Co-infection of enteropathogens in puppies with enteritis: a hospital based study

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ABSTRACT

Infectious gastroenteritis is a common problem in young puppies. The detection of co-infections in dogs is critical for determining treatment response and prognosis of disease. A total 35 fecal samples from diarrheic dogs were screened for parvovirus, parasitic and bacterial infections. Results showed that 74.28% diarrheic samples of dogs were found positive for various enteropathogens. Among the enteropathogens, 51.42%, 14.28%, 11.42%, 14.28% and 5.71% samples were positive for canine parvovirus-2, Escherichia coli, Isospora canins, Toxocara canis, and Ancylostoma caninum, respectively. Among the positive samples, 42.31% samples were found positive for single pathogen and 57.69% samples were found positive co-infection with multiple pathogens. From the results of present study, it is concluded that multiple entropathogens should be investigated in puppies presenting with diarrhea for better treatment outcome.
Key words: Enteropethogenc, co-infection, dog, diarrhoea,

INTRODUCTION

Acute diarrhoea is a common problem in young puppies, which can cause fatal dehydration and other complications (Hubbard *et al.*, 2007). Infectious gastroenteritis is one of the leading reasons of canine hospitalization but because of its pathogenic diversity and the simultaneous existence of viral, bacterial, and protozoan co-infections, canine infectious diarrhoea has been regarded as a difficulty for veterinarians. The detection of co-infections in dogs is critical for determining prognosis and planning methods for treatment and prevention. The clinical significance of numerous pathogens co-existing in dog faeces samples is yet unknown, since this may merely reflect common exposure. However, pathogens can also interact with one another to determine or exacerbate illness. To further understand the potential impact of co-infections, it is crucial to test for numerous pathogens in the faeces of both healthy and diarrheal puppies. Breed, gender, immunisation history, age, season, breeder-origin, and/or kennel stay are potential risk factors for acute infectious diarrhoea in pups (Duijvestijn *et al.*, 2016¸ Chethan *et al.*, 2021). Among different infectious agent of acute gastroenteritis, canine parvovirus (CPV) is considered as the most important pathogen in dogs. (Sharma *et al.*, 2019). About 40-50% diarrheic cause in canine are associated with CPV infection (Agnihotri *et al.*, 2018; Dorlikar, 2018; Sharma *et al.*, 2019, Surendhar *et al.*, 2019). The aim of this study was to investigate the frequencies of pathogens and co-infections in owned dogs with diarrhoea that visited a veterinary facility.

MATERIAL AND METHOD

Study design and sample collection

The study was carried out at Referral Veterinary Polyclinic and Teaching Veterinary Clinical Complex (RVP-TVCC), Indian Veterinary Research Institute (IVRI), Izatnagar (UP). The canine cases presented to out-patient department (OPD) of Medicine section of the RVP & TVCC were included for the current study. Fecal samples collected from dogs with the history of diarrhoea/ hemorrhagic diarrhoea. Fecal samples from the dogs with enteritis were collected directly from rectum using sterile sample collection swab (Sterile, Hiculture collecting device, Himedia, Mumbai, India) in separate tubes containing Hanks balance salt solution (HBSS) to avoid any cross contamination between samples. Each sample was transported to laboratory in ice box to maintain cold chain.

Sample processing

The samples of faeces were immediately processed for regular bacterial culture and tested for the presence of parasite eggs and oocysts using faecal flotation test. Fecal samples screened for viral antigen by rapid CPV antigen detection
kit (Bionote, Korea) as per recommended procedure of manufacturer

Bacterial culture

Escherichia coli

Distinct colonies were isolated and identified using the traditional three-way streak plate approach (Cappuccino and Sherman, 2001). Each sample was incubated for 24 hours at 37 degrees Celsius in both aerobic and anaerobic conditions using two sheep blood agar plates and MacConkey agar plates. If many oxidase-negative and lactose-positive â- hemolytic colonies were produced by the culture it was sub cultured in *E.coli* selective media eosin methylene blue agar (EMB).

Fecal floatation

Helminth eggs and/or protozoa were checked in faecal samples by flotation method using saturated Zinc Sulphate (ZnSO4) as a flotation solution (sg.1.34 g/cm^2). The slides were evaluated at 100X and identified.

Screening for CPV-2

Initially, CPV-2 antigen in fecal materials was screened for rapid CPV antigen detection kit (Bionote, Korea) as per recommended procedure of manufacturer. In brief, the fecal swabs were dipped into the assay diluent, mixed and kept for 2-3 minutes. After that, 4 drops mixture was placed on the sample hole of the test device by disposable dropper and waited for development of distanced line on 'C' for control and 'T' for test line. The test was considered invalid when 'C' line was not developed.

RESULTS

In total 35 fecal samples screened for different entero-pathogens and their co-infection in puppies suffering from acute gastroenteritis. Potential enteropathogens were identified in 26/35 (74.28%) diarrheic samples in the present study, most of which contained multiples pathogens. Single infections were observed in 11/26 (42.31%) positive samples and coinfection in 15/26 (57.69%) possible samples. Dual, and triple infections were observed among the co-infections (Table 1). The association of viral and parasitic infections was the most prevalent type of co-infection in the diarrheic group (33.33%), with CPV-2 co-infection. Viral and bacterial co-infections

accounted for 26.67% of these associations, Viral, bacterial and parasitic for 26.67% and bacterial, and protozoan co-infections for

13.33% (Table 2). Canine parvovirus-2, *Escherichia coli*, *Isospora canins, Toxocara canis,* and *Ancylostoma caninum* were positive in 18, 5, 4, 5, and 2 diarrheic fecal samples. (Table 3 & Figure 1, 2,3) 4/15 (26.67) Bacterial and parasitic 2/15

Table 1: Occurrences of single or co-infection in diarrheic feces of puppies

Infection Diarrheic dogs (35) n (%) Negative 9/ 35 (25.71)

Fig 1: Diagnosis of CPV-2 in fecal samples by Rapid Ag detection kit (Bionote, Republic Korea)(a. Fecal sample neagative for CPV-2 b.Fecal sample positive for CPV-2)

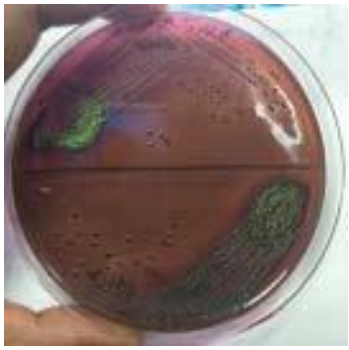

Fig 2: *Escherichia coli* showing mettalic sheen in EMB agar

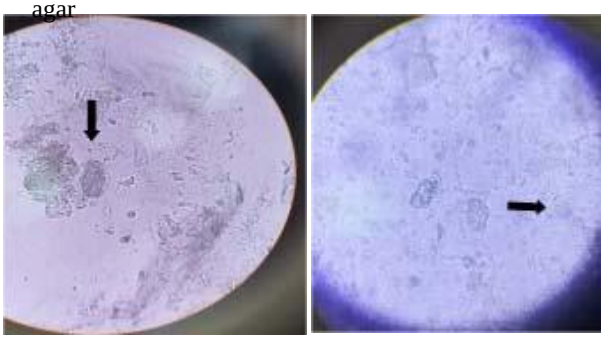

Fig 3: *Isospora canins,* and *Ancylostoma caninum* oocyst and egg under microscope (10x)

Table 2: Virus, bacteria and protozoan association in diarrheic and control feces of dogs

BacteriaActerial Positive	26/35 (74.28)
Single	11/26 (42.31)
Co-infection	15/26 (57.69)
Dual	11/15 (73.33)
Triple	4/15 (26.67)
Viral and parasitic	2/15 (13.33)
Viral, bacterial, and parasitic	4/15 (26.67)

Co-infection	Diarrheic dogs (35) n (%)
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Table 3: Individual infectious agents of canine diarrhea samples of dogs from bareilly

Canine parvovirus-2	18
Escherichia coli	5
Isospora canins	4
Toxocara canis	5
Ancylostoma caninum	2

DISCUSSION

The rate of co-infection observed here in diarrheic dogs (45.1%) was also higher than those in the other countries tested. Pathogen co-occurrence in the pups was anticipated. In in 26/35 (74.28%) of the puppies with diarrhoea were identified to be shed pathogen. Puppies have been documented to have mixed infections with many enteropathogens in the past (Dupont *et al.*, 2013; Grellet *et al.*, 2014; Gizzi *et al.*, 2014). However, variations in research populations, chosen infections, and detection techniques may explain for variations in our findings. We used methods that are often employed as standard operating procedures in diagnostic laboratories to screen our samples.

Together, co-infecting pathogens may result in more severe diarrhoea than infections with each pathogen separately (Grimprel *et al.*, 2008), and many enteric viruses, bacterial pathogens, and parasites likely contribute to illness both alone and in combination. The pathogens present in a co-infection may interact synergistically, such as through the host's immune system, increasing the quantity and/or virulence of the other, leading to increased pathogenesis and a larger burden of illness overall (Bhavnani *et al.*, 2012). Interspecific pathogen interactions can thereby change the dynamics of the pathogen, the health of the host, and the efficacy of control measures (Lello *et al.*,2008; Pedersen *et al.*, 2007).

CONCLUSION

Multiple enteropathogens were detected in the faeces of diarrhoeic puppie. Relevance of enteropathogen detection needs to be interpreted with caution. In puppies suffering from (severe) acute diarrhoea routine screening should focus on CPV, *Escherichia coli*, *Isospora canins*, *Toxocara canis*, and *Ancylostoma caninum* as they frequently together.

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