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Research Article

Detection of Shiga Toxin-Producing *Escherichia coli* (STEC) from a Case of Diarrhoea in Domestic Cat

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ABSTRACT

A case of diarrhoea in a cat was investigated to assess the involvement of certain enteropathogens of bacterial and viral origin. Standard microbiological and molecular techniques were employed to identify these enteropathogens. Escherichia coli could be isolated from the faecal sample, while Clostridium species and Feline Pan Leukopenia virus could not be detected. Further, the E. coli isolate was found to be positive for the presence of stx1 gene, which is one of the major virulence factor of Shiga toxin-producing Escherichia coli (STEC). The therapeutic regimen included Amikacin and fluid therapy along with other supportive medications, which could effectively manage the occurrence of diarrhea in the cat.

Keywords: *Cat, STEC, Feline Parvo virus, Feline Panleukopenia, Clostridium.*

INTRODUCTION

Acute and chronic diarrhoea are commonly encountered in small animal practice. Although diarrhoea is a primary sign of intestinal disease, it may also be a manifestation of other systemic diseases (Battersby & Harvey, 2006). The causes of diarhhoea, therefore, may be both gastrointestinal *viz.* dietary causes, gastrointestinal infection, inflammation or neoplasia or extra-gastrointestinal diseases. Various potential enteropathogens like bacteria, virus and parasites have been found in diarrhoeic and non-diarrhoeic feline faeces (Paris et al., 2014). Among the enteropathogens of bacterial origin Diarrheagenic *Escherichia coli* (DEC), *Clostridium*, *Campylobacter* and *Salmonella* species are important as causal agents of diarrhea in cats (Weese, 2011; Marks et al., 2011; & Silva & Lobato, 2015). While feline bocaviruses (FBoV), feline astroviruses (FeAstV), FRV (feline rotavirus) and feline protoparvovirus virus (FPV) (formerly Feline panleukopenia virus) are some of the most prevalent feline enteric viruses (Ng et al., 2014; Otto et al., 2015; & Zhang et al., 2014).

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Multiple factors are apparently responsible for causing diarrhea and may also involve a combined action of different enteric pathogens (Queen et al, 2012). However, most of the time studies concerning enteric pathogens from cats have focused mainly on a single pathogen (Morato et al, 2009; & Pu˜no-Sarmiento et al., 2013). Therefore, in this particular study few of the most commonly encountered enteropathogens were screened in order to ascertain the cause of the diarrhea in the cat.

MATERIALS AND METHODS

Case history and clinical observations

In this study, a male cat of 9 months of age weighing 1.5 kg was presented at the Veterinary clinical complex, (VCC) Khanapara, Guwahati, Assam with the complaints of partial anorexia, reduced activity, occasional vomiting, and profuse foul smelling watery diarrhea along with blood tinge since 7 days. On clinical inspection, the cat was moderately dehydrated exhibiting 2-4 seconds during skin turgor test, mucus membranes of the eyes were dry and slightly congested with mild sunken eyes. However, other clinical parameters like body temperature, respiration rate and pulse rate were found to be normal. The owner also reported that the cat had undergone routine vaccination and de-worming. Further, the owner also reported that the episodes of diarrhea are continuing since 1 month.

The diarrhoeic feaces and faecal swabs were collected and processed for identification of selected enteropathogens such as Feline panleukopenia (FP) virus, *Escherichia coli* and *Clostridium* species to determine the cause of diarrhoea in the cat.

Bacterial isolation:

Escherichia coli:

The faecal sample was inoculated onto Blood agar and Mac Conkey's Lactose agar plate and incubated at 37^0 C for 24-48 hours (de Paula et al., 2019). Presumptive diagnosis was done on the basis of cultural and staining characteristics. The colonies were examined every day and the colonies showing bright pink colour on Mac Conkey's Lactose agar were subcultured to Eosin Methylene Blue agar. Biochemical tests were performed for confirmation.

Detection of virulence genes:

For DNA extraction the suspected colonies were grown in LB broth and subjected to Hot cold lysis method. PCR was performed using the primers and thermal cycling conditions recommended by Hinenoya et al. (2009) for *stx1*, *stx2* and *eae* genes. The details of the primers used are given in Table no. 1:

Table 1: Details of the primer sequences and amplified product size for detection of specific virulence genes

Sequence (5'-3')	Product size (bp)	Gene
F: CAACACTGGATGATCTCAG R: CCCCCTCAACTGCTAATA	349	*stx1*
F: ATCAGTCGTCACTCACTGGT R: CTGCTGTCACAGTGACAAA	110	*stx2*
F: AAACAGGTGAAACTGTTGCC R: CTCTGCAGATTAACCTCTGC	454	*eaeA*

***Clostridium* species:**

The samples were inoculated onto Blood agar plates and incubated at 37^0C for 48-72 hours anaerobically (Goldstein et al., 2012).

<u>PCR detection of Feline Panleukopenia virus:</u>

The viral DNA was extracted using the Qiagen kit for faecal samples following the manufacturer's instructions.

FPV specific primers (P3: 5'-AAA GAG TAG TTG TAA ATA ATT-3', P4:5' -CCT ATA TAACCA AAA GTT AGT AG-3') designed

by Zhang et al. (2010) were used in this study. A thermal cycling profile of 30 cycles of denaturation at 94 ^{0}C for 30 s, annealing at 55 ^{0}C for 45 s and extension at 72 ^{0}C for 45 s was employed (Parthiban et al., 2014).

TREATMENT AND DISCUSSION

Therapeutic management was initiated by administering Ringer's lactate @ 15ml/kg intravenously and dextrose normal saline (5%) @ 20ml/kg intravenously alternatively at 12 hours interval in order to compensate the fluid and electrolyte loss to avoid hypovolemic shock.

Amikacin @15 mg/kg bodyweight intramuscularly twice in a day for 3 days were injected.Amikacin belongs to amino glycosides group of drugs and is known to be effective against most resistant *E.coli* and other ESBL producing bacteria (Kuti et al., 2018).

Other supportive medication included Pantaprazole @0.5mg/kg bodyweight intravenously to reduce acid secretion along with anti-emetic Ondansetron @0.1mg/kg bodyweight intravenously to stop emesis. By 2nd day onwards the cat showed good response to the treatment, the frequency and volume of diarrheic stool reduced progressively. By 4th day the consistency of stool improved and the cat also developed tendency to eat. Finally, by 6th day onwards the cat resumed its normal appetite with no complaint of vomition or loose stool. For the next few days, the owner was also advised to limit the quantity of food. Commercially available edible probiotics for cat were also advised to be incorporated with the feed in order to rebuild healthy gut flora.

Microbiological investigation resulted in the isolation of *E coli* from the faecal sample. *Clostridium* species could not be detected as was evident from the absence of growth on the blood agar plates. Also, no specific amplification was observed in the PCR for confirmation of FPV.

E coli was identified on the basis of morphology, staining reaction and colony characteristics. Distinctive metallic sheen was observed on EMB agar and biochemical confirmation was done by employing the IMViC test. Further, PCR analysis showed that the isolate was positive for *stx 1*. However, *stx 2* and *eae1* genes could not be detected.

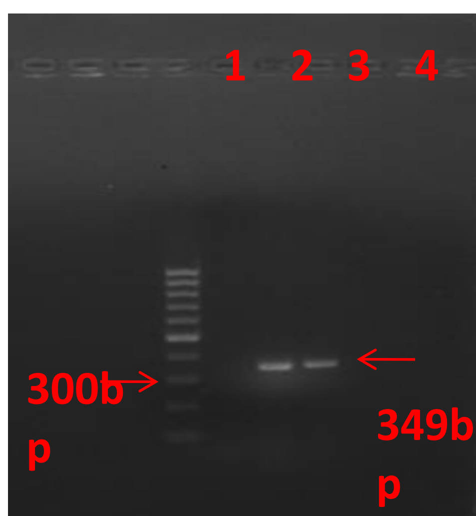

Fig. Agarose gel electrophoresis showing showing positive amplification of *stx 1* gene
Lane 1: 100 bp ladder, Lane 2: Negative control, Lane 3: Positive control, Lane 4: Sample

Pathogens of bacterial origin such as *E. coli* are found in the normal enteric microflora (Kerr et al., 2014), therefore, it is suggested that a positive culture alone is not sufficient for diagnosis and specific toxins or genes need to be identified to ascertain the pathogenicity of an individual *E. coli* strain (Hall, 2009). In our study, it was observed that the *E. coli*

isolate possessed the *stx 1* gene. It is established that main virulence factor of Shiga toxin-producing *E. coli* (STEC) is the production of Shiga toxin-1 (stx-1) and/or Shiga toxin-2 (stx-2) or its variants (Kaper et al., 2004; & Bentancor et al., 2007). An alarming fact about Shiga toxin-producing Escherichia coli (STEC) is that it is a zoonotic pathotype which is associated with human gastrointestinal disease and more specifically life-threatening conditions like haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) (Gonzalez & Cerqueira, 2020).

Ruminants, especially cattle, are considered to be the main reservoirs for STEC (Parma et al., 2000; & Meichtri et al., 2004) and transmission to humans is known to occur through contaminated food *viz.* minced meat or nonpasteurized milk (Rivas et al., 1996; & Mead & Griffin, 1998). There are also evidences of zoonotic human infections which have been caused by contact with companion and domestic animals (Luna et al., 2018). Further, dogs and cats too have been found carrying STEC strains. In context of this study, different workers have shown that the prevalence of STEC in pets is highly variable (Beutin, 1999; & Bentancor et al., 2007). In an earlier study, Abaas et al. (1989) reported a 40% prevalence for STEC in clinically healthy cats and 95% in cats with diarrhea while Beutin et al. (1993) showed a prevalence of around 4% and 14% in healthy and symptomatic pets, respectively in Germany. Thereafter, Bentancor et al. (2007) confirmed 2.6% of the cat samples as STEC carrying the stx2 sequence.

Researchers opine that the eventual role of cats as bacterial reservoirs is yet to be completely elucidated (Bentancor et al., 2007), even though there has been a realization regarding potential zoonotic transmissions and human health risks associated with owning cats (Spain et al., 2001). In this study, we examined involvement of probable enteropathogens in causing diarhhoea either alone or in combination. The isolation of STEC from the diarrhoeic faeces of the cat provides information regarding the presence of virulent strains among companion animals. Such reports are sparse from this part of the country and this finding, therefore, highlights the need to carry out detailed studies in the future with large number of samples to obtain a realistic estimate of the prevalence of such bacteria in the pet population. This in turn will help in chalking out risk-management strategies for reducing transmission to humans and other animals.

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