SHORT COMMUNICATION

Secondary Bacterial Infections Associated with Canine Parvo Viral Enteritis and the Risk of Transmission to Pet Handlers: A One Health Perspective

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Abstract

Complications due to secondary bacterial pathogens are common in canine viral enteritis especially canine parvo viral enteritis (CPVE), leading to sepsis and high mortality. A cross sectional survey was conducted in dogs to identify the distribution of secondary bacterial pathogens in dogs with PVE and the potential risk of transmission to the in-contact pet owners under one health perspective. Faecal samples from the PVE cases were subjected to isolation, identification and confirmation of bacterial isolates by biochemical tests. The prevalence was higher for *E. coli* followed by *Klebsiella pneumoniae,* non-typhoidal Salmonella (NTS) and *Proteus vulgaris* with 45.0, 15.0, 20.0 and 10.0 %, respectively, and a co-existence of these pathogens was also recorded. Understanding the distribution of these enteric bacterial pathogens could underscore the risk of sepsis leading to high mortality in PVE cases, multi-drug resistance resulting in failure of treatment and zoonotic transmission to the in-contact pet owners.

Keywords: Canine, *E. coli*, *Klebsiella*, Parvo viral enteritis, *Salmonella*, Zoonoses.

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Introduction

Dogs and cats can contribute significantly to the spread of pathogens in the home and environment by their excretions that can infect humans and other animals in the surrounding area (Galán-Relaño *et al*., 2023). The bacteria such as *E. coli* and *Salmonella enterica* are reported to be the important secondary enteric pathogens (Giacomelli *et al.,* 2015) in canine viral enteritis. Their role is significant in PVE, since immunosuppression in PVE leads to secondary bacterial infections resulting in sepsis and a high case-fatality. Nevertheless, neither the distribution of these pathogens as primary etiology in enteric cases nor their role as complicating secondary pathogens in enteric cases have been scarcely reported in India.

In humans, *E. coli* and non-typhoidal Salmonella (NTS) are reported to be the leading causes of food poisoning and may lead to septicaemia, meningitis, haemolytic uraemic syndrome (HUS), and thrombocytopenia and haemolytic anaemia (Bais *et al*., 2022). Further, dogs can act as reservoirs for *E. coli* and *Salmonella* (Kim and Park, 2022) and thus can act as potential sources of infection to the pet handlers, leading to food borne bacterial illness. *Klebsiella pneumoniae* is a commensal organism in dogs and cats (Roberts *et al.,* 2000) and an important nosocomial and urinary tract infections in humans and animals (Glickman, 1981). Proteus is widespread in gastrointestinal tract of both animals and humans and hence reported to be an opportunistic nosocomial pathogen (El-Tarabili *et al*., 2022). In addition, these secondary enteric pathogens are major multi-drug resistant (MDR) pathogens, leading to treatment failures both in dogs and human cases (Bisola Bello *et al*., 2024). Nevertheless, in India, there is a paucity in the surveillance of these pathogens in dogs under the One-Health Stewardship of Food Borne Zoonoses. Hence the objective of this study was to report the occurrence of common secondary bacterial pathogens in PVE cases, and role of diarrhoeic cases in the transmission of zoonotic bacteria to the in-contact pet owners, under One Health Stewardship of zoonoses and food safety.

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Materials and Methods

A total of 20 unvaccinated dogs presented with signs suggestive of PVE such as characteristic haemorrhagic and catarrhal diarrhoea with offensive odour, vomiting, lymphopenia or neutropenia with leukopenia were selected for this study. The faecal swabs were supplemented for

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enrichment in selenite-F broth ((Hi-Media), peptone water for 24 h at 37°C, then the broth was streaked on xylose lysine deoxycholate (XLD) agar (Hi-Media), MacConkey agar (Hi-Media), eosine methylene blue (EMB) agar (Hi-Media) and triple sugar iron (TSI) slant (Hi-Media) by using a sterile bacterial loop, and then incubated at 37°C for 24-48 h. The colonies of the isolates were inoculated into peptone and MR-VP broth and Citrate slant for performing IMViC test (Indole test, Methyl red test, Voges-proskaur test and citrate utilization test). Bacterial identification was performed based on the culture characteristics and morphological characteristics with Gram's staining (Quinn *et al.,* 2002).

Results and Discussion

E. coli and *Klebsiella* spp. were identified by yellow colonies on XLD media indicating fermentation of xylose, lactose and sucrose; On EMB media, *E. coli was* identified by bluish black colonies with greenish metallic sheen and *K .pneumoniae* by purple 'Fish eye' colonies suggestive of *K. pneumoniae*. Biochemical identification of both was made by acidic/ acidic reaction (yellow slant/ yellow butt) and gas (+) in TSI reaction. The IMViC reactions for confirmation of *E. coli* and *K. pneumoniae* were ++-- and --++, respectively (Fig. 1, 2).

Non-typhoidal Salmonella (NTS) were identified by pink colonies with black centre on XLD media indicating fermentation of xylose, lysine decarboxylation and H_2S (+). Biochemical identification was made by alkaline/acidic reaction (red slant/ yellow butt) non-lactose fermentation, and H_2S (+) in TSI reaction. The IMViC reactions for confirmation of NTS were -+-+ (Fig. 3).

Proteus spp. was identified by yellow colonies with black centre on XLD media indicating fermentation of xylose and H_2S production. Biochemical identification was made by alkaline/acidic reaction (red slant/ yellow butt) indicating non-lactose fermentation and H_2S (+) in TSI reaction; *P. vulgaris* was confirmed by the IMViC reactions ++-+ (Fig. 4).

The prevalence of the bacterial pathogens isolated from the faecal samples of dogs affected with PVE is presented in Table 1. A high prevalence (45%) was recorded for *E. coli* followed by *K. pneumoniae,* NTS and *P. vulgaris*, and co-infections of NTS with *K. pneumoniae* and *E. coli* with *P. vulgaris* were recorded at 10% and 5% level.

Table 1: Prevalence of bacterial pathogens associated with canine parvo viral enteritis

Bacterial pathogens	No. of Samples	Isolates	% positivity
E. coli	20	09	45.0
K. pneumoniae	20	04	20.0
Non-Typhoidal Salmonella	20	03	15.0
P. vulgaris	20	02	10.0
NTS+ *K. pneumoniae*	20	02	10.0
E. coli + *P. vulgaris*	20	01	5.0

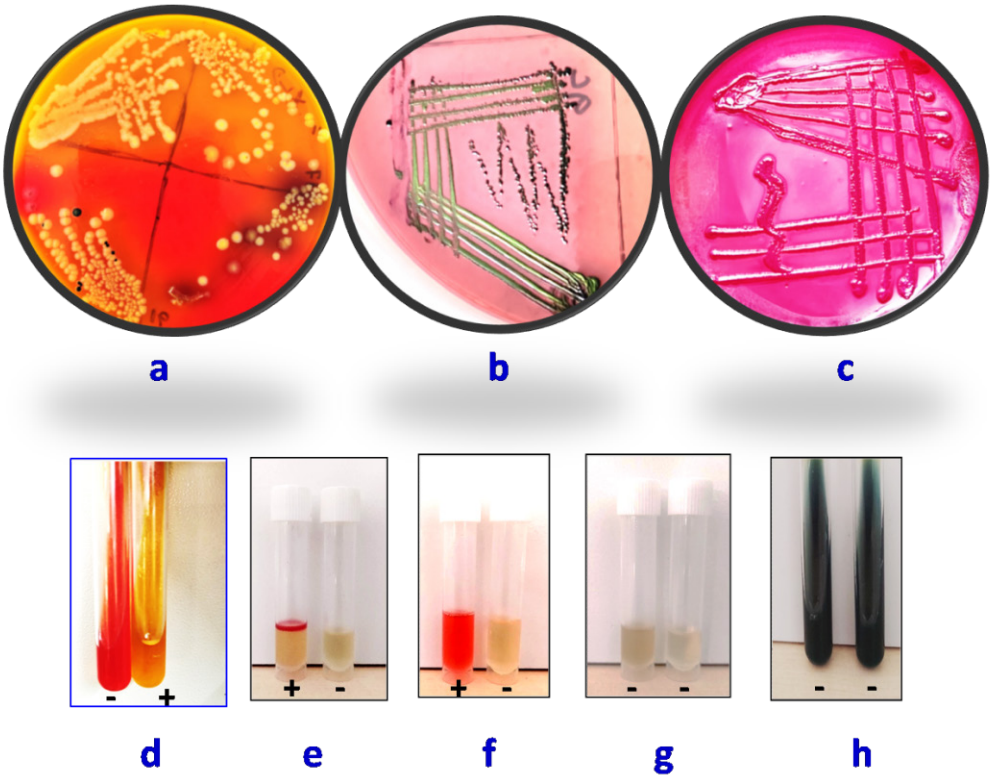


Fig. 1: (a) Yellow colonies on XLD agar (b) Bluish black colonies with greenish metallic sheen suggestive on EMB agar (c) Pink colonies on MacConkey agar (d) TSI reaction- Yellow slant/ Yellow butt and (e) Indole reaction (f) Methyl red reaction (g) Voges-Proskauer reaction and (h) Citrate fermentation

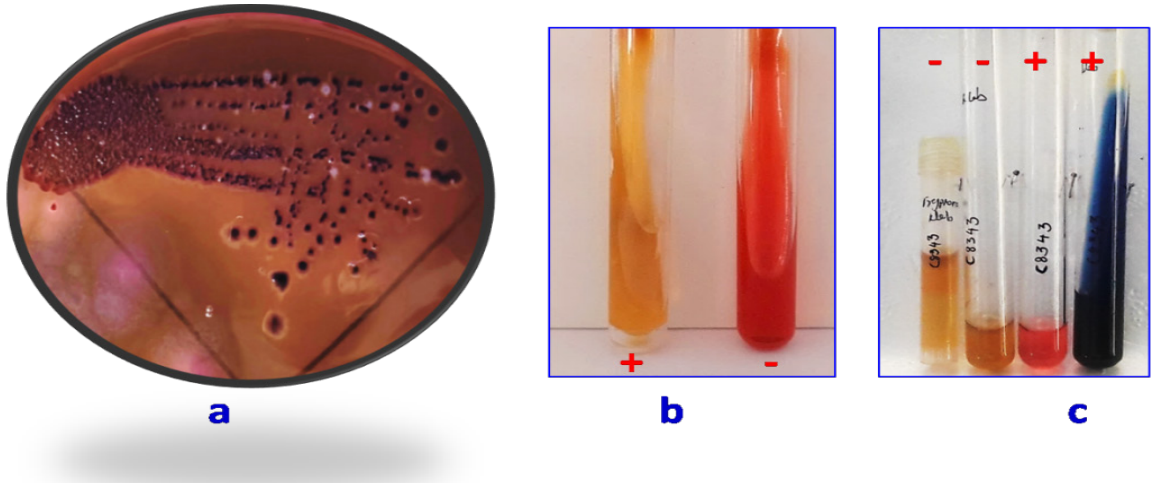


Fig. 2: (a) Bluish black colonies with 'Fish eye' appearance on EMB agar (b) TSI reactions- Yellow slant/ Yellow butt, Gas (+) and (c) IMViC reactions

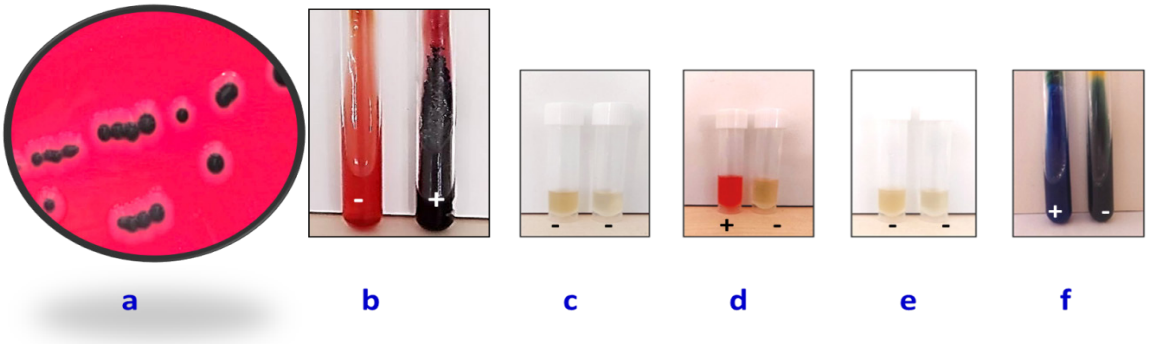


Fig. 3: (a) Pink colonies with black centre on XLD agar (b) TSI reaction- Red slant/Yellow butt, H_2S (+) (c) Indole reaction (d) Methyl red reaction (e) Voges-Proskauer reaction and (f) Citrate fermentation

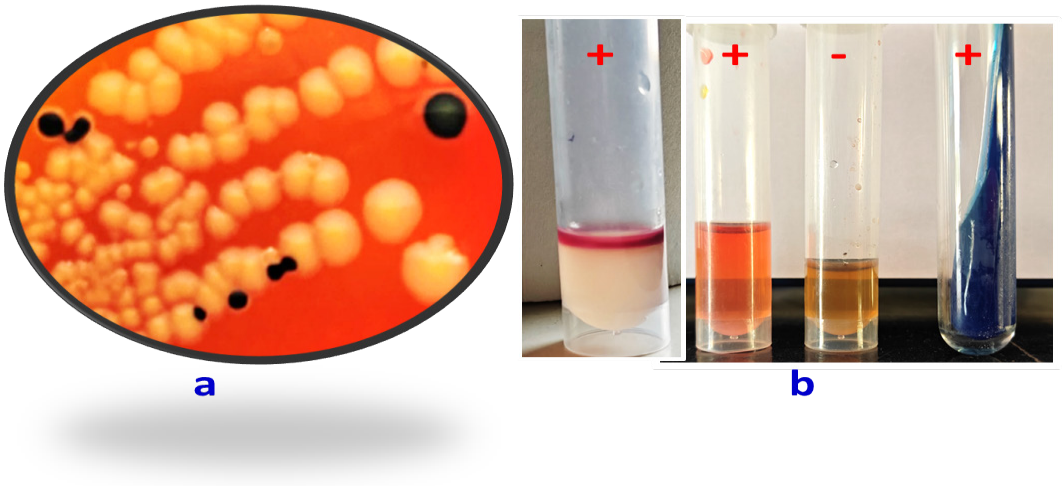


Fig. 4: (a) Yellow colonies with black centre on XLD agar (b) IMViC reactions

The prevalence of *E. coli* observed in this study was lower than that observed by Bais *et al.* (2022) and Banik *et al.* (2015), who reported a higher prevalence of 66.66 % and 84.2 % in diarrhoeic dogs in Mathura and West Bengal, respectively. Further serotyping of the isolates may help in distribution of pathogenic serotypes that may gain significance and threat to pet handlers.

The prevalence of NTS observed in this study was concurrent to that of Upadhyay and Mansi (2023), who recorded the prevalence to be 15.2 % in feces of diarrhoeic dogs in Uttara Khand. In contrast, Ravish *et al.* (2024), Mandawat *et al.* (2025) and Verma *et al.* (2011) reported a prevalence of 33.3%, 8.0% and 0.4% in feces of enteric dogs in Haryana, Jaipur and UP, respectively.

Concurrent to the study, the prevalence of K. *pneumoniae* in diarrhoeic cases of dogs was reported to be 14.0% (Ribeiro *et al.*, 2022), 24.26% (Sharif *et al.*, 2017) and 25.9% (Banerjee *et al.*, 2024) in India. Roberts *et al.* (2000) recorded an outbreak

due to *K. pneumoniae* with haemorrhagic enteritis, sepsis and mortality in a kennel. Published report on prevalence of *Proteus* spp. in enteric infections of dogs in India was not available. However, the prevalence of *P. vulgaris* observed in this study was higher than that observed by El-Tarabili *et al.* (2022) in Egypt, who reported a prevalence of 2.8 % in diarrhoeic dogs.

In conclusion, the isolation and identification of secondary enteric pathogens from faeces of enteric cases could underscore the possibility of complications in dogs with viral enteritis especially of immunosuppressive origin and risk of sepsis. In addition, these pathogens may be transmitted to pet handlers through food and may lead to food safety hazard. Hence knowledge on distribution of these bacterial pathogens in dogs could be useful in the management of zoonoses, antimicrobial resistance and food safety under One Health.

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