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# EXPLORING THE BEST POSSIBLE ROUTE OF INFECTION OF VEROTXIN PRODUCING *ESCHERICHIA COLI* IN DEVELOPMENT OF EXPERIMENTAL GASTROENTERITIS AMONG STREPTOMYCIN TREATED MURINE MODEL

FAHAREEN-BINTA-MOSHARRAF[1], CHOWDHURY RAFIQUL AHSAN, JAMALUN NESSA[*] AND MAHMUDA YASMIN

*Department of Microbiology, University of Dhaka, Dhaka-1000, Bangladesh*

## ABSTRACT

The prevalence of *Escherichia coli* O157:H7 induced bacteremia generate a critical problem in modern medical therapy for bacterial infections. This study sought to find out the best possible route and dose of *E. coli* 0157:H7 infection in experimental murine model by periodic stool and blood culture count of relevant bacterial strains. Streptomycin treated mouse model were used for investigating the clinical manifestation exerted by *stx1A* and *stx2A* positive *E. coli* O157:H7 with increasing doses applied through three alternative routes (oral, intramuscular and intraperitoneal). The highest titer of orally added *E. coli* 0157:H7 among five test doses started showing symptoms at the earliest time and reached moribund condition about 48 hours just before being dead. The oral way of *E.coli* O157:H7 at the dose of 100 µl suspension containing $1 \times 10^9$CFU $ml^{-1}$ was taken as the most potent concentration in producing bacterial fatality and hence was selected as the minimum lethal dose (MLD).

Key words: *E. coli* 0157:H7, Bacteremia, Blood and stool culture, Murine model

## INTRODUCTION

*Escherichia coli* O157:H7 is an enter hemorrhagic strain of the bacterium *E. coli* which causes food borne illness. Infection often leads to hemorrhagic diarrhoea, and occasionally to kidney failure, especially in young children. Enter hemorrhagic *Escherichia coli* (EHEC) is an important group of multiple food and water-borne pathogens. EHEC, including *E. coli* O157:H7 comprise a subset of Shiga toxin-producing *E. coli* (STEC). *E. coli* O157:H7 has become a worldwide threat to public health and is one of today's most troubling food-borne pathogens. *E. coli* 0157:H7 has been known to be a human pathogen for nearly 30 years (Nataro *et al*. 1998).

The centre for disease control (CDC) estimated that about 85% of *E. coli* O157:H7 infections are food borne in origin. In fact, consumption of any food or beverage that becomes contaminated by animal (especially cattle) manure can result in contracting the

[*] Corresponding author: <jamalun_nessa@hotmail.com>.
[1] Microbiology program, Department of Mathematics and Natural Sciences, BRAC University, 66 Mohakhali Dhaka 1212, Bangladesh.

disease. Foods that have been sources of contamination include ground beef, venison, sausages, dried (non-cooked) salami, unpasteurized milk and cheese, unpasteurized apple juice and cider (Khan *et al.* 2003). The CDC recently reported *E. coli* O157:H7 as the fourth most prevalent bacterial diarrhoeal pathogen after *Campylobacter* sp., *Salmonella* sp. and *Shigella* sp. (Bell *et al.* 1994).

The pathogenicity of *E. coli* 0157:H7 is associated with several virulence factors including shiga toxins or verotoxins 1 and 2 encoded by *stx1A* and *stx2A* genes, respectively. This potent cytotoxin is the major factor that might lead to many symptoms or even to death in patients infected with EHEC.

There are no currently available vaccines to prevent diseases due to EHEC, but a number of experimental approaches are being investigated in animals. Numerous *in vitro* assays and animal models have been developed in an effort to imitate various aspects of *E. coli* O157:H7 mediated disease production in humans. These models exist in two varieties: those solely focused on the effects of Stx (in the absence of bacteria) and those that explore *E. coli* O157:H7 infection. Small animals that have served as models for EHEC infection and disease include mice, rats and rabbits. Larger animals that have been so used for experimentation, even though less frequently, include chickens, pigs, cows, dogs, baboons and macaques (Bell *et al.* 1994).

This work investigated the clinical manifestation exerted by *stx1A* and *stx2A* positive *E. coli* O157:H7 in various doses applied through three alternative routes (oral, intramuscular and intraperitoneal) into healthy conventional rodents over a period to evaluate the level of *E. coli* O157:H7 in feces and blood of infected mice at various time period and compared it to their health status. The objective of the study was to identify the best possible route of *E.coli* infection in experimental rodent model by statistical analysis, hence determining the minimal lethal dose (MLD) of infection.

## MATERIALS AND METHODS

*E. coli* O157:H7 (clinical strain) obtained from ICDDR,B (International Center for Diarrheal Disease Research) was used.

Bacterial chromosomal DNA was extracted and purified according to GuSCN DNA extraction method (Khan *et al.* 2003) to detect the presence of *stx1A* and *stx 2A*. Polymerase chain reactionbased detection of *stx*1A and *stx*2A gene from extracted DNA of bacteria was performed by following the method of Brown (2006). PCR was performed in a thermal cycler with program required for each system.

Forty Swiss albino mice (*Mus musculus*) of 6 - 8 weeks age were randomly selected on the basis of experimental scheme and to verify the reproducibility of the outcome each set of investigation was performed in duplication.

To demonstrate the infective capacity of *E. coli* O157:H7 in murine model, three sets of mice (set-1, set-2, and set-3) each containing six animals were administrated with increasing dose of *E. coli* O157:H7 culture (Table 1) through three different routes of entry: oral, intramuscular and intraperitoneal. Over the course of infection the animals were observed daily for their activity level, water intake and amount of food consumption (March *et al.* 2004). All of the three groups of mice (set 1, 2 and 3) were streptomycin treated and starved from feed and water for 24 hours.

Treated mice of all the three sets (set 1, 2 and 3) challenged with different dose of *E. coli* 0157:H7 (Table 1) along with the untreated (the mice fed with 1% sucrose solution i.e. negative control) ones were kept under thorough watch for the further 144 hours to detect any visible change.

The health status of the mice along with the increasing dose of *E.coli* O157:H7 was recorded. The dose responsible for the death or maximum worst situation (moribund or terminally ill) of murine model was determined to establish the MLD.

To verify if the cause of death due to *E. coli* O157:H7 bacteremia, blood samples were collected from infected mice by cardiac puncture and then directly spread on CT-SMAC plate. Freshly voided fecal samples were collected over time along with the progression of disease symptoms and also spread on CT-SMAC plate (Merril *et al.* 1996).

Presumptive isolation of *E. coli* 0157:H7 in both fecal and blood sample was done by direct plating of serially diluted fecal and blood sample onto CT-SMAC and incubating for 18 - 24 hours at 37°C. From CT-SMAC plates, suspected colorless *E. coli* O157:H7 isolates were confirmed by cultural properties on EMB and MUG media and by standard biochemical tests.

## RESULTS AND DISCUSSION

The PCR result revealed that clinical strain of *E. coli* O157:H7 used in this research was positive for *stx1A* and *stx2A* identical to the *Shigella dysenteriae stx* positive strains (Khan *et al*. 2003).

The six mice in each group (An/Bn) were labeled through 1 to 6 (i.e. A1/B1 up to A6/B6). The mice received various doses of *E. coli* 0157:H7 according to the list (Table 1) and examined for the symptoms developed for further 144 hours. It is to be noted that the mice were treated with streptomycin antibiotic to reduce the normal facultative resident flora of intestine which would have interfered with the clinical picture.

**Table 1. Various titers of orally administered *E. coli* 0157:H7.**

| Animal model | Mouse identification number (ID No.) in each set | Dose ($CFUml^{-1}$) scheme | |
|---|---|---|---|
| Mice set-1 | A1/B1 | Dose 1 | Negative control (1% aqueous sucrose solution) |
| | A2/B2 | Dose 2 | $1 \times 10^5$ |
| | A3/B3 | Dose 3 | $1 \times 10^6$ |
| | A4/B4 | Dose 4 | $1 \times 10^7$ |
| | A5/B5 | Dose 5 | $1 \times 10^8$ |
| | A6/B6 | Dose 6 | $1 \times 10^9$ |

Mice challenged with $10^5$ and $10^6$ CFU $ml^{-1}$ of *E. coli* 0157:H7 developed no visible symptoms while examined for 144 hours since infection, whereas dose 4 ($10^7$ CFU $ml^{-1}$) and dose 5 ($10^8$ CFU $ml^{-1}$) produced specific symptoms (slight illness like lethargy and ruffeled fur) at specific times but eventually the experimental animals recovered from illness. A significant increase of both stool and blood count was observed which was related to health status. The highest titer of orally added *E. coli* 0157:H7 ($10^9$ CFU $ml^{-1}$) showed severe symptoms like ruffled fur, and hunched back, exudative accumulation around partially closed eyes tends to moribund state (Lemuel *et al*. 2004). These symptoms started to manifest at 14th hour, increased with time and had reached moribund condition near at 48 hour with an increasing stool and blood count of *E. coli* O157:H7 just before being dead. The level of *E. coli* O157:H7 found in the feces and blood (Fig. 1) samples collected from infected mice from time to time was found to be proportionately correlated to their health status.

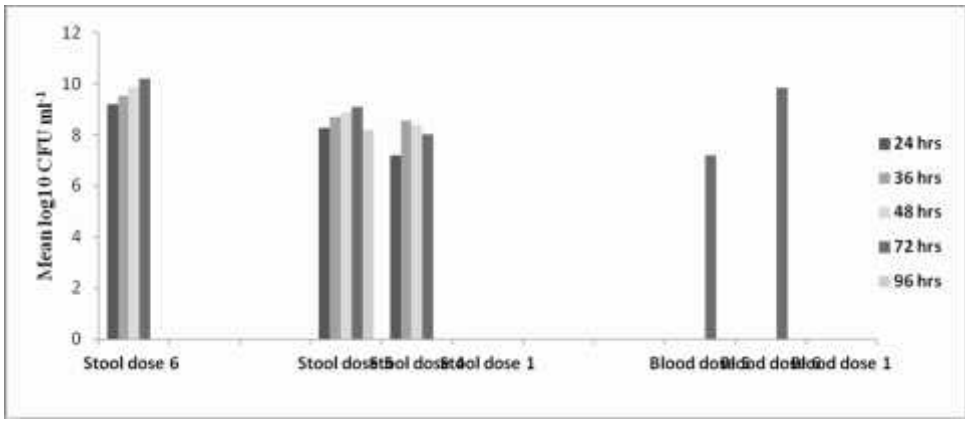


Fig. 1. Kinetics of infection monitored by fecal and blood counts for orally administrated *E. coli* 0157:H7 at different doses.

To determine the survival pattern of Swiss albino mice of set-2 and 3 (group An/Bn), increasing dose of *E. coli* 0157:H7 were injected through intramuscular and intraperitoneal route following Table 1. For both routes $10^5$ and $10^6$ CFU $ml^{-1}$ of *E. coli*

0157:H7 developed no remarkable symptoms while examined for 144 hours since infection, but in case of dose 4 ($10^7$ CFU $ml^{-1}$) intramuscularly infected mice gave no visible symptoms whereas intraperitoneally infected mice produced minor symptoms. For dose 5 ($10^8$ CFU $ml^{-1}$) and dose 6 ($10^9$ CFU $ml^{-1}$) mice produced minor symptoms like lethargy at different point of time which was similar for both the routes. Both fecal and blood count of *E. coli* 0157:H7 increased for these routes (Figs 2 and 3) but significant decrease of bacterial counts was observed following next 36 hours of infection.

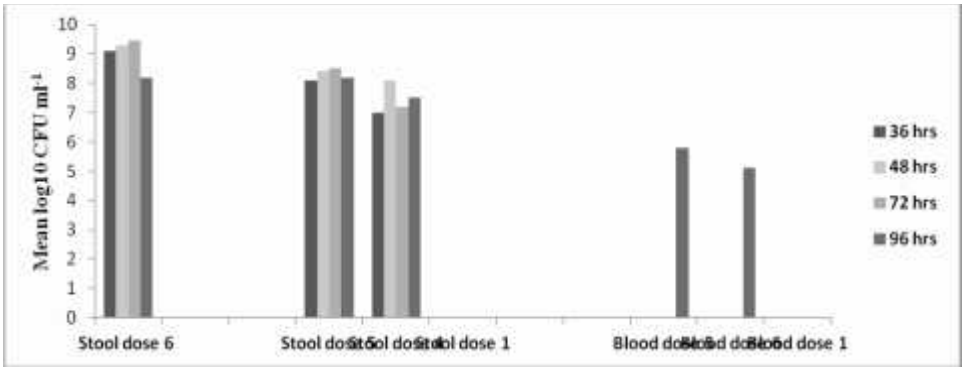


Fig. 2. The kinetics of infection monitored by fecal and blood counts for intramuscularly administrated *E. coli* 0157:H7 at different dose.

The disease severity of mice was found to be correlated with the augmented dose of *E. coli* O157:H7. All the three routes of bacterial inoculation shared this observation. However, the oral way of *E. coli* O157:H7 addition at a dose of $1 \times 10^9$CFU $ml^{-1}$ was proved to the most potent in developing bacterial pathogenesis and hence selected as the MLD.

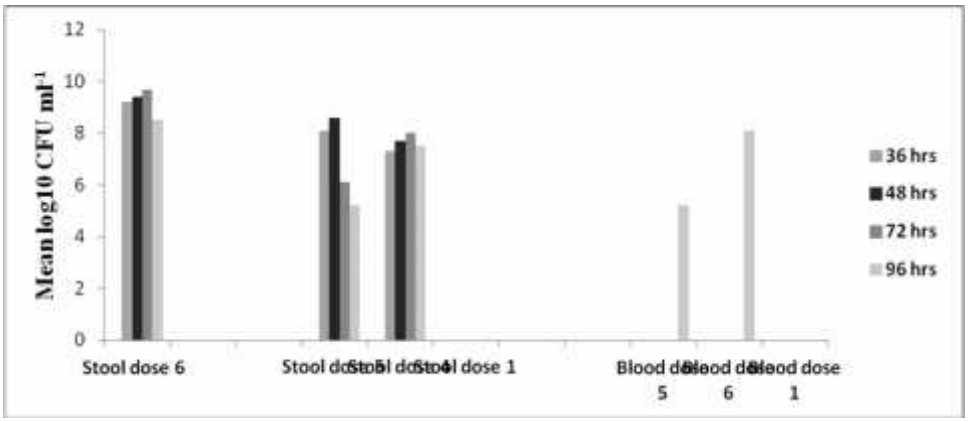


Fig. 3. Kinetics of infection monitored by fecal and blood counts for intraperitoneally administered *E. coli* 0157:H7 at different doses.

The experiment was not prolonged after 6 days to avoid the likelihood of contamination with other prevailing microorganisms, which would have interfered with the clinical picture. To verify if the cause of death was truly due to *E. coli* bacteremia, blood and stool samples were streaked on CT-SMAC agar and compared to the original

inoculum, clinical isolate of *E. coli* O157:H7. The untreated mice group developed no symptoms and acquired adequate body weight within the studied time frame.

As a result of the increased rate of HC and HUS over the last several years and the lack of therapies for treatment of HUS, further research is necessary to define mechanisms involved in the pathogenesis of *E. coli* O157:H7 and to identify potential disease prevention strategies and therapeutics. The application of animal model systems is vital to achieve these goals. Despite the fact that no one animal model recapitulates all features of *E. coli* O157:H7 infection, mouse models have been explored in this research for understanding *E. coli* O157:H7 pathogenesis better and by doing so helping to pinpoint the means by which *E. coli* O157:H7 infection and/or disease can be controlled or prevented.

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