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Short Communication

Prevalence of potentially disease producing *Escherichia coli* in ducks of organized farms/ backyards and their associated environments in different agro-climatic zones of West Bengal

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Abstract

The present study was undertaken to assess the prevalence of *Escherichia coli* possessing different virulence genes in healthy ducks reared in organized farms, the backyards and their associated environments covering various agro-climatic zones of West Bengal. A total of 272 samples were collected and screened for *E. coli* based on cultural, morphological and biochemical properties followed by PCR for confirmation of 16S rRNA gene. A total of 342 isolates were generated. The virulence genes, viz., *stx1*, *stx2*, *eaeA* and *ehxA* were detected in 79 (23.10%), 51 (14.91%), 43 (12.57%) and 77 (22.51%) isolates, respectively. The present study revealed that healthy ducks might play a role in transmitting disease producing *E. coli* to other animal species including human through direct contact and/or the food chain, that warrants serious attention and strategic intervention.

Keywords: Duck, *E. coli*, EHEC, STEC

Highlights

- Virulence genes (*stx, stx2, eaeA* and *ehxA)* were observed in *E. coli* isolated from apparently healthy ducks.
- The healthy ducks are carriers of potentially disease producing *E. coli.*
- Prevalence of *E. coli* having virulence genes was highest in the Undulating Red and Laterite zones of West Bengal.

Shiga toxin-producing *Escherichia coli* (STEC) also known as verotoxin-producing *E. coli* is a subdivision of an important pathogenic group called Enterohaemorrhagic *E. coli* (EHEC). STEC are food borne pathogens that can cause bloody diarrhoea to several other diseases such as haemorrhagic colitis (HC), thrombotic thrombocytopenic purpura (TTP) and life-threatening haemolytic uremic syndrome (HUS) (Paton and Paton, 1998). Shiga toxins which inhibit protein synthesis of the host cell leading to cell death are divided into two groups – Shiga toxin 1 and Shiga toxin 2 (Karmali *et al*., 2010). The toxins are phage-encoded cytotoxins, the most commonly assayed virulence factors of STEC that are encoded by the *stx1* and *stx2* genes, respectively. The protein intimin, which is encoded by the chromosomal gene *eae*, are responsible for the intimate attachment of the bacteria to intestinal epithelial cells and the production of attaching and effacing (AE) lesion (Paton and Paton, 1998). Some of the STEC strains exhibit haemolysis in washed sheep blood agar because of enterohaemolysin production, encoded by the *ehxA* gene (Beutin *et al*., 1993). STEC have been found in various animals, including cattle, sheep, goats, pigs, cats, dogs, chickens and gulls. In terms of human infection, cattle and pigs are the most significant animal species. The STEC strains commonly obtained from healthy animals can cause early episodes of diarrhoea in young animals, followed by asymptomatic colonization (Rivera *et al*., 2012). When these strains are present with a greater frequency in farm animals, humans are presumably exposed more often to such strains. There are limited data available from India regarding the isolation of STEC and other pathotypes of *E. coli* from ducks reared in the organized and unorganized sectors. Environmental samples, viz. feed, water and soil including faecal samples from duck farms, are not routinely evaluated for these pathogens.

In this study, a total of 272 samples comprising of the tracheal swabs (n=127), cloacal swabs (n=127) and environmental samples (n=18) were collected from indigenous ducks (n=25), Pekin ducks (n=24) and Khaki Campbell ducks (n=78) reared in the backyard and organized farms, respectively. These samples were collected on a random basis from different districts covering various agro-climatic zones of West Bengal. The sample collection area included farms of Pathar Pratima, South 24 Parganas district (Coastal Saline Zone), Mohanpur, Nadia district (Gangetic Alluvial Zone) and

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Table 1. Prevalence of different virulence genes in *Escherichia coli* isolated from various agro-climatic zones of West Bengal

Sample Source	Zones	*E. coli* isolates	Isolates having virulence gene(s)	*Stx1* +ve	*Stx2* +ve	*eaeA* +ve	*ehxA* +ve	*Stx1* + *Stx2* +ve	*Stx1* + *eaeA* +ve	*Stx1* + *ehxA* +ve	*Stx2* + *eaeA* +ve	*Stx2* + *ehxA* +ve	*eaeA* + *ehxA* +ve	*Stx1*+ *Stx2* + *ehxA* +ve	*Stx1* + *eaeA* + *ehxA* +ve
Mohanpur	Gangetic Alluvial Zone	166	55	22	20	22	21	06	07	06	04	01	02	02	01
Pathar Pratima	Saline Coastal Zone	84	37	18	10	09	21	01	01	08	02	01	02	02	01
Raipur	Undulating Red and Laterite Zone	92	62	39	21	12	35	10	00	16	01	04	04	01	04
Total		342	154	79	51	43	77	17	08	30	07	06	08	05	06

Raipur, Bankura district (Undulating Red and Laterite Zone). All the samples, i.e., cloacal swabs, tracheal swabs and environmental samples (feed, drinking water used for duck feeding, and soil) were collected aseptically with sterile cotton swabs and in sterile plastic zipper/vials (HiMedia, India). Then, the swabs were kept in a sterile sample collecting vial containing peptone water as a transport medium (HiMedia, India). The collected samples were labelled properly and placed above the ice pack in a thermo flask and brought to the laboratory for further processing. The collected samples were enriched in nutrient broth (HiMedia, India) at 37°C overnight. After that, the samples were cultured on MacConkey agar (HiMedia, India) and incubated at 37°C overnight for isolation of *E. coli* colonies. The positive colonies showing a rose-pink colour were selected and subcultured on Eosin methylene blue (EMB) agar (HiMedia, India) and further incubated overnight at 37°C. The next day colonies with metallic sheen were identified, and a single colony was transferred onto a nutrient agar (HiMedia, India) slant for further biochemical and molecular confirmation. Biochemical identification of the isolates was performed as per Quinn *et al*. (1994) with modifications as per the media/reagent manufacturer's recommendations. DNA was extracted from all the biochemically confirmed isolates and subjected to PCR for molecular confirmation as per Wang *et al*. (1996). All the molecular confirmed *E. coli* isolates confirmed by PCR were screened for the presence of *stx1*, *stx2*, *eaeA* and *ehxA* with multiplex PCR amplified product sizes of 180, 255, 384 and 534 bp, respectively (Paton and Paton, 1998). The amplified product was visualized by a gel documentation system after electrophoresis in 1.5 % (w/v) agarose gel (HiMedia, India) containing ethidium bromide (0.5 µg/mL) (SRL, India).

A total of 342 *E. coli* isolates comprised of 156 isolates from cloacal swab, 127 from tracheal swab and 59 from environmental sample (feed, n=15, water, n=20, soil, n=24) were generated from 272 samples. Out of 342 isolates,154 (45.03%) were positive for Shiga toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC) and Enterohemorrhagic *E. coli* (EHEC) genes, which consisted of *stx1* (n=79), *stx2* (n=51), *eaeA* (n=43), *ehxA* (n=77) (Table1). Among them, 17 (11.04%) were positive for both *stx1* and *stx2*; 08 (5.19%) were positive for both *stx1* and *eaeA*; 30 (19.48%) were positive for both *stx1* and *ehxA*; 07 (4.54%) were positive for both *stx2* and *eaeA*; 06 (3.89%) were positive for both *stx2* and *ehxA*; 08 (5.19%) were positive for both *eaeA* and *ehxA*; 06 (3.89%) isolates were positive for all *stx1*, *eaeA* and *ehxA*; and 05 (3.25%) were positive for all *stx1*, *stx2* and *ehxA*.

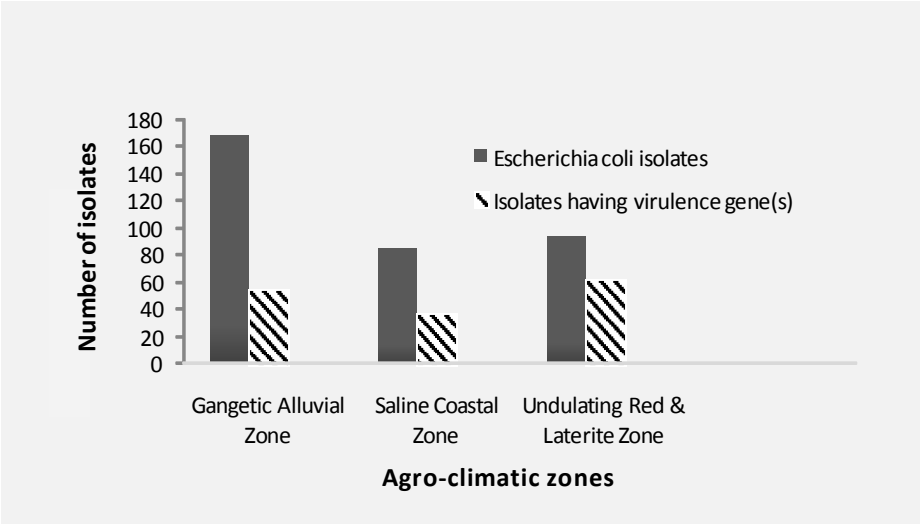

Fig. 1. Agro-climatic zone-wise prevalence of virulence genes in *Escherichia coli* isolates

Zone-wise analysis showed 166 isolates were from the Gangetic Alluvial Zone, 84 from Saline Coastal Zone and 92 from Undulating Red and Laterite Zone. A total of 55(33.13%) isolates from the Gangetic Alluvial Zone, 37(44.05%) from Saline Coastal Zone and 62 (67.39%) from Undulating Red and Laterite Zone were positive for different virulence genes, viz., STEC, EPEC and EHEC genes (Fig. 1). The results indicated that the prevalence of *E. coli* having virulence genes were higher in Undulating Red and Laterite zone followed by Coastal Saline Zone and Gangetic Alluvial Zone.

Earlier studies reported a comparatively low prevalence (2-4%) of STEC in ducks reared in China (Wang *et al*., 2010) and North India (Farooq *et al*., 2009). However, our findings were corroborated by the higher prevalence of STEC (24-87%) in ducks reported from West Bengal (India) (Banerjee and Acharya, 2020), Egypt (Elsayed *et al*., 2021) and Malayasia (Adzitey *et al*., 2012). However, the results of the present study differed from Wani *et al*. (2004) in which samples were collected from chicken and pigeons lacking *stx1* or *stx2* genes. Moreover, the presence of *ehxA* in the samples of the present study may be considered unique as no previous studies of the state reported the same. Prevalence of STEC in duck and poultry samples varied due to geographical location, season, farm size, number of birds in the farm, farm hygiene and management practices, variation in sampling and differences in detection methodologies used (Mohammadi *et al*., 2013).

It is apprehended that these virulence genes may be transmitted to human beings and other animals through environmental contamination by ducks, i.e., contamination of land and water bodies with duck faecal droppings or through direct consumption of duck meat. In the study, the samples were collected from apparently healthy ducks without any signs and symptoms of diseases. Hence, we infer that healthy ducks are potential carriers of virulence genes and are considered transmitting agents of disease potential *E. coli* to other animals including human. The findings might be considered as primary information while estimating the prevalence of virulence genes of *E. coli* in apparently healthy ducks and their environment in West Bengal. Further studies are needed to reveal the actual threat of potentially disease producing *E. coli* and formulate strategies to minimize the risk of infections in humans.

Conflict of interests: Authors declare that they have no conflict of interests.

Authors' contributions: AP: Acquisition and analysis of data, manuscript preparation; IS: Interpretation of data, editing of the manuscript; KB, SD: Editing of the manuscript; SNJ: Concept, design, supervision of work and fund generation. All the authors approved the manuscript.

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