# Shiga Like Toxin 1 (STX-1) Detection From *Escherichia coli* O157:H7 Local Isolates

I W. Suardana[1], K. J. P. Pinatih[2], and D. A. Widiasih[3]

[1]Veterinary Public Health Laboratory Faculty of Veterinary Medicine Udayana University
Bali-Indonesia
E-mail: wayan_suardana@unud.ac.id
[2]Microbiology Clinic Laboratory Faculty of Medicine Udayana University
Bali-Indonesia
E-mail: kjanuartha@yahoo.com
[3]Veterinary Public Health Laboratory Gadjah Mada University
Yogyakarta-Indonesia
E-mail: dawidiasih@yahoo.com

**Abstract**
Shiga-like toxin (Stx) otherwise known as verotoxin and verocytotoxin is a toxin produced by some strains of *Escherichia coli* particularly by strain O157: H7. This toxin is an AB5 toxin type which is known to have similarities with the toxins produced by *Shigella dysenteriae*. Stx from *E. coli* O157: H7 can be distinguished into stx 1 and stx 2. Stx1is usually associated with most outbreaks and detrimental sporadic cases of illness in humans. In this research, we observed the titer of Shiga toxin (Stx 1 / VT1) from local isolates isolated from cattle and human feces by using vero toxin *Escherichia coli*-reverse passive latex agglutination test (VTEC-RPLA) method. The results showed local isolates KL-48 (2) of human origin, SM-25 (1) of cattle feces origin and DS 21 (4) of beef origin positively produced VT1 2 units of titer, meanwhile the isolate SM 7 (1) was negative. Titer of toxin Stx1 produced from local isolates was known to be lower when compared to the control isolate ATCC 43894 with 8 units of titer.

***Key words* : *E.coli* O157:H7, VT1, local isolates, zoonoses.**

## I. INTRODUCTION

Infection through food due to Shiga toxin from *Escherichia coli* (STEC) was reported as the most prominent cause of several outbreak cases [1,2]. One characteristic of STEC is the existence of a kind of cytotoxin known as Verotoxin or Shiga like toxin (Stx) [3]. *Escherichia coli* O157:H7 is one of the main serotypes behind hemolytic uremic syndrome (HUS) cases, aside of other contributing serotypes like O26:H11, O103:H2 and O111:H- serotypes that range from as much as 20% until 25% [4]. Cattle are essential reservoir of Shiga toxin *Escherichia coli* (STEC) that includes *E.coli* O157:H7 serotype as the producer of STEC bacteria. Surveys showed that around 1-5% of the number of cattle would release *E.coli* O157:H7 in their feces with a contamination level of $< 10^2$ cfu/g until $10^5$ cfu/g [5].

Generally, *E.coli* is considered as normal flora inside animal's digestive system (cattle) that can contaminate both the meat and the surrounding environment of the slaughterhouse during the butchery process. Cattle meat that had initially been contaminated along with improper cooking process became the source of infections for a number of food poisoning cases, including the ones caused by STEC [3]. Although there are other mediums for transmitting STEC outbreak cases into humans, cattle feces are still regarded as the most common source of contamination [3]. Research development in order to detect STEC rapidly has initiated since 1987 [6]. Mohammad *et al.,* (1985 *in* Samadpour *et al.,* 2002) with his direct counting technique towards produced toxin for instance, managed to detect 28 out of 172 samples (16%) of cattle feces as STEC positive. Detection by using probe Stx-I and II DNA by Samadpour *et al* in the year of 1990, was also successful in detecting 9 out of 28 (32%) of calves in feces as STEC positive [7].

After considering the fact of how there is no information about Shiga toxin produced by local isolate *E.coli* O157:H7, particularly Shiga like toxin 1 (Stx-1), research about how to detect Stx-1 from *E.coli* O157:H7 toxin especially the one from local isolates isolated from cattle feces and meat is deemed as necessary.

## II. METHODS

### A. Isolate Preparation

As many as 5 *E.coli* O157:H7 isolates that include KL-48(2), SM-25(1), SM-7(1) and DS-21(4) along with control

isolate ATCC 43894, part of the researcher's collection, were taken from glycerol stock to be replanted in Brain Heart Infusion (BHI) gelatine medium. Grown isolates were subsequently analysed.

### *B. E.coli O157 Serotype*

Grown isolates in BHI medium were later planted at selective sorbitol MacConkey gelatine media (SMAC) (Oxoid CM 0813). The research also used *E. coli* O157:H7 ATCC 43894 as positive control. After incubated at $37^0$C for 24 hours, colonies of *E.coli* were identified as *E.coli* O157. Their characteristics were clear colonies, colourless, or negative sorbitol [8].

### *C. Agglutination Test with E.coli O157 Latex Agglutination Test*

In order to confirm that the positive colonies from SMAC media were *E.coli O157*, they were then tested again along with the positive control isolates by using *E.coli* O157 latex agglutination test (Oxoid DR620 M), with the following methods: as much as 2-3 ose of *E.coli* positive isolates from isolate stocks and presumptive *E.coli* O157 from SMAC media were put into 1 ml of physiological NaCl and heated at $100^0$C for 1-2 hours. After the heating, as much as 1 drop of isolates was reacted with 1 drop of reacted latex. Result of the positive test was indicated by the occurrence of precipitation, according to the available positive controls [8].

### *D. Shiga Toxin-1 Test with VTEC-RPLA*

Shiga toxin Stx-1 test was carried out by mixing 0.5 ml of solvent into each prepared vial kit. Next, a plate consisted of 3 columns with each column consisted of 8 pits. In each pit, 25 µl of solvent was added. Starting from pit 1, 25 µl of tested sample was added and then diluted in series until pit 7. Meanwhile, pit 8 contained only solvent. Next step, 25 µl of VT 1 test latex was added into every pit column 1. The mixture on the plate was then mixed by shaking it. Then, the plate was sealed and incubated in room temperature for 24 hours. Positive result was indicated by with the occurrence of sediment/ precipitation at the bottom of the plate.

### *E. Data Analysis*

Data obtained from the research would be presented descriptively in the form of Tables [9].

## III. Results and Discussion

### *A. Isolation Results and E.coli O157:H7 Isolate Identification*

Testing result of 5 *E.coli* O157 isolates on sorbitol MacConkey gelatine media (SMAC), showed the shape of colourless colonies and this indicated that the grown colonies did not ferment the sorbitol. Further test with Latex Agglutination Test as confirmation showed positive agglutination reaction towards O157 anti serum test. Based on the identification result, the whole isolates were then considered as *E.coli* O157:H7 and were then deemed as decent to be used in further tests.

### *B. Shiga Like Toxin-1 Production (VT-1) with VTEC-RPLA Agglutination Test*

Test result of reverse passive latex agglutination test (VTEC-RPLA) towards VT-1 from *E.coli* O157:H7 local isolates was shown on Table I.

TABLE I
RESULTS OF VT-1 *E.COLI* O157:H7 ISOLATES TEST WITH REVERSE PASSIVE LATEX AGGLUTINATION TEST METHOD (VTEC-RPLA)

| Number | Samples Codes | Isolate Origins | VT-1 | Titer VT-1* |
|---|---|---|---|---|
| 1. | ATCC 43894 | Control | +++ | 8 |
| 2. | KL-48(2) | Human | + | 2 |
| 3. | SM-25(1) | Cattle | + | 2 |
| 4. | SM-7(1) | Cattle | - | - |
| 5. | DS-21(4) | Meat | + | 2 |

Notes:
- : Agglutination didn't take place
+ : 25% agglutination from isolate volume
++ : 50% agglutination from isolate volume
+++ : 75% agglutination from isolate volume

Table 1 presented how the identification towards 4 *E.coli* O157:H7 local isolates showed that only 3 local isolates were positively identified to produce Shiga like toxin-1 (VT-1). The existence of *E.coli* O157:H7 that did not produce Stx was also reported by Avery *et al.,* (2002). Out of 24 tested isolates, 19 isolates showed positive results towards Stx2, 2 positive isolates towards Stx 1 and 2, as well as 3 negative isolates to both Stx 1 and Stx 2 [10]. Foley *et al.,* (2004) also discovered that not all *E.coli* O157:H7 isolates could produce both types of verocytotoxin. There were chances that 1 isolate would produce both (Stx 1 and Stx 2). However, there would be a number of strains that produced only 1 type of toxin which would be either Stx 1 or Stx 2 [2].

LeJeune *et al.,* (2004) mentioned that main virulent factor of *E.coli* O157:H7 was the existence of prophage that coded Shiga like toxin. Besides that, it was further explained that the bigger the production of Shiga like toxin for the bacteria, the more severe the illness would be when infected into humans [11]. The same conclusion was also mentioned in Fey *et al*., (2000) that stated how Shiga like toxin 1 and 2 were main virulent factors of *E.coli* O157:H7 and were directly correlated to the cases of hemorrhagic colitis and hemolytic uremic syndrome (HUS). This was particularly due to their interactions with endothelial cells in infected areas, including the glomerulus, artery, and kidney. Based on the research result along with the existing theoretical perspectives, it could be concluded that the 3 local isolates were most likely pathogen and would be interesting enough to be further researched [12].

## IV. Conclusion

Based on the detection result of Shiga like toxin-1 (Stx-1) with VTEC-RPLA method, it was shown that 3 out of 4 tested isolates which were KL-48(2), SM 25(1), dan DS-21(4) were proven to produce Stx-1 toxin with 2 unit titer individually. As a result, they could potentially be further researched.

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