RESEARCH ARTICLE

Molecular Characterization of *Escherichia coli* Associated Haemorrhagic Gastroenteritis and Concurrent Sepsis in Canine Population in Patna

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Abstract

The present study was conducted to determine the molecular incidence of *Escherichia coli*-associated haemorrhagic gastroenteritis (HGE) and its concurrent occurrence with sepsis in canine clinical cases presented to the Veterinary Clinical Complex, Bihar Veterinary College, Patna. Screening of suspected cases was initially performed based on characteristic clinical symptoms, following which fecal samples were subjected to molecular confirmation using PCR. Of the 150 fecal samples examined, 71 (47.33%) were found positive for *E. coli*. Among the affected dogs, Mongrels exhibited the highest incidence (35.21%, n=25) of the PCR-confirmed cases. Age-wise younger dogs were more susceptible, with puppies aged 3-6 months showing the highest incidence at 45.07% (n=32). A sex-wise greater prevalence was noted in males (63.38%, n=45) compared to females. Additionally, among the 71 PCR-confirmed HGE cases, 42 dogs were positive for systemic inflammatory response syndrome (SIRS), yielding an overall SIRS occurrence of 59.2%. These findings highlight the significant involvement of *E. coli* in canine HGE and underscore its association with systemic inflammatory complications.

Key words: Dog, *Escherichia coli*, Haemorrhagic gastroenteritis, Molecular incidence, Sepsis.

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Introduction

The occurrence of haemorrhagic gastroenteritis has been estimated as 12.7% in a retrospective study (Alves *et al*., 1998), but the prevalence of gastroenteritis in dogs was found to be 12.24 % by Kataria *et al*. (2020). An earlier epidemiological study by Jani (2004) indicated a higher prevalence of enteritis in dogs (19.3%). According to Rakha *et al*. (2015), among all clinical disorders, digestive issues are most prevalent in dogs, accounting for 56.50% of cases. With regards to gastrointestinal disturbances, gastroenteritis is the most prevalent issue, affecting all age groups and breeds of the canine population. Anorexia, vomiting, diarrhea (which can be haemorrhagic), dehydration, fatigue, and occasionally fever are its characteristic symptoms. Weight loss or stunting is frequently noted in the case of those dogs that are more seriously affected (Bhat *et al*., 2013). It leads to dehydration and an imbalance in electrolytes. A life-threatening illness, haemorrhagic gastroenteritis (HGE), is brought on by a number of factors, including bacterial and viral infections, endoparasitic infestations, irritating medications, an improper diet, and the consumption of toxic substances (Enany *et al*., 2021). Over four decades ago, the first published report of canine haemorrhagic gastroenteritis appeared in the veterinary literature (Post and Feldman, 1978).

Bacterial haemorrhagic diarrhoea might be due to several mechanisms that promote the translocation of indigenous bacteria from the gastrointestinal system, such as intestinal bacterial overgrowth, deficiencies in host immune defences, and intestinal mucosal barrier damage (Berg, 1999). Further decreased splanchnic blood flow, which is likely present in hypovolemic haemorrhagic enteritis patients, is an additional factor predisposing to bacterial translocation (Reisinger *et al*., 2024). A tentative diagnosis is made mostly on the basis of the patient's history, symptoms, and typical signalment; fecal culture is rarely used. According to Zoran (2008), a definitive diagnosis necessitates fecal culture, PCR detection of bacterial toxins or DNA, or proof that there is bacterial toxin present in the feces. This study was planned to know the molecular incidence of *Escherichia coli* associated haemorrhagic

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Table 1: Primer used in the study

Primer sequence	Target gene	Product size (bp)	Reference
F:GAAGAAGCTTGCTTCTTTGCTGAC R:GCCCGGGGATTTCACATCTGACTT	*16S rRNA E. coli*	544	Sabat *et al*. (2000)

gastroenteritis and concurrent sepsis in canine population in Patna, Bihar

Materials and Methods

The study was conducted at the Veterinary Clinical Complex, Bihar Veterinary College, BASU, Patna, Bihar, India, where canine patients presented with gastrointestinal disorders were examined. The study focused on cases suspected of haemorrhagic gastroenteritis (HGE) and was carried out over the period of one year from June 2024 to May 2025.

Case Selection and Clinical Examination

Dogs (n=150) presented with symptoms suggestive of HGE such as acute onset of vomiting, haemorrhagic diarrhea, dehydration, lethargy, and abdominal discomfort were screened clinically. Detailed case history including age, breed, and sex, was recorded. Animals exhibiting characteristic clinical signs were selected for further diagnostic evaluation.

Sample Collection

1-2 gram fresh fecal samples were collected aseptically from each clinically suspected dog, placed into sterile containers, properly labelled, and transported to the laboratory under chilled conditions for immediate processing.

Approximately 1 mL of whole blood sample was collected from cephalic or saphenous vein of each animal in ethylene diamine tetraacetic acid (EDTA) vial for leukogram estimation using manual procedure.

Molecular Characterization of *E. coli* by PCR

The PCR was carried out for *E. coli* detection targeting species specific *16S rRNA* gene. Bacterial lysate was used as template DNA prepared by snap chill method. The reaction mixture was made comprising 2.5 µL of 10x *Taq* buffer, 0.5 µL of dNTPs (2 mM), 2 µL of each primer (Table 1), 1 U of *Taq* DNA polymerase from Thermo Scientific, USA, 5 µL of template DNA, and nuclease-free water was added to reach a final volume of 25 µL. The amplification procedure comprised a 3 min initial denaturation step at 94°C, 40 cycles of denaturation at 94°C for 30 sec, annealing at 72°C for 45 sec, and elongation at 72°C for 45 sec, and a single cycle of final extension at 72°C for 10 min (Sabat *et al*., 2000).

Incidence and Sepsis Study

The incidence of haemorrhagic gastroenteritis (HGE) was studied in dogs of different breeds, sexes, and age groups. Systemic Inflammatory Response Syndrome (SIRS) in canines was diagnosed based on clinical and haematological evaluation. According to Sykes (2014), the presence of at least two of the four criteria was considered indicative of SIRS*, viz.,* body temperature <100.04°F or >102.92°F; heart rate >140 beats/min; respiratory rate >30 breaths/min; and white blood cell count <6,000 cells/µL or >16,000 cells/µL, or the presence of >3% band neutrophils.

Results and Discussion

Overall Incidence of PCR Confirmed *E. coli* Associated HGE in Dogs

Total 150 dogs were tentatively considered positive for haemorrhagic gastroenteritis (HGE) based on the clinical symptoms. These dogs were further screened using polymerase chain reaction for the presence of *Escherichia coli*. Out of the 150 suspected cases, 71 dogs were confirmed positive by PCR, indicating an overall incidence of 47.33% for PCR-confirmed *E. coli*-associated HGE in the study population (Fig. 1). By comparing the findings with previous studies, we can better understand the public health risks and the global trends in antimicrobial resistance (AMR). The study indicated higher rate of occurrence of *E. coli* in HGE infected dogs and aligned more closely with the findings of Enany *et al*. (2021), who reported 44.23% (n=46) dogs with *E. coli* by PCR. El-Hofy *et al*. (2025) in Egyptian Nisnas showed *E. coli* incidence of 47.5% (n=19) in HGE affected dogs. These findings were also consistent with KuKanich *et al.* (2020), who indicated that *E. coli* isolated in pets had an incidence of 43% in dogs and cats. The results indicate a greater accuracy of polymerase chain reaction in diagnosing the disease. An experienced clinician can make a tentative diagnosis just on the basis of clinical signs and start the treatment in order to reduce the consequences of delayed diagnosis.

Breed-wise Incidence

The breed-wise distribution of PCR-confirmed *E. coli*-associated HGE among 71 positive dogs revealed variations in susceptibility across different breeds (Table 2). Mongrel dogs showed the highest incidence, accounting for 35.21% of the positive cases. This was followed by Labrador Retrievers (21.12%) and Pomeranians (18.31%). German

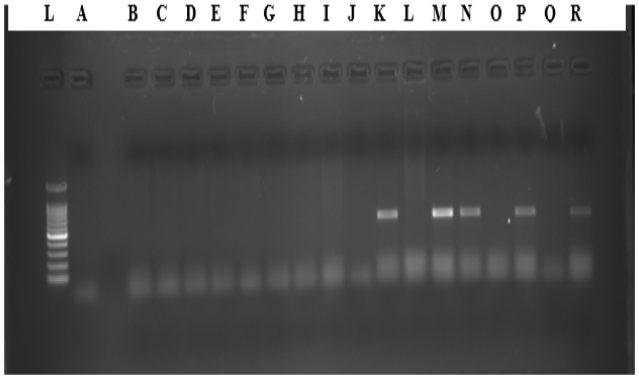


Fig. 1: PCR amplification of species specific *16S rRNA* gene to confirm *E. coli*. L: Ladder (100 bp), A: Negative control, K, M, N, P and R: Positive amplification of 544 bp.

Shepherds contributed 11.26% of the positive cases, while Golden Retrievers, Rottweilers, and Pugs showed relatively lower incidences of 7.04%, 4.23%, and 2.81%, respectively. These results align with previous studies indicating a higher susceptibility to enteric infections in Mongrels, possibly due to genetic diversity and lack of selective breeding for disease resistance (Kouamo *et al*., 2021). Purebred dogs such as German Shepherds and Golden Retrievers showed lower incidences, which may be attributed to selective breeding practices aimed at enhancing disease resistance breed-specific factors, including genetic predispositions and environmental exposures, which play significant roles in disease susceptibility (Shima *et al.,* 2021). Wael (2011) reported high incidence in Labrador retriever followed by German shepherd.

Table 2: Breed-wise incidence

Breed	No. of positive dogs (n=71)	Incidence (%)
Mongrel	25	35.21
Labrador retriever	15	21.12
Pomeranian	13	18.31
German shepherd	8	11.26
Rottweiler	3	4.23
Golden retriever	5	7.04
Pug	2	2.81

Age-wise Incidence

The distribution of PCR-confirmed *E. coli*-associated HGE among different age groups showed that younger dogs were more commonly affected (Table 3). Puppies aged 3-6 months exhibited the highest incidence, accounting for 45.07% of the positive cases. Dogs younger than 3 months contributed 33.80% of the cases, indicating that dogs under 6 months of age collectively represented the majority of infections (78.87%). Dogs aged 6-12 months accounted for 15.49% of positive cases, while dogs older than 12 months showed the lowest incidence of 5.63%. These findings corroborated with the earlier reports (Alves *et al*., 1998; Gizzi *et al*., 2014). However, Mortier *et al*. (2015) reported high incidence in above 6 years dogs. The current finding is consistent with existing literature highlighting the vulnerability of puppies to enteric infections due to immature immune systems and incomplete vaccination status (Kouamo *et al*., 2021). This might be due to hyperactivity of young ones than adults and their curious nature. They may unnecessarily try to eat or feel things around them which may ultimately lead to infection or ingestion of some foreign objects leading to GIT disturbances or due to weaning stress (Guard *et al*., 2015). Young pups have poor immune status and high epithelial cell turnover rate of the intestines with higher mitotic index of the enterocytes of intestinal crypts that enhance bacterial growth. Also, at this stage of life they usually come out from their shelter places and start roaming their neighbourhood more frequently, thus chances of ingesting garbage and acquiring infection is more as compared to adults (Strombeck and Guilford, 1991). The lower incidence in dogs over 12 months (5.63%) may reflect the development of acquired immunity and the protective effects of vaccination. Contrarily, some studies reported a more uniform age distribution, suggesting that factors such as environmental conditions and pathogen exposure levels can influence age-related susceptibility (Kataria *et al*., 2020).

Table 3: Age-wise incidence

Age	No. of positive dogs (n=71)	Incidence (%)
< 3 months	24	33.80
3-6 months	32	45.07
6-12 months	11	15.49
> 12 months	4	5.63

Sex-wise Incidence

The sex-wise distribution of PCR-confirmed a higher prevalence of *E. coli*-associated HGE in male dogs (63.38%, 45/71) compared to females (36.61% 26/71). This finding concurred with Bhat *et al*. (2015) and Rawat and Sumathi (2017), who reported higher rate of incidence in males than females. This male predisposition has been observed in several studies and is often attributed to behavioural and physiological factors, including higher roaming activity and stress-induced immunosuppression, which may increase exposure to pathogens (Chakraborty *et al*., 2019). However, other reports have found no significant sex-based differences, suggesting that environmental and management factors might play a more prominent role than biological sex in disease susceptibility (Alves *et al*., 1998).

Occurrence of Sepsis in *Escherichia coli* Associated HGE in Dogs

Total 71 PCR-positive dogs were screened for the presence of Systemic Inflammatory Response Syndrome (SIRS) based on temperature, heart rate, respiratory rate and white blood cell counts. Out of 71 HGE-positive dogs, 42 dogs were SIRS positive. Hence, the overall occurrence of SIRS was 59.2%. This high prevalence indicates that a substantial proportion of HGE-affected dogs develop systemic inflammation, reflecting the progression from localized gastrointestinal infection to a systemic response. The pathophysiology of SIRS in these dogs involves bacterial translocation across a compromised intestinal barrier, endotoxin release, and dysregulated immune responses that trigger widespread pro- and anti-inflammatory cytokine cascades (Golcher, 2018; Smith, 2019). Haemorrhagic diarrhea, vomiting, and dehydration further exacerbate hypoperfusion and tissue hypoxia, potentiating organ dysfunction. These findings were consistent with observations of Saputra *et al*. (2017), who reported SIRS prevalence in 55-60% of dogs with naturally occurring bacterial enteritis, and Dickson and Lehmann (2019) demonstrated Gram-negative bacterial infections in dogs frequently associated with endotoxemia and exaggerated systemic inflammatory responses leading to sepsis and organ dysfunction. Similarly, Randell *et al*. (2001) noted that intestinal barrier compromise and endotoxin absorption in canine gastroenteritis contribute to leukocytosis, tachycardia, and tachypnea, all hallmark features of SIRS. The underlying mechanisms involve activation of neutrophils and monocytes, increased capillary permeability, and release of inflammatory mediators such as TNF-α, IL-1β, and IL-6, which collectively drive systemic manifestations of sepsis (Dinarello, 2000).

Conclusion

The present study demonstrated a substantial molecular incidence of *Escherichia coli* in canine haemorrhagic gastroenteritis (HGE) cases, with younger dogs and males showing greater susceptibility. The high proportion of SIRS-positive cases among PCR-confirmed dogs emphasizes the strong association between *E. coli*-associated HGE and systemic inflammatory complications, including progression toward sepsis. These findings underscore the importance of early molecular diagnosis and prompt therapeutic intervention to improve clinical outcomes and reduce disease-associated morbidity in affected dogs.

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