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Molecular detection of *Escherichia coli* and Shiga toxin-producing genes *stx1* and *stx2* from diarrheal samples in dogs

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Abstract
Diarrhoea is a widespread public health concern that can be caused by several pathogens, such as *Salmonella, Shigella, Escherichia coli*, etc. *Escherichia coli* is found in both animals and humans. Multidrug-resistant *E. coli* bacteria with varying antibiotic resistance profiles, especially for Extended-spectrum beta-lactamases (ESBL), can be found in pet dogs. Infant diarrhoea, hemorrhagic colitis, thrombotic thrombo cytic purpura, and hemolytic uremic syndrome are all linked to Shiga-like toxin-producing *E. coli* (SLT-EC). This study aimed to molecularly identify *E. coli* from diarrheal samples of dogs from Anand, GJ and detect Shiga toxin-producing genes *stx1* and *stx2* using PCR technique. 58 out of 68 rectal samples were found to have the presence of *E. coli* using PCR using species-specific primers. In this study, the overall prevalence of *E. coli* was 85.29%.

Keywords: *E. coli*, Shiga toxin, diarrhoea, dog, gene, *stx1*, *stx2*, PCR

Introduction
One significant source of zoonotic illnesses is dogs. Numerous bacterial and viral infections can be transmitted from dogs to people. Numerous factors can contribute to acute infectious diarrhoea, which is an international health issue. Among the most prevalent pathogens are Shigella, *Escherichia coli* and *rotavirus* [1].

Animals and humans' typical associated organisms contain *Escherichia coli*, a member of the Enterobacteriaceae family. Yet, several clinical cases of diarrhoea in both people and animals have been connected to specific strains of *E. coli*. Several *Escherichia coli* strains cause extraintestinal and gastrointestinal diseases in dogs and cats by acting as pathogens [2].

The concurrent existence of antibiotic-resistant *E. coli* in dogs and their owners, especially for Extended-spectrum beta-lactamases (ESBL), has been demonstrated to be a possible source of multidrug-resistant *E. coli* strains in the home. [3]. Infant diarrhea, hemorrhagic colitis, thrombotic thrombo cytic purpura, and hemolytic uremic syndrome are all linked to Shiga-like toxin-producing *E. coli* (SLT-EC). *E. coli* strains that generate the Shiga toxins produced by the *stx1* and *stx2* genes are known as strains that produce Shiga toxins (STEC) [4].

For this, molecular identification of *E. coli* from diarrhoeal samples is essential for rapid diagnosis and control of the disease in dogs. The current work is planned with these objectives: (1) Collection of rectal swabs from diarrhoeal cases in dogs, (2) The DNA extraction using the boiling method from diarrheal samples in dogs and (3) Molecular detection of *E. coli* and Shiga toxin-producing genes *stx1* and *stx2* from diarrheal samples in dogs.

Review of Literature
Staats *et al*. (2003) [5] obtained specimens from 60 greyhounds with severe diarrhoea and 76 greyhounds without diarrhoea. The *stx1* gene was detected in 3% of non-diarrheic samples and 15% of diarrheic samples, whereas the *stx2* gene was discovered in 36% of non-diarrheic and 23% of diarrheic samples.

Shiga toxin was detected in 48% of diarrheal and 25% of non-diarrheal *in vitro* cultivated samples. Fratamico *et al*. (2009) [6] collected 32 isolates, 19 of which carried the *stx1* gene, 10 had the *stx2* gene, and 17 had the *eae* gene. Paula and Marin (2009) [7] found the presence of Shiga toxin-producing genes in 12 strains (13.0%) from a total of 92 *E. coli* bacteria from 25 diarrheic using PCR. Zahraei Salehi *et al.* (2011) [4] collected rectal swabs from 100 dogs, 50 of which had diarrhoea and gastroenteritis symptoms and 50 of which did not. 10 isolates were obtained from 100 samples. Four isolates (4%) tested *stx+* and were classified as STEC, with two being *stx+/eae+*.

Kaur *et al*. (2014) [8] performed DNA extraction by boiling method. According to Hasan *et al*. (2016) [9], the total proportion of *E. coli* with and without gastroenteritis was 17.3% in 144 faecal samples. One isolate contained the *stx1* gene, six isolates included the *eaeA* gene, and three isolates contained both the *stx1* and *eaeA* genes. In addition, a single isolate possessed the *stx2* and *eaeA* genes.

Ali *et al*. (2018) [10] took 120 rectal swabs aseptically from diarrheal dogs in private clinics and veterinary facilities in Egypt. Out of 120 samples, *E. coli* was found in 68 samples. During molecular analysis, one and two samples were determined to have the *eaeA* and *stx2* genes. Jitendra *et al*. (2020) [11] performed PCR using a DNA template prepared from faecal suspension from dogs in PBS using the boiling method.

Materials and Methods

The present study was undertaken to ascertain molecular detection of *E. coli* and Shiga toxin-producing genes *stx1* and *stx2* from diarrheal samples in dogs. For the present study rectal swabs were collected from diarrhoeal cases presented to Veterinary Clinical Complex (TVCC), Veterinary College, Anand, Gujarat, India. A total of 68 rectal swabs were collected (Table 1). The rectal swab samples were transferred to a sterile disposable plastic specimen vial. The specimen vial was labelled properly according to the case number. The samples were transferred to the Microbiology Laboratory of the Department of Veterinary Microbiology, Kamdhenu University under refrigeration conditions.

Table 1: Details of rectal swab samples collected from TVCC Anand

Sex	Age (months)				Type of diarrhoea		
	0-3	3-6	6-12	>12	Bloody	Watery	
Male	24	14	00	00	34	04	
Female	16	06	08	00	24	06	
Breed	Doberman	Pug	Rottweiler	German Shephard	Pomeranian	Labrador	Non-descript
	06	02	02	06	04	30	18

The samples were collected in normal saline solution in sterile tubes. The liquid was placed in a water bath and boiled for 10 minutes. The supernatant was centrifuged at 10,000 rpm for 10 minutes and used as a template DNA in subsequent PCR experiments before being frozen at -20 °C until needed. An ND-1000 spectrophotometer (Nanodrop Technologies Inc., USA) was used to quantify DNA, with one absorbance unit at 260 nm wavelength equaling 50 µg DNA per ml.

The concentration of DNA (µg per ml) = Optical Density at 260 × Dilution factor × 50

Where 50 is the concentration of DNA at one O.D.

Primers designed particularly for *E. coli* and Shiga toxin-producing genes *stx1* and *stx2* were reported by Riffon *et al.* (2001) [12] and Gannon *et al.* (1992) [13].

Table 2: The PCR reaction mixture's composition

Components	Quantity (µL)
2X PCR Master mix	12.5
Forward Primer (10 pmole/µL)	1.0
Reverse Primer (10 pmole/µL)	1.0
Nuclease free water	5.5
DNA template	5.0
Total	25.0

Table 3: List of oligonucleotide primers used in PCR

Name of the target organism	Primer Sequence (5'-3')		Size of amplified products (bp)	References
E. coli	Eco220	ATCAACCGAGATTCCCCCAGT	232	Riffon *et al.* (2001)
	Eco455	TCACTATCGGTCAGTCAGGAG		
Stx1	*Stx1* F	ACACTGGATGATCTCAGTGG	614	Gannon *et al.* (1992)
	Stx1 R	CTCAATCCCCCTCCATTGTG		
Stx2	*Stx2* F	CCATGACAACGGACAGCAGTT	779	Gannon *et al.* (1992)
	Stx2 R	CCTGTCAACTGAGCACTTTG		

Table 4: Thermal cycling steps and conditions for several primers in PCR

Primers (forward and Reverse)	Cycling Conditions				
	Initial denaturation	Denaturation	Annealing	Extension	Final Extension
stx1 F *stx1* R	94 °C 5 min	94 °C 1 min	60 °C 1 min	72 °C 2 min	72 °C 5 min
		Repeated for 35 cycles			
stx2 F *stx2* R	94 °C 5 min	94 °C 1 min	60 °C 1 min	72 °C 2 min	72 °C 5 min
		Repeated for 35 cycles			
Eco 223 Eco 455 (*E. coli.*)	95 °C 5 min	94 °C 45 sec	64 °C 45 sec	72 °C 90 sec	72 °C 10 min
		Repeated for 35 cycles			

To validate targeted PCR amplification, 5µl PCR products were electrophoresed on a 2.0 per cent agarose gel with ethidium bromide (0.5 mg/ml) at 80 V in 0.5X TBE buffer using a 100bp DNA ladder. At the 30-minute interval, the amplified product was observed under UV light as a of the predicted size, which was documented using the gel documentation technique.

Results and Discussion

Around 68 rectal swab samples from diarrhoeal cases presented at the Veterinary Clinical Complex (TVCC), Veterinary College, Anand, Gujarat, India were considered in this study. The DNA of samples was extracted to detect the presence of *E. coli* and Shiga toxin-producing genes *stx1* and *stx2*. Out of a total of 68 rectal swab samples screened, 58 (85.29%) samples were identified as positive for *E. coli* by molecular identification using the PCR technique.

All 58 *E. coli* strains were evaluated for PCR-based characterization using DNA sequence coding for the 23S rRNA gene, and all tested to be positive by a 232 bp amplification. (Fig. 1). The present study has a high prevalence with the report of Ali *et al*. (2018) [10], *Hasan et al.* (2016) [9] and Zahraei Salehi *et al*. (2011) [4] isolated 56.67%, 17.3% and 10%, respectively. Here, higher prevalence is due to other concurrent viral diseases like parvoviral gastroenteritis. So, *E. coli* might be because by secondary bacterial infection.

All 58 isolates were found negative for *stx*1 and *stx*2 genes because DNA was isolated directly from rectal swab samples by boiling methods.

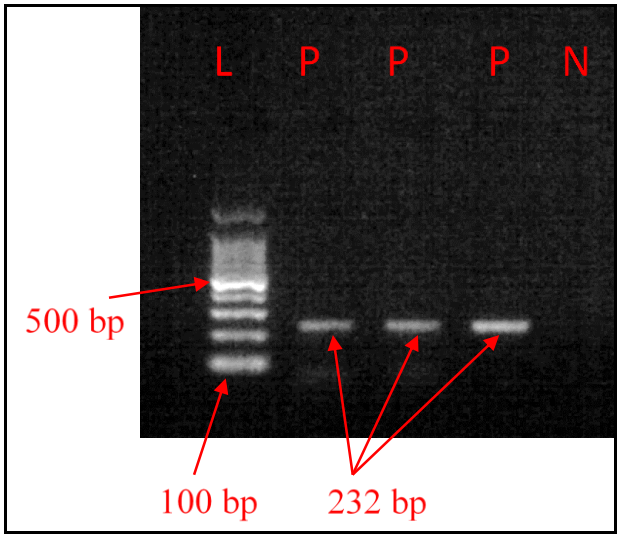

Fig 1: Agarose gel electrophoresis of a 232-bp increased PCR product of *E. coli*

L: Ladder, P: Positive sample, N: Negative sample

Summary and Conclusion

The present work comprised molecular identification of *E. coli* from rectal swab samples of diarrheal cases of dogs, and molecular detection of Shiga toxin-producing genes *stx1* and *stx2* of *samples* by PCR technique. Around 68 rectal swabs from TVCC, Anand, Gujarat, India were screened to know the prevalence status of *E. coli* in diarrheal cases of dogs. *E. coli* was confirmed by PCR using species-specific primer pairs in around 58 samples. The overall prevalence of *E. coli* in the study was 85.29%. DNA extraction directly from the sample using the boiling method is found useful.

References

1. Thielman NM, Guerrant RL. Acute Infectious Diarrhea. N Engl J Med. 2004 Jan 1;350(1):38-47. Available from: https://doi.org/10.1056/NEJMcp031534
2. Beutin L. *Escherichia coli* is a pathogen in dogs and cats. Vet Res. 1999;30(2-3):285-98.
3. Carvalho AC, Barbosa AV, Arais LR, Ribeiro PF, Carneiro VC, Cerqueira AMF. Resistance patterns, ESBL genes, and genetic relatedness of *Escherichia coli* from dogs and owners. Braz J Microbiol. 2016;47(1):150-158. Available from: https://doi.org/10.1016/j.bjm.2015.11.005
4. Salehi ZT, Badouei AM, Gohari MI. Molecular detection and antibacterial susceptibility of enteropathogenic *Escherichia coli* (EPEC) and shigatoxigenic *Escherichia coli* (STEC) strains isolated from healthy and diarrhoeic dogs. Comp Clin Pathol. 2011;20(6):585-589. Available from: https://doi.org/10.1007/s00580-010-1037-y
5. Staats JJ, Chengappa M, DeBey MC, Fickbohm B, Oberst RD. Detection of *Escherichia coli* Shiga toxin (stx) and enterotoxin (estA and elt) genes in fecal samples from non-diarrheic and diarrheic greyhounds. Vet Microbiol. 2003;94(4):303-312. Available from: https://doi.org/10.1016/S0378-1135(03)00134-2
6. Fratamico PM, DebRoy C, Miyamoto T, Liu Y. PCR detection of enterohemorrhagic *Escherichia coli* O145 in food by targeting genes in the *E. coli* O145 O-antigen gene cluster and the shiga toxin 1 and shiga toxin 2 genes. Foodborne Pathog Dis. 2009;6(5):605-611. Available from: https://doi.org/10.1089/fpd.2008.0254
7. Paula CJS, Marin JM. Multidrug-resistant Shiga toxin-producing *Escherichia coli* in dogs with diarrhea. Arq Bras Med Vet Zootec. 2009;61(2):511-514. Available from: https://doi.org/10.1590/S0102-09352009000200032
8. Kaur G, Chandra M, Dwivedi PN, Sharma NS. Antigenic typing of canine parvovirus using differential PCR. VirusDis. 2014;25(4):481-487. Available from: https://doi.org/10.1007/s13337-014-0232-x
9. Hasan MS, Yousif AA, Alwan MJ. Detection of Virulent Genes in *E. coli* O157:H7 Isolated from Puppies and Adult Dogs by Polymerase Chain Reaction. Res J Vet Pract. 2016;4(1):1-6. Available from: https://doi.org/10.14737/journal.rjvp/2016/4.1.1.6
10. Ali A, el shehadi M, Eraqi M. Molecular Studies on *Escherichia coli* Isolated from Diarrheic Dog. Int J Pharm Sci Rev Res. 2018;50(1):1-8.
11. Jitendra T, Choudhary S, Jain G, Bihani D. Comparative study for efficacy of Immunochromatography antigen assay (ICA) test kit with Polymerase chain reaction (PCR) to detect canine parvovirus tle. J Entomol Zool Stud. 2020;8(4):416-419.
12. Riffon R, Sayasith K, Khalil H, Dubreuil P, Drolet M, Lagacé J. Development of a Rapid and Sensitive Test for Identification of Major Pathogens in Bovine Mastitis by PCR. J Clin Microbiol. 2001;39(7):2584-9. Available from: https://doi.org/10.1128/JCM.39.7.2584-2589.2001
13. Gannon V, King R, Kim J, Thomas EJG. Rapid and Sensitive Method for Detection of Shiga-Like Toxin-Producing *Escherichia coli* in Ground Beef Using the Polymerase Chain Reaction. Appl Environ Microbiol. 1992 Dec;58(12):3809-3815.