Original Research Article
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Detection of *Escherichia coli* in Dogs and Cats of Different Locations of Braj, Mathura

Usha Bais [(1)], Udit Jain [(1)] *, Gourab Basak [(2)], Singh Parul [(1)], Barkha Sharma [(3)]

[(1)] Department of Veterinary Public Health, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, [(2)]Department of VPHE, Apollo College of Veterinary Medicine, Jaipur, Rajasthan-302031, [(3)] Department of Veterinary Epidemiology, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura

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Abstract

Escherichia coli is a ubiquitous and enteric commensal organism of warm-blooded animals. Nevertheless, its pathogenic strains possess higher risks for both animals and public health as these are zoonotic in nature. It causes a range of pathogenesis including severe enteric manifestation in all age groups of humans and animals. Mostly, this may contract through ingestion of contaminated food and water. In this study, the detection of *E. coli* from dogs and cats comprising different health statuses in Braj was carried out using cultural and biochemical tests. Overall, the percentage of *E. coli* detection was 61.43% from the faecal samples collected in which 61.66% and 60% were from dogs and cats respectively.

Key-words: Braj, *E. coli*, Dog, Cat

Introduction:

Dogs and cats live in close contact with humans. Dog and cat faeces may contain several types of microorganisms potentially pathogenic for humans. Bacteria that are pathogens for the intestinal tract and cause diarrhoea include *Campylobacter*, *Salmonella*, *Yersinia* and *Escherichia coli.* The presence of pet animal faeces in urban settings due to the habit of pet owners not removing pet faeces from the street may represent a problem for hygiene and public health. These animals may harbour and shed pathogens while remaining healthy or exhibiting only mild signs of infection. Infection can occur through direct contact with animals, faeco-oral route, human-to-human transmission, the food chain (contaminated milk or meat products including chicken, fish, lamb and shellfish), animal-to-animal and by contaminated drinking water (Fegan and Desmarchelier, 1999).

E. coli possesses a public health impact on humans by causing gastroenteritis including haemorrhagic colitis and haemolytic uremic syndrome (mostly the verotoxic strains); characterized by abdominal cramps, initially watery and later bloody diarrhoea, occasional fever and leucocytosis. Post-diarrhoeal haemolytic uremic syndrome, a clinical condition defined by acute renal injury, thrombocytopenia and microangiopathic haemolytic anaemia. The symptoms last for three to seven or even more and get not influenced by antimicrobial agents (Hassanshahian and Shahi, 2014).

There were no epidemiological surveys on the detection of zoonotic enteric bacteria in healthy, diarrhoeic and diseased dogs and cats in Braj. Braj, which is also known as Vraj, Vraja, Brij or Brijbhumi, is an Indian region situated in both the banks of river Yamuna, centred by Mathura and Vrindavan in the state of Uttar Pradesh. The area also includes Palwal and Ballabhgarh areas of Haryana, Bharatpur, Karauli and Dholpur regions of Rajasthan and Morena district of Madhya Pradesh (Figure 1). The term 'Braj' had been derived from the Sanskrit word '*Vraja* (व्रज)' which was first mentioned in Rigveda, meaning pasture, shelter or resort or cow grazing ground sBraj region is a Hindu pilgrimage devoted to Lord Radha and Krishna (Prasad, 2015). Therefore, the purpose of this study was to determine the status of *E. coli* among dogs and cats of this region.

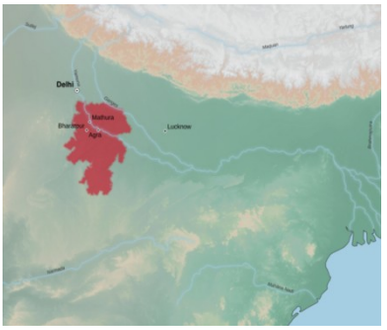

Figure 1: The map showing Braj region which is indicated in red(https://en.wikipedia.org/wiki/File:Braj_Bhasha_language_map.png)

Materials and Methods:

Collection of Samples: The study was carried out in some of the places of Braj region, *viz*., Aligarh city, Holi gate and civil line areas of Mathura city, Kothari Veterinary Hospital of DUVASU and the adjoining Aurangabad village of DUVASU, Mathura (Figure 2). The latitude and longitude of these places of sample collection are summarised in Table 1 chronologically.

Table 1: Locations of sample collection sites

Place	Latitude and Longitude
Aligarh	27.8974° N, 78.0880° E
Holi gate	27.4991° N, 77.6847° E
Civil line	27.4735° N, 77.7023° E
Kothari Veterinary Hospital	27.4603° N, 77.6997° E
Aurangabad	27.4451° N, 77.7080° E

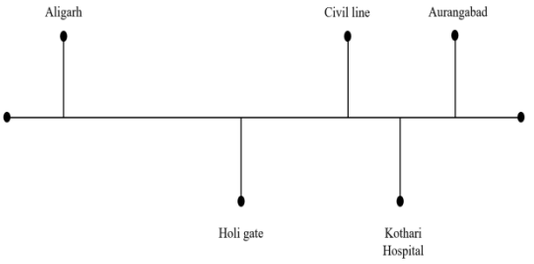

Figure 2: Different sample collection sites of Braj

For the current study, faecal samples from dogs and cats were collected from these places of Braj. The animals selected were healthy and diarrhoeic; also diseased but non-diarrhoeic dogs were also considered for the study which was presented in the Kothari hospital. A total of 210 faecal samples were collected, of which 180 samples were from dogs (60 samples each from three categories, *viz*., healthy, diarrhoeic, diseased but non-diarrhoeic) and 30 samples were of cat faeces (25 healthy and 05 diarrhoeic). The detailed number of samples collected from various places is listed in Table 2.

The faecal samples were collected aseptically in individual polythene bags (UV sterilized) by rectal swabbing with a sterile swab stick which were then brought to the laboratory in ice-cooled condition and processed within 3 hours of collection.

Isolation of *Escherichia coli*

Enrichment: For primary isolation of *E. coli*, the swab sticks were directly enriched in 9mL of Trypticase Soya Broth (TSB) containing acriflavin @ 10mg/L in order to reduce the growth of gram-positive organisms and incubated at 37°C for 6 hours.

Selective plating: The inoculum from the enrichment medium was selectively streaked on Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 hours. The colonies showing characteristic metallic sheen were indicative of *E.coli*. These isolated colonies were further purified on MLA (McConkey Lactose Agar) and subsequently checked for the purity of the isolates using Gram staining. The purified cultures were further stored in a Nutrient Agar slant for further identification by biochemical tests for more confirmation.

Biochemical characterization: The biochemical properties of *E. coli* were carried out using Biochemical Test Kit (Hi-media, Mumbai) for Indole, Methyl red, Voges-Proskauer and citrate utilization and eight carbohydrate utilization tests. A single isolated colony from Nutrient agar was inoculated in 5 mL Brain Heart Infusion (BHI) broth and incubated at 37°C for 4-6 hours until inoculum turbidity was 0.5 McFarland. Subsequently, the kit was opened aseptically and each well of the kit was inoculated with 50 µL of inoculum by surface inoculation method and incubated at 37°C for 18-24 hours.

Table 2: Details of sample collection from various places in the Braj region

Sample		Place of Collection	Number of samples collected		Total
Dog faeces	Healthy	Aurangabad	16	60	**180**
		Holi gate	11		
		Civil line	13		
		Kothari Veterinary Hospital	20		
	Diarrhoeic	Kothari Veterinary Hospital		60	
	Hospitalized but non-diarrheic (diseased)	Kothari Veterinary Hospital		60	
Cat faeces	Healthy	Aligarh	7	25	**30**
		Aurangabad	6		
		Holi gate	9		
		Kothari Veterinary Hospital	3		
	Diarrhoeic	Kothari Veterinary Hospital		05	

Results and Discussion:

Isolation and identification of *E. coli* isolates: The colonies that revealed a clear greenish metallic sheen on EMB media, which was used as differential and selective plating media, were tentatively considered presumptive *E. coli* organisms (Figure 3). A single colony from each plate on further streaking on MLA media gave pink colour colonies. These pink-coloured single colonies under the microscope were gram-negative rods with parallel sides and rounded ends arranged singly, in pairs and were motile in nature.

The *E. coli* isolates were confirmed by biochemical tests which showed the following characteristics as presented in Table 3, Figure 4.

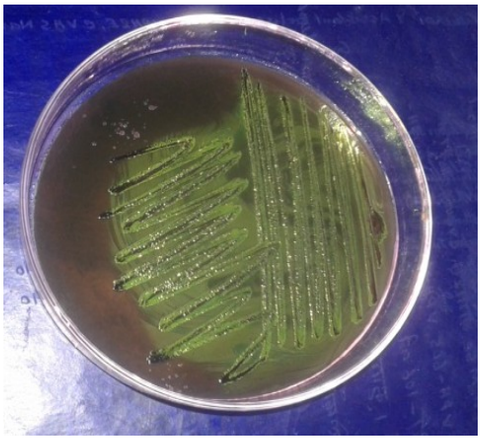

Figure 3: Presumptive *E. coli* colonies showing green metallic sheen on the selective EMB agar

Table 3: Biochemical reactions of *E. coli* isolates

Sl. No.	Tests	The original colour of the medium of the kit	Colour of kit after incubation	Interpretation
1	Indole	Colourless	Reddish pink	+
2	Methyl red	Colourless	Red	+
3	Voges-Proskauer	Colourless/light yellow	Colourless/slight copper	_
4	Citrate utilization	Green	Green	_
5	Glucose	Pinkish red/Red	Yellow	+
6	Adonitol	Pinkish red/Red	Red	_
7	Arabinose	Pinkish red/Red	Yellow	+
8	Lactose	Pinkish red/Red	Yellow	+
9	Sorbitol	Pinkish red/Red	Yellow	+
10	Mannitol	Pinkish red/Red	Yellow	+
11	Rhamnose	Pinkish red/Red	Yellow/Red	VR
12	Sucrose	Pinkish red/Red	Yellow/Red	VR

VR- variable range

Table 4: Percentage of *E. coli* isolates from various sources

Sources	Type of sample	No. of samples	Positive for *E. coli*	Percentage of *E. coli*
Veterinary hospitals and households of Braj region	Dog faeces	180	111	61.66%
	Cat faeces	30	18	60.00%
	Total	**210**	**129**	**61.43%**

Table 5: Percentage of *E. coli* isolates from faecal samples of dogs

Sl. No.	Status of animal	No. of Samples	Positive for *E. coli*	Percentage of *E. coli*
1	Healthy	60	35	58.33%
2	Diarrhoeic	60	40	66.66%
3	Diseased	60	36	60.00%
	Total	**180**	**111**	**61.66%**

Table 6: Percentage of *E. coli* isolates from faecal samples of cats

S. No.	Status of animal	No. of Samples	Positive for *E. coli*	Percentage of *E. coli*
1	Healthy	25	15	60.00%
2	Diarrhoeic	5	3	60.00%
	Total	**30**	**18**	**60.00%**

Figure 4: Biochemical characteristics shown by *E. coli*

Overall Percentage of *E. coli* isolates from various sources: Out of 210 samples collected from various sources, *viz*., 180 faecal samples of dogs (60 healthy, 60 diarrhoeic and 60 diseased but non-diarrhoeic) and 30 cat faeces (25 healthy and 05 diarrhoeic); 129 *E. coli* isolates were obtained. The overall percentage of *E. coli* isolates detected was 61.43%, of which 61.66% was from dogs and 60% from cats. The details are given in Table 4.

Percentage of *E. coli* isolates from dog faecal samples: The overall percentage of *E. coli* from dogs was found to be 61.66 % (111/180).This finding was nearly similar to the findings of Tramuta et al. (2014), i.e., 56.00 % and Johnson et al. (2000), i.e., 58% and lower to the finding of Ahmed et al. (2015),i.e., 88%. The details are given in Table 5.

Percentage of *E. coli* isolates from cat faecal samples: The overall percent of *E. coli* from cats was 60.00% (18/30) which was nearly similar to the findings of Ho et al. (2011), *i.e*., 58.9%. The details are given in Table 6.

Conclusion:

This study revealed the fact that the presence of *Escherichia coli* which could be of verotoxic strains in dogs and cats could be a silent hazard for humans and other animals. More than 50% of the dogs and cats harboured the enteric pathogen of zoonotic importance which is a clear indication of risk and sources of faecal contamination to foods and water. As a result, proper sanitation and disposal of faecal materials should be carried out, especially in cases of household pets.

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***Corresponding author's email ID:** druditjain@hotmail.com

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