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CLINICAL MANIFESTATIONS IN RABBITS EXPERIMENTALLY INFECTED WITH *ESCHERICHIA COLI* O157:H7 ISOLATES OF DIFFERENT VIRULENCE GENE PROFILES

O. E. OJO[1*], A. T. P. AJUWAPE[2], O. L. AJAYI[3], E. B. OTESILE[4], AND A. I. ADETOSOYE [2]

[1]Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Abeokuta. Postcode 110001. Nigeria
[2]Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[3]Department of Veterinary Pathology, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Abeokuta, Nigeria
[4] Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Abeokuta, Nigeria

***Corresponding author**: oeoefemi@yahoo.com **Tel:** +2347030425778

ABSTRACT

Escherichia coli O157:H7 is a major cause of zoonotic food-borne infections transmissible from asymptomatic animals to humans following consumption of contaminated foods. Pathogenicity of *E. coli* O157:H7 is attributed to possession of virulence genes such as *eaeA* and *stx* responsible for intimate adhesion to enterocytes and production of cytolethal shiga toxins. The pathogenic potentials of five *E. coli* O157:H7 isolates of different virulence gene profiles recovered from the faeces of slaughter cattle was compared in rabbit model. Five groups (A-E) of five rabbits were each inoculated orally with 5 x 10^9 colony forming units of an *E. coli* O157:H7 isolate possessing one of the virulence gene profiles: *stx*$_1$ / *stx*$_2$ / *eaeA* / *hlyA* (group A), *stx*$_1$ /*stx*$_2$ (B), *stx*$_1$ (C), *stx*$_2$ (D), and *eaeA* / *hlyA* (E). Group F (control) received sterile broth. The mean onset and duration of clinical manifestations varied significantly among the experimental groups being earliest and shortest in group infected with *E. coli* O157:H7 possessing *stx*$_1$ / *stx*$_2$ /*eaeA* / *hlyA*. Infected rabbits showed clinical signs including dullness, profuse non-bloody diarrhoea, weakness, anorexia and epistaxis starting from two days post infection (*p.i.*). Epistaxis was observed only in rabbits inoculated with isolates that possessed *stx*$_2$ either alone or in combination with other virulence genes. Mortality of 100% was recorded in isolates with *stx*$_1$ /*stx*$_2$ / *eaeA* / *hlyA*, *stx*$_1$ / *stx*$_2$ and *stx*$_2$ and 60% with *stx*$_1$ and *eaeA* / *hlyA*. Test organisms were detected in the faeces of inoculated animals as from two days p.i. and persisted in survivors for 19 to 30 days *p.i.* This study showed that *E. coli* O157:H7 isolates from cattle produced fatal illness in experimental rabbits and that virulence gene profile significantly influenced the onset, duration and severity of clinical manifestation of infection in the experimental animals.

Keyword: Clinical manifestation *E. coli* O157:H7, experimental infection, rabbits, virulence, gene profile.

INTRODUCTION

Escherichia coli O157:H7 is a leading cause of acute gastroenteritis leading to fatal haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) in humans (Riley *et al.,* 1983; Rangel *et al.,* 2005). Although food animals especially ruminants are natural reservoirs of *E. coli* O157:H7 (Zschöck *et al*., 2000; Ojo *et al.,* 2010), the pathogenicity of the organism in food animals is low as clinical infection appears to occur only occasionally in young animals while no serious disease condition is recorded in adults (Stoffregens *et al.,* 2004; Twardon *et al,* 2005; Dean-Nystrom *et al.,* 2008). In contrast, humans are very susceptible to *E. coli* O157:H7 infection. Fewer than 10 bacterial cells of *E. coli* O157:H7 are able to establish clinical infection in humans (Twardon *et al.,* 2005). Most cases of *E. coli* O157:H7 infections in humans are due to the consumption of foods and water contaminated with faeces of asymptomatic carrier animals (Tauxe 1997; Mead *et al.,* 1999).

The preponderance of *E. coli* O157:H7 in the faeces of ruminants and non-adherence to principles of hygiene during processing and marketing of meat facilitate contamination (Ojo *et al.,* 2010). Consequently, consumers are at risk of possible exposure to this dangerous pathogen. However, infections with *E. coli* O157:H7 strains do not always lead to clinical disease and in cases of clinical infections, presenting signs may differ according to the strains involved. The severity of *E. coli* O157:H7 infections in humans is influenced by the possession of virulence genes including shiga toxin 1 and 2 (*stx*$_1$ and *stx*$_2$), intimin (*eaeA*) and enterohaemolysin (*hlyA*) genes (Paton and Paton, 1998). The presence of these virulence genes either alone or in combination in *E. coli* O157:H7 strains determines the clinical outcome of infection (Dean-Nystrom *et al,* 2003). Pathogenic STEC colonizes the large intestine, adheres to the enterocytes and produces shiga toxins. *Escherichia coli* O157:H7 adherence to intestinal epithelia is enhanced by the possession of intimin (Paton and Paton, 1998). Shiga toxins are transported across the epithelial cells and into the blood circulation from where they are disseminated to other organs (Paton and Paton, 1998). The toxins have profound effect on the endothelial cells of blood vessels and cause endothelial damage. Toxins are responsible for non-bloody to bloody diarrhoea, abdominal cramp and in some cases renal damage leading to HUS (Paton and Paton, 1998).

The objective of the present study is to verify the pathogenic potentials of five *E. coli* O157:H7 field isolates of different virulence gene profiles and compare clinical outcome of their infection in a rabbit experimental model. These isolates were originally recovered from non-diarrhoeic faeces of slaughter cattle at an abattoir in Ibadan, Nigeria.

MATERIAL AND METHODS

This experiment conformed to study protocols specified by the European Union Directive 86/609 and Council for International Organizations of Medical Sciences (CIOMS). It was approved, monitored and ethically certified by the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

Bacterial isolates:

The five *E. coli* O157:H7 isolates possessing *stx*$_1$/*stx*$_2$/*eaeA*/*hlyA*, *stx*$_1$/*stx*$_2$, *stx*$_1$, *stx*$_2$, and *eaeA*/*hlyA* were selected based on their virulence genes profiles and were all originally isolated from non-diarrhoeic faeces of slaughter cattle in an abattoir in Ibadan, Nigeria (Ojo *et al,* 2010). The methods of detec-

tion of virulence genes and confirmation of *E. coli* O157:H7 were previous described (Ojo *et al.,* 2010).

Preparation of inocula:

Escherichia coli O157:H7 isolates previously preserved on nutrient agar slopes were inoculated onto sorbitol MacConkey agar (SMAC). Two to three pure colonies on SMAC were transferred into 10ml TSB and incubated for 18-24 hours at 37 ^{O}C. The TSB culture was centrifuged and the packed bacterial cells in form of sediment at the bottom of the test tube washed twice with phosphate-buffered saline (PBS). The washed bacterial cells were re-suspended in sterile TSB to a final concentration of 5 x 10^9 colony forming units per millilitre (cfu/ml) determined by pour plate count method on SMAC after a tenfold serial dilution of the bacterial suspension in TSB.

Infection of Animals:

Thirty weaned New Zealand rabbits of four weeks of age weighing 450 to 500g were divided into six groups (A-F) of five rabbits per group. The rabbits were apparently healthy, *E. coli* O157:H7-culture negative and had well-formed faeces. Each rabbit was sedated as previously described (Sherman *et al.,* 1988) and orally infected with 5 x 10^9 (cfu) of an *E. coli* O157:H7 isolate suspended in 1ml TSB. Group A rabbits were infected with an *E. coli* isolate possessing *stx*$_1$/*stx*$_2$/*eaeA*/*hlyA*, group B (*stx*$_1$/*stx*$_2$), group C (*stx*$_1$), group D (*stx*$_2$), and group E (*eaeA*/*hlyA*) while control group F received sterile TSB.

Inoculated rabbits were observed 12-hourly for signs of illness. An animal was considered to be diarrhoeic if the perineum or/and the hind limbs were soiled with faeces. Rectal swabs were collected from inoculated rabbits daily (throughout the 40 days of the experiment) for the isolation and identification of *E. coli* O157:H7 using SMAC, *E. coli* O157 latex agglutination kit (DR0620M, Oxoid, Basingstoke, UK) and H7 antiserum (Difco®, USA).

Statistical analysis:

Mean values (± standard deviation) were calculated for animals that showed clinical signs in each group. The differences in mean values across groups were compared by Duncan's one way ANOVA at $p<0.05$ probability level using the Statistical Package for Social Sciences (SPSS) version 16 software (2007).

RESULTS

Clinical findings:

In this study, all experimental rabbits inoculated with *E. coli* O157:H7 showed signs of illness. Observed clinical signs were diarrhoea, dullness, anorexia, weakness, epistaxis and death (Table 1). The mean onset of clinical signs varied significantly among the experimental groups (Table 2). Diarrhoea started significantly earliest (3.4±1.1 dpi) ($p<0.05$) in group inoculated with *stx*$_1$/*stx*$_2$/*eaeA*/hlyA and latest (7.2±2.4 dpi) in group infected with *stx*$_1$. The diarrhoea started as passage of loose faeces but progressed to profuse watery non-bloody diarrhoea in all the groups. The mean onset of dullness also differed significantly ($p>0.05$) among some of the experimental groups, being earliest among rabbits with *stx*$_1$/*stx*$_2$/*eaeA*/*hlyA* (3.2 ± 0.5 dpi) and *stx*$_2$ (3.6 ± 0.5 dpi) but latest in groups with *stx*$_1$ (5.2 ± 0.8 dpi) and *eaeA*/hlyA (5.3 ± 1.0 dpi). Similarly, anorexia started significantly earliest ($p<0.05$) in rabbits with *stx*$_1$/*stx*$_2$/*eaeA*/*hlyA* (3.4 ± 0.5 dpi) than in all the other groups. There was no significant difference ($p>0.05$) in the mean onset of anorexia among groups with *stx*$_1$ (9.0 ±

1.4 dpi), *stx*$_2$ (8.8 ± 1.3 dpi) and *eaeA/hlyA* (8.3 ± 1.2 dpi). Three rabbits from group with *stx*$_1$ and two from group with *eaeA/hlyA* did not develop anorexia. Weakness occurred significantly earliest ($p<0.05$) among rabbits with *stx*$_1$/*stx*$_2$/*eaeA/hlyA* (5.6 ± 0.5 dpi) than in groups with *stx*$_1$/*stx*$_2$ (7.6 ± 1.7 dpi), *eaeA / hlyA* (9.5 ± 0.7 dpi), *stx*$_2$ (10.6 ± 1.3 dpi). Only one rabbit from the group with *stx*$_1$ showed signs of weakness. Epistaxis occurred in two rabbits from group with *stx*$_1$/*stx*$_2$/*eaeA/hlyA* at 6.5 ± 0.7 dpi and in one rabbit each from groups with *stx*$_1$/*stx*$_2$ and *stx*$_2$ at 7 dpi and 9 dpi respectively. All rabbits with epistaxis died within 24 hours.

Diarrhoea continued till death in rabbits infected with *stx*$_1$/*stx*$_2$/*eaeA/hlyA* and *stx*$_1$/*stx*$_2$ as well as in one rabbit each from the other three infected groups. In rabbits infected with *stx*$_2$-O157H7, diarrhoea stopped 2 to 5 days prior to death. Two rabbits each from groups with *stx*$_1$ and *eaeA/hlyA* survived the diarrhoea episode. The mean durations of diarrhoea and weakness were not significantly ($p>0.05$) different among the rabbit groups (Table 3). However, the duration of dullness was significantly shorter ($p<0.05$) in rabbits with *stx*$_1$/*stx*$_2$/*eaeA/hlyA* (3.8±0.4 days) than in groups with *stx*$_1$ (6.6±3.2 days) and *stx*$_2$ (8.6±1.3 days) but not significantly different from rabbits with *stx*$_1$/*stx*$_2$ (4.6±1.1 days) and *eaeA/hlyA* (5.7±1.5 days). The duration of anorexia was significantly shorter in group with *eaeA/hlyA* (2.7±0.6 days) than in the group with *stx*$_1$ (4.5±0.7 days). There was no significant difference ($p>0.05$) in the duration of anorexia among groups infected with *stx*$_1$/*stx*$_2$/*eaeA/hlyA* (3.6±0.5 days), *stx*$_1$/*stx*$_2$ (3.4±0.9 days) and *stx*$_2$ (3.4±0.9 days).

Mortality was 100% in groups with *stx*$_1$/*stx*$_2$/*eaeA/hlyA*, *stx*$_1$/*stx*$_2$ and *stx*$_2$ but 60% in groups with *stx*$_1$ and *stx*$_1$. Death occurred significantly earliest ($p<0.05$) in rabbits from group with *stx*$_1$/*stx*$_2$/*eaeA/hlyA* (7.0 ± 0.7 dpi) than in groups with *stx*$_1$/*stx*$_2$ (9.2 ± 1.6 dpi), *eaeA/hlyA* (11.0 ± 1.0 dpi), *stx*$_2$ (12. 2± 1.6 dpi) and *stx*$_1$ (13.6 ± 1.5 dpi).

Neither signs of illness nor mortality were observed among the rabbits in the control group throughout the period of the experiment.

Recovery of E. coli O157:H7 from experimentally infected rabbits:

E. coli O157:H7 was detected in faeces of all the inoculated animals as from 48 hours p.i. The shedding continued till the rabbits died. The two surviving animals from the group infected with *stx*$_1$/*stx*$_2$-O157:H7 shed the organism for 16 and 19 days post-diarrhoea while surviving animals from group infected with *eaeA/hlyA*-O157:H7 continued to shed the organisms until the experiment was terminated (30 days post-diarrhoea). None of the faecal samples collected from the control group yielded *E. coli* O157:H7.

Table 1.Clinical signs observed in rabbits experimentally inoculated with *E. coli* O157:H7 of different virulence gene profiles

Observed clinical signs	Frequency of occurrence per group					
	Group A (stx1/stx2/eaeA/hlyA)	Group B (stx1//stx2)	Group C (stx1)	Group D (stx2)	Group E (eaeA/hlyA)	Group F (Control)
Dullness	3/5	5/5	5/5	5/5	3/5	0/5
Anorexia	5/5	5/5	2/5	5/5	3/5	0/5
Diarrhoea	5/5	5/5	5/5	5/5	5/5	0/5
Weakness	5/5	4/5	1/5	5/5	2/5	0/5
Epistaxis	2/5	1/5	0/5	1/5	0/5	0/5
Death	5/5	5/5	3/5	5/5	3/5	0/5

Table 2: Onset of clinical signs in rabbits experimentally infected with *E. coli* O157:H7 isolates of different virulence gene profiles

Clinical signs	Mean ± standard deviation (range) days post infection in rabbits showing Signs within groups*					
	Group A (stx1/stx2/eaeA/hlyA)	Group B (stx1/stx2)	Group C (stx1)	Group D (stx2)	Group E (eaeA/hlyA)	All infected rabbits
Diarrhoea	3.4 ± 1.1a (2.0 – 5.0)	4.6 ± 0.9a, b (4.0 – 8.0)	7.2 ± 2.4c (4.0 – 10.0)	5.6 ± 1.7a, b, c (4.0 – 10.0)	6.2 ± 2.5b, c (4.0 – 10.0)	5.4 ±2.2 (2 – 10)
Dullness	3.2 ± 0.5a (3.0 – 4.0)	4.6 ± 1.3b, c (3.0 – 6.0)	5.2 ± 0.8c (4.0 – 6.0)	3.6 ± 0.5a, b (3.0 – 4.0)	5.3 ± 1.0c (4.0 – 6.0)	4.3 ± 1.2 (3 – 6)
Anorexia	3.4 ± 0.5a (3.0 – 4.0)	5.8 ± 0.8b (5.0 - 7.0)	9.0 ± 1.4c (8.0 – 10.0)	8.8 ± 1.3c (7.0 – 10.0)	8.3 ± 1.2c (7.0 – 9.0)	6.7 ± 3.6 (3 – 10)
Weakness	5.6 ± 0.5a (5.0 – 6.0)	7.6 ± 1.7b (6.0 – 9.0)	14.0 ± 0.0 (14.0)	10.6 ± 1.3c (9.0 – 12.0)	9.5 ± 0.7c (9.0 – 10.0)	8.5 ± 2.7 (5 - 14)
Epistaxis	6.3 ± 0.6 (6.0 – 7.0)	7.0 ± 0.0 (7.0)	-	9.0 ± 0.0 (9.0)	-	7.3 ± 1.3 (6 - 9)
Death	7.0 ± 0.7a (6.0 – 8.0)	9.2 ± 1.6b (7.0 – 11.0)	13.6 ± 1.5d (12.0 – 15.0)	12. 2± 1.6c, d (11.0 – 14.0)	11.0 ± 1.0c (10.0 – 12.0)	10.3 ± 2.7 (6 - 15)

* The mean values were determined only for the rabbits that manifested clinical signs out of the five rabbits inoculated in each group. Values with the same superscripts are similar while those with different superscripts differ significantly at $p<0.05$.

Table 3: Duration of clinical signs in rabbits experimentally infected with *E. coli* O157:H7 isolates of different virulence gene profiles

Clinical signs	Mean ± standard deviation (range) days post infection in rabbits showing Signs within groups*					
	Group A (stx1/stx2/eaeA/hlyA)	Group B (stx1/stx2)	Group C (stx1)	Group D (stx2)	Group E (eaeA/hlyA)	All infected rabbits
Diarrhoea	3.6±0.9a (3.0-5.0)	4.6±1.1a (3.0-7.0)	4.0±1.6a (2.0-6.0)	4.4±1.1a (3.0-6.0)	3.8±1.8a (1.0-6.0)	4.2±1.8 (3.0-7.0)
Dullness	3.8±0.4a (3.0-4.0)	4.6±1.1a, b (3.0-6.0)	6.6±3.2b, c (3.0-10.0)	8.6±1.3d (7.0-10.0)	5.7±1.5a, b (4.0-7.0)	5.9±2.4 (3.0 – 10.0)
Anorexia	3.6±0.5a, b (3.0-4.0)	3.4±0.9a, b (2.0-4.0)	4.5±0.7b (4.0-5.0)	3.4±0.9a, b (2.0-4.0)	2.7±0.6a (2.0-3.0)	3.7±1.1 (2.0-5.0)
Weakness	1.6±0.5a (1.0-2.0)	1.3±0.5a (1.0-2.0)	1.0	1.6±0.5a (1.0-2.0)	1.5±0.7a (1.0-2.0)	1.6±1.3 (1.0-2.0)
Epistaxis	1.0±0.0 (1.0)	1.0	-	1.0	-	1.0±0 ($\leq$1.0)
Survival time	7.0±0.7a (6.0-8.0)	9.2±1.6b (7.0-8.0)	13.6±1.5d (12.0-15.0)	12.2±1.6c, d (11.0-14.0)	11.0±1.0c (10.0-12.0)	10.3 ± 2.7 (6 - 15)

* The mean values were determined only for the rabbits that manifested clinical signs out of the five rabbits inoculated in each group. Values with the same superscripts are similar while those with different superscripts differ significantly at $p<0.05$.

DISCUSSION

Escherichia coli O157:H7 is an important zoonotic pathogen associated with food-borne gastroenteritis in humans. Natural infections often manifest as non-bloody to bloody diarrhoea.

In this study, all the *E. coli* O157:H7 isolates induced severe non-bloody diarrhoea in weaned rabbits regardless of the virulence genes profile. The mean onset of diarrhoea was shortest in rabbits inoculated with *stx*$_1$/*stx*$_2$/*eaeA*/*hlyA*-O157:H7 (2.6±0.6 dpi) and longest in those inoculated with *stx*$_1$-O157:H7 (6.5±2.5 dpi). Earlier studies have shown that *E. coli* O157:H7 induced non-bloody diarrhoea in rabbits (Pai *et al*, 1986; Sherman *et al*, 1988; Raji *et al*, 2009). Raji *et al* (2009) reported that day-old infant rabbits inoculated with *E. coli* O157:H7 developed severe diarrhoea 2 days post infection. In another study, *E. coli* O157:H7 induced diarrhoea in experimentally infected post-weaning rabbits as from 5 days p.i. (Sherman *et al*, 1988). In humans, *E. coli* O157:H7 infection typically begins with an onset of non-bloody watery diarrhoea between 2 and 12 days p.i. which may last 1 to 3 days (Tarr *et al,* 2005). In most cases, this progresses to bloody diarrhoea which is the hallmark of *E. coli* O157:H7 infection in humans. However, it appears oral inoculation of *E. coli* O157:H7 does not induce bloody diarrhoea in rabbits as observed in this study and those by other workers (Pai *et al*, 1986; Sherman *et al*, 1988; Raji *et al*, 2009).

Other clinical manifestations observed in rabbits experimentally infected with *E. coli* O157:H7 in this study included dullness, anorexia, weakness and epistaxis. The onset of these signs varied significantly in rabbits according to the virulence profile of the infecting *E. coli* O157:H7. However, the duration of clinical signs did not vary as much as the onset. Variations were only observed in the durations of dullness and anorexia. In this study, *E. coli* O157:H7 possessing *stx*$_1$/*stx*$_2$/*eaeA*/*hlyA* induced clinical signs earlier than any of the other tested isolate. This showed that rabbits succumbed significantly earliest to infection with *E. coli* O157:H7 that possessed all the four virulence genes (*stx*$_1$/*stx*$_2$/*eaeA*/*hlyA*-O157:H7) compared to other isolates. In the present study, epistaxis was observed in rabbits inoculated with *E. coli* O157:H7 isolates possessing *stx*$_2$ gene either alone or in combination with other genes (that is, *stx*$_1$/*stx*$_2$/*eaeA*/*hlyA*, *stx*$_1$/*stx*$_2$ and *stx*$_2$ virulence profiles). We are not aware of any previous report of epistaxis in rabbits experimentally or naturally infected with *E. coli* O157:H7. The present study provides useful information on the comparative onset and duration of clinical parameters such as dullness, anorexia, weakness and epistaxis in addition to diarrhoea induced by *E. coli* O157:H7 of different virulence gene profiles which appear to us as being absent in previous studies.

In the present study, 100% mortality was recorded in experimental groups inoculated with *stx*$_1$/*stx*$_2$/*eaeA*/*hlyA*-O157:H7, *stx*$_1$/*stx*$_2$ -O157:H7 and *sstx*$_2$-O157:H7 but 60% in groups inoculated with *stx*$_1$-O157:H7 and *eaeA*/*hlyA*-O157:H7. Survival time was longest in group inoculated with *stx*$_1$-O157:H7 (13.7±1.5 dpi) and shortest in group inoculated with *stx*$_1$/*stx*$_2$/*eaeA*/*hlyA*-O157:H7 (7.0 ± 0.7). Previous studies have shown that *E. coli* O157:H7 caused mortality in infant rabbits 4 days *p.i.* (Raji *et al.,* 2009). Pai *et al* (1986) also reported mortality in infant rabbits inoculated with *E. coli* O157:H7. The earlier onset of mortality observed by Raji *et al.* (2009) than in the present study could be

due to the age difference in the experimental rabbits used. It has been observed that the susceptibility of rabbits to *E. coli* O157:H7 is dose and age-dependent (Pai *et al,* 1986). The quantity of toxin produced by inoculated *E. coli* O157:H7 could also be a factor contributing to the outcome of infection (Wagner *et al.,* 2002; Gamage *et al.,* 2003). However, in contrast to observation in the present study, Ritchie *et al.* (2003) reported that *E. coli* O157:H7 did not produce mortality in experimentally inoculated infant rabbits. The present study showed that virulence gene profile of inoculated *E. coli* O157:H7 significantly influenced the survival of infected rabbits.

The present study showed that rabbits infected with *stx*-deficient *E. coli* O157 isolate (*eaeA*/*hlyA*-O157:H7) developed diarrhoea with 60% mortality. This *E. coli* strain, with no gene for shiga toxin production, cause significant clinical manifestation in rabbits similar to observations in humans suggesting that stx does not appear to be crucial in the manifestation of clinical diseases following *E. coli* O157:H7 infection (Schmidt *et al.,* 1999). *Escherichia coli* O157:H7 strains possessing *eaeA* and *hlyA* genes without *stx* genes can be grouped into a similar pathogroup as enteropathogenic *E. coli* (EPEC) as opposed to STEC/EHEC (Schmidt *et al.,* 1999). Non-shiga toxin producing *E. coli* strains have been associated with human outbreaks of diarrhoea and HUS (Schmidt *et al.,* 1999; Allerberger *et al.,* 2000).

The results of this study suggest that all the virulence genes present in the *E. coli* O157 isolates investigated are important in pathogenicity. However, the severity and duration of *E. coli* O157 infection may be influenced by differences in the combination of the virulence genes. Clinical manifestations observed in the present study were most profound with *stx*$_1$/*stx*$_2$/*eaeA*/*hlyA*-O157:H7 isolate followed by *stx*$_1$/*stx*$_2$-O157:H7, *eaeA*/*hlyA*-O157:H7, *stx*$_2$-O157:H7 and least with *stx*$_1$-O157:H7. Synergy among the virulence factors may contribute to make the animals succumb early to infection and influence the severity of infection. It is conceivable that the *E. coli* O157:H7 isolates used in this study may possess unidentified virulence factors that may contribute to their pathogenicity. A combination of bacterial and host factors may contribute to the spectrum and severity of lesions observed in *E. coli*-infected rabbits (Garcia *et al,* 2002). The *E. coli* O157:H7 isolates used in this study which were recovered from the faeces of apparently healthy cattle in abattoir, induced systemic disturbances similar to those observed in STEC infections in humans. This emphasizes the importance of strict adherence to biosecurity measures and hygienic practices during meat processing and marketing to forestall transmission of these pathogens to humans and other susceptible animals.

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