Feline panleukopenia is transmitted through oronasal route from contaminated food, fomites and air. Viral multiplication in actively dividing cells (such as, bone marrow, lymphoid tissue, intestinal epithelium, cerebellum and retina of neonate kittens) cause lysis of cells resulting in panleukopenia, diarrhoea, ataxia, incoordination and vision impairment in young animals (Steinel *et al.*, 2001; Truyen *et al.*, 2009; Parrish, 1995), and abortion, still birth and fetal resorption in infected pregnant queens (Jordan and Sever, 1994).

Recently, a leopard cub (*Panthera pardus*) and a Bengal tiger cub (*Panthera tigris tigris*) housed at two distantly located Zoological Gardens in Sri Lanka had developed haemorrhagic diarrhoea, and the aetiology was confirmed as FPLV by polymerase chain reaction (PCR) and sequencing. This communication discusses the clinical presentations, and pathological changes observed in these two felids due to FPLV.

Case 1: A ten-month old unvaccinated female leopard cub housed at Pinnawala open zoo had developed lethargy, acute inappetance, haemorrhagic diarrhoea and vomiting

in mid September 2016. Blood on the first day of the clinical onset revealed a total leukocyte count (WBC) of $22.47 \times 10^3/\mu\text{l}$, without significant changes in the differential counts other than the presence of 4% of band neutrophils. The hematocrit was 35.3% and she was moderately dehydrated at the time of blood sampling for laboratory analysis. Differential diagnoses included, enteritis caused by CPV, FPLV, *Salmonella enterica* or *E. coli* (enterohaemorrhagic *E. coli*). The faecal sample was negative for *Salmonella enterica*. The PCR amplifying the VP2 gene of parvovirus, (F 5'-TTACTAAGAACAGGTGATGAA-3' and R- 5'-ATTTGGATAAACTGGTGGT-3') confirmed parvoviral DNA in faeces (Figure 1).

The leopard cub was housed in isolation and treated with ceftriaxone (50 mg/ Kg BW, IV, 12 h), metranidazole (20 mg/ Kg BW, IV, 12h), metoclopramide (0.5 mg/ Kg BW, IV, 24h), catosal (1 ml containing, 100mg of 1-[n-Butylamino]-1- methylethyl phosphonous acid [Butaphosphan] and 0.05mg of vitamin B₁₂). In addition, intravenous fluids (0.9% NaCl and lactated ringers) were administered to correct dehydration and electrolyte loss, and 25% dextrose and amino acids were administered as energy and protein supplements. No oral food was introduced until vomiting ceased. She responded well to treatment and recovered after 4 days.

Case 2: A three month old unvaccinated female Bengal tiger cub housed at National Zoological Gardens, Dehiwala developed haemorrhagic diarrhoea and vomiting. The faecal sample revealed eggs of *Ancylostoma* species, and hence she was treated with an oral anthelmintic (Drontal plus at 10 mg/ Kg BW). Fluid therapy could not be given due to difficulties in separating the cub from the dam, and the tiger cub died the following day.

Necropsy revealed a dehydrated carcass with severe hyperaemia on the serosae of the duodenum and jejunum, and petechial haemorrhages on the rest of the intestine. The dilated intestines contained haemorrhagic watery feces which tested positive for parvoviral DNA (Figure 1),

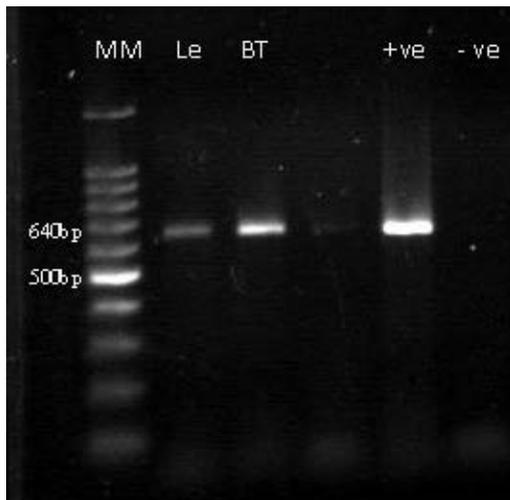


Figure 1: Agarose gel electrophoresis of amplified PCR products of VP2 gene. MM: 100bp molecular ladder, Le: Leopard-faecal sample, BT: Bengal Tiger-faecal sample, +ve: positive control and ve: negative control

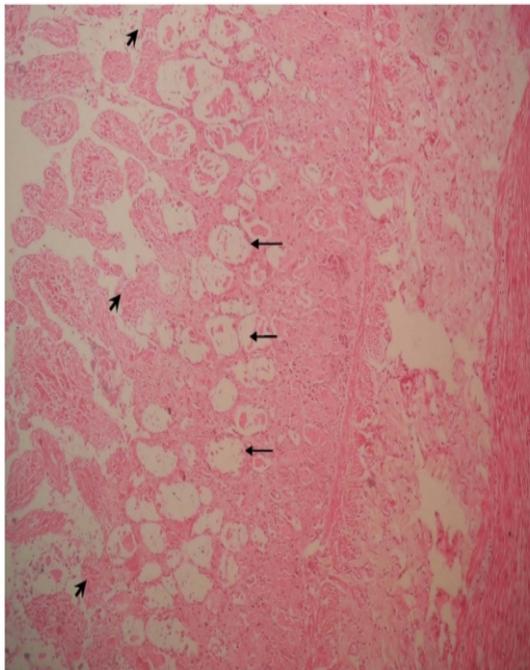


Figure 2 a. H & E stained section of the duodenum (10X10) of Bengal tiger infected with FPLV. Loss of epithelium of intestinal crypt (long arrows) and destruction of intestinal villi (short arrows)

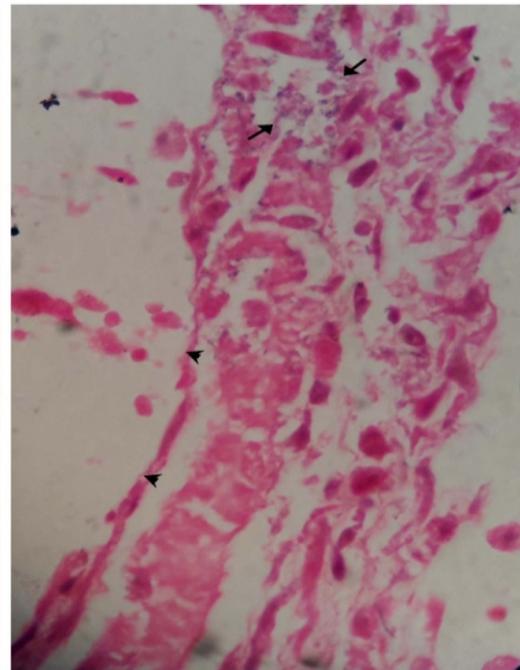


Figure 2 b. H & E stained section of the duodenum of Bengal tiger infected with FPLV (10X100). Destruction of epithelium of intestinal crypt (arrow heads) and bacterial colonization in the mucosae (long arrows)

but not for Salmonella. Mesenteric lymph nodes and spleen were enlarged. Histopathology revealed shortening and collapsing of intestinal villi with extensive loss of epithelium in the intestinal crypts. In addition, (Figure 2a and 2b) marked bacterial colonization was seen in the mucosae and sub mucosae of the intestines (Figure 2b).

DISCUSSION

In addition to domestic and wild felids, FPLV can also affect racoon, mink and fox (Truyen *et al.*, 2009). It is important to note that the FPLV is not the only parvo virus affecting cats, and new variants of canine parvo virus (CPV2a and b) are also capable of causing haemorrhagic

enteritis in both domestic and wild cats (Truyen *et al.*, 1996). Passaging of FPLV and CPV2 through multiple hosts has caused mutations in the gene encoding viral capsid. Resulting changes in the capsid has broaden the host range of the virus (Truyen *et al.*, 2009).

In order to identify the species of the parvo virus, PCR products were sequenced. The PCR assay used in the present study can screen for both CPV and FPLV. The resulting PCR amplicons were sequenced for species identification. Sequences were compared using BLASTn against the GenBank (<http://www.ncbi.nlm.nih.gov/>) non-redundant nucleotide collection database to identify the closest match. The results confirmed that the two sequences were identical and highly similar (96% coverage and 98% identity) to the Feline panleukopenia virus strain 42/06-G8, isolated from a cat with FPL in Italy (gene bank accession EU498699.1). Though many clinical cases with signs similar to FPL were observed among domestic cats in Sri Lanka, none of those cases were confirmed as FPLV by molecular or any other diagnostic methods. Therefore we are unable to confirm that the FPLV strain in wild felines is similar to the FPLV circulating among domestic cats in Sri Lanka.

Previous reports have shown that the FPL may develop as a clinical or sub clinical infection. Severity of the infection may depend on various factors including maternal derived antibodies, age, the immune status of the individual and underlying diseases. The concurrent worm infestation could have contributed to the severity of the infection in the Bengal tiger. Both *Ancylostoma* and FPLV damage the intestinal epithelium facilitating the intestinal microbes to translocate to blood and various organs causing septicaemia and endotoxaemia. In a previous study, a germ free cat had developed only mild transient infection following FPLV infections (Carlson *et al.*, 1977), indicating that the systemic entry of intestinal bacteria determine the severity and the prognosis of FPLV infection. Furthermore, the young age of the animal (<3 months) would have contributed to the severe outcome. Previous reports have shown FPLV infected kittens to have a very high (90%) mortality (Truyen *et al.*, 2009)

Histopathology of the tiger cub showed epithelial cell destruction mainly in the intestinal crypts. Cells in crypts of Lieberkuhn have high replication rate and the FPLV initially start to grow in these cells. With the progression of the infection, villi atrophy occur due to absence of new cells to replace the old epithelium (Goddard and Leisewitz, 2010). The most common symptoms of FPL; ie., diarrhoea, vomiting and dehydration occur due to damaged absorptive surface of the intestines.

Since there is no specific treatment available for FPL infection, affected animals should be managed and treated according to the symptoms. A broad spectrum antimicrobial, antiemetic and appropriate fluid and energy corrections should be included in the treatment and management plan to avoid complications due to septicaemia, endotoxaemia or severe dehydration. It is also vital to isolate the suspected patients as FPL is highly

contagious. When FPL infection is suspected in animals in captivity, extra precautions should be taken to prevent the spread of the infection. In order to prevent FPL in Zoological Gardens, all members of the cat family should be properly vaccinated. Three vaccines, at 8-9 weeks, 11-12 weeks and 16-20 weeks of age are recommended for kittens when there is a high infection pressure. A booster vaccine should be given, when animal is one year old followed by boosters at intervals of three year or more.

ACKNOWLEDGEMENT

Authors would like to thank Ayoma Hettiarachchi, K. Thanajayan, Chandima Mallawa, Ushantha Hewagamage for their support.

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