



UNIVERSITI PUTRA MALAYSIA

***ISOLATION OF NON-0157 SHIGA TOXIN-PRODUCING ESCHERICHIA COLI
FROM CATTLE AND GOATS AND DETECTION OF THEIR VIRULENCE GENES***

TAY ZAR AYE CHO

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DETECTION OF THEIR VIRULENCE GENES**

By

TAY ZAR AYE CHO

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Veterinary Science**

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DEDICATION

This work is dedicated to my family.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfillment of the requirement for the degree of the Master of Veterinary
Science

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Shigatoxin-producing *Escherichia coli* (STEC) have emerged as the important food borne pathogen since after the first documented outbreak of O157:H7 STEC in 1982. Clinical symptoms range widely from uncomplicated diarrhea to life threatening hemolytic uremic syndrome (HUS). Shigatoxin 1 and Shigatoxin 2 (Stx1 and Stx2) play the major role in causing diseases in humans. Genes coding for attaching and effacing lesion (*eae*) and enterohemolysin production (*ehlyA*) also contribute to the potency of STEC in causing the disease. Many of the STEC outbreaks were linked to the consumption of raw or undercooked meat contaminated with STEC. Farm animals, particularly cattle, sheep and goats, serve as major reservoir hosts for human STEC transmission. Although serogroup *E. coli* O157 is reported

as a major strain in causing STEC infections, other serogroups have also been reported in many outbreaks worldwide and they are categorized as non-O157 STEC. Serogroups O8, O26, O91, O103, O111, O113, O128 and O145 are considered as the most common among non-O157 STEC in causing severe human infections. Unlike O157, which can be differentiated easily from other *E. coli* due to its late fermentation of sorbitol, there is no culture method to differentiate all non-O157 STEC strains from commensal *E. coli*. It was reported that enterohemolysin (E-Hly) production is a characteristic feature of many of the human pathogenic non-O157 STEC strains. Studies showed that E-Hly production could be seen on special blood agar made with washed sheep blood erythrocytes. The common *E. coli* isolation media such as MacConkey agar or Sorbitol MacConkey (SMAC) agar were also suggested for isolation. In Malaysia, both O157 and non-O157 STEC have been reported from hospitalized patients, market beef and meat products. However, report on non-O157 STEC in farm animals is lacking. The aims of the present study were to investigate the presence of non-O157 STEC in cattle and goats, to determine a reliable culture based isolation method for non-O157 STEC for use in routine laboratory diagnosis and to detect the virulence genes of non-O157 STEC isolates.

Recto-anal swab samples (RAMS) were collected from 144 cattle from seven farms and 87 goats from four farms during a six-month period. The samples were cultured on two agar media, namely Sorbitol MacConkey (SMAC) agar

and washed sheep blood agar (WSBA) and identified as *E. coli* using biochemical tests. Confirmed *E. coli* isolates were serogrouped against seven monovalent 'O' antisera namely, O8, O26, O91, O103, O111, O128 and O145. Colorless colonies from SMAC agar and E-Hly positive colonies from WSBA were tested for O157. Each seropositive isolate was investigated for the presence of Shigatoxin 1 and 2 (Stx1 and Stx2) using Duopath Verotoxins test kit (DV test, Merck, Germany).

Isolates from 19 of 144 (13%) cattle and seven of 87 (8%) goats were positive in serogrouping. Using DV test, non-O157 STEC were identified from 71% of cattle farms and 75% of goat farms. Non-O157 STEC serogroups O8, O103 and O128 were isolated from 6.9% of cattle and serogroups O8 and O128 from 4.6% of goats. All non-O157 STEC produced Stx1 alone except one isolate from goat that produced both Stx1 and Stx2. Ninety three percent (93%) of total non-O157 STEC were isolated from SMAC agar and 77% from WSBA. Serogroup O157 were also isolated from two cattle at one farm but failed to produce Stx1/Stx2 on DV test.

With multiplex PCR (m-PCR), 58% of seropositive isolates from cattle and 71% from goats were found to harbor *stx1* with or without other virulence genes. *stx2* was detected in 46% and 25% of non-O157 STEC isolates from cattle and goats respectively. *eaeA* was also found in 46% of cattle and 25% of goat isolates. All non-O157 STEC isolates from goats and 60% non-O157 STEC

isolates from cattle were positive for *ehlyA*. The most common patterns of virulence genes combinations were *stx₁*+ *eaeA* + *ehlyA* for cattle and *stx₁* + *ehlyA* for goats. O157 isolates were also detected by m-PCR and found to be positive for *stx₂*, *eaeA* and *ehlyA*.

The present study highlighted the occurrence of common zoonotic serogroups of non-O157 in cattle and goats in Malaysia. Serogroups included in this study were considered as most common human pathogenic strains of non-O157 STEC worldwide. Isolation rates on SMAC agar and WSBA were not statistically significant although SMAC agar gave higher isolation rate. WSBA failed to isolate E-Hly negative strains of non-O157 STEC. This could be due to not all STEC produce E-Hly and all E-Hly producing *E. coli* are not STEC. There was a strong agreement between DV test and m-PCR in detecting the presence of Shigatoxin. However, isolates that showed *stx₂* positive in m-PCR failed to produce Stx2 on DV test. It could be probably that Stx2 produced by those strains were below the detection limit of DV test or the strains failed to produce Stx2 although they possessed *stx₂*. In the present study, 93% of non-O157 STEC from cattle and goats possessed more than one virulence genes with 40% harboring *stx₂* and *eaeA* which are associated with disease severity in humans. Thus it is of public health significance.

For future studies, sampling areas should be extended to other parts of Malaysia to determine the nationwide prevalence of non-O157 STEC. The number of common non-O157 STECserogroups should also be increased as there are more than 100 different serotypes of non-O157 STEC reported worldwide.



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PENGASINGAN *ESCHERICHIA COLI* PANGHASIL SHIGA TOKSIN BUKAN O157 (STEC NON-O157) PADA LEMBU DAN KAMBING DAN PENGESANAN GEN VIRULENS

Oleh

TAY ZAR AYE CHO

November, 2011

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Escherichia coli (STEC) penghasil Shiga toksin telah muncul sebagai patogen bawaan makanan penting sejak wabak pertama STEC O157:H7 yang didokumenkan pada tahun 1982. Tanda-tanda klinikal didapati adalah pelbagai, daripada cirit-birit biasa sehingga sindrom hemolitik uremic (HUS), yang mengancam. Shiga toxin 1 dan Shiga toxin 2 (Stx1 and Stx2) memainkan peranan utama dalam menyebabkan penyakit pada manusia. Gen pengkodan untuk lesi "attaching and effacing" (*eae*) dan pengeluaran enterohemolysin (*ehlyA*) juga menyumbang kepada potensi STEC dalam menyebabkan penyakit tersebut. Kebanyakan wabak STEC telah dikaitkan dengan memakan daging mentah atau kurang dimasak yang tercemar dengan STEC. Haiwan ternakan, terutamanya lembu, bebiri dan kambing,

terdapat sebagai punca utama yang dapat mendedahkan STEC kepada manusia. Walaupun serokumpulan *E. coli* O157 dilaporkan sebagai strain utama dalam menyebabkan jangkitan STEC, serokumpulan yang lain juga telah dilaporkan dalam banyak wabak di seluruh dunia dan mereka dikategorikan sebagai STEC bukan O157. Serokumpulan O8, O26, O91, O103, O111, O113, O128 dan O145 dianggap sebagai yang paling lazim di kalangan STEC bukan O157 yang menyebabkan jangkitan manusia yang teruk. Tidak seperti O157, yang boleh dibezakan dengan mudah daripada *E. coli* yang lain kerana penapaian lewat sorbitol, tidak ada kaedah kultur untuk membezakan strain semua STEC bukan O157 daripada *E. coli* komensal. Telah dilaporkan bahawa enterohemolysin (E-Hly) merupakan ciri-khusus pada kebanyakan STEC bukan O157 strain yang patogenik untuk manusia yang mana boleh dilihat pada agar darah khusus yang dibuat dengan eritrosit darah bebiri dibasuh. Di Malaysia, kedua-dua STEC O157 dan bukan O157 telah dilaporkan daripada pesakit di hospital, daging lembu pasaran dan produk daging. Walau bagaimanapun, laporan mengenai STEC bukan O157 pada haiwan ternakan adalah kurang. Tujuan kajian ini adalah untuk menyiasat kehadiran STEC bukan O157 pada lembu dan kambing, untuk menentukan kaedah kultur pengasingan STEC bukan O157 untuk digunakan dalam diagnosis makmal rutin dan untuk mengesan gen virulens STEC bukan O157 yang diasingkan.

Sampel swab rectum-anus (RAMS) dikumpulkan daripada 144 lembu daripada tujuh ladang dan 87 kambing daripada empat ladang dalam tempoh enam bulan. Sampel dikultur atas dua media agar, iaitu agar Sorbitol MacConkey (SMAC) dan agar darah bebiri dibasuh (WSBA) dan dikenal pasti sebagai *E. coli* dengan menggunakan ujian biokimia. Isolat *E. coli* yang disahkan diuji serokumpulan dengan tujuh antisera 'O' monovalent iaitu O8, O26, O91, O103, O111, O128 dan O145. Koloni yang tidak berwarna pada agar SMAC dan koloni E-Hly positif dari pada WSBA telah diuji untuk O157. Setiap isolat seropositif disiasat untuk kehadiran Shiga toxin 1 dan 2 (Stx1 dan Stx2) dengan menggunakan kit ujian Duopath Verotoxins (DV test, Merck, Jerman).

Tiga belas (13%) isolat daripada lembu dan lapan (8%) daripada kambing didapati positif pada ujian serokumpulan. Menggunakan ujian DV, STEC bukan O157 telah dikenal pasti pada 71% lembu dan 75% kambing. Serokumpulan bukan O157 iaitu O8, O103 dan O128 telah diasingkan daripada 6.9% lembu dan serokumpulan O8 dan O128 daripada 4.6% kambing. Semua STEC bukan O157 menghasilkan Stx1 sahaja kecuali satu isolat daripada kambing yang meng dihasilkan kedua-dua Stx1 dan Stx2. Sembilan puluh tiga peratus (93%) daripada jumlah STEC bukan O157 telah diasingkan daripada agar SMAC dan 77% dari WSBA. Serokumpulan O157 juga diasingkan daripada dua lembu daripada satu ladang tetapi gagal untuk menghasilkan Stx1/Stx2 pada ujian DV.

Dengan multiplex PCR (m-PCR), 58% daripada isolat seropositif daripada lembu dan 71% dari kambing didapati menghasilkan *stx*₁ dengan atau tanpa gen virulens yang lain. *stx*₂ telah dikesan pada 46% dan 25% STEC bukan O157 yang masing-masing diasingkan daripada lembu dan kambing. *eaeA* juga di dapati pada 46% isolat lembu dan 25% isolat kambing. Semua STEC bukan O157 yang diasingkan daripada kambing dan 60% STEC bukan O157 yang diasingkan daripada lembu adalah positif untuk *ehlyA*. Corak yang paling biasa bagi kombinasi gen virulens adalah *stx*₁ + *eaeA* + *ehlyA* untuk lembu dan *stx*₁ + *ehlyA* untuk kambing. Isolat O157 juga dikesan oleh m-PCR dan didapati positif untuk *stx*₂, *eaeA* dan *ehlyA*.

Kajian ini memperlihatkan kehadiran serokumpulan STEC bukan O157 yang lazim dan bersifat zoonosis pada lembu dan kambing di Malaysia. Serokumpulan yang dimasukkan dalam kajian ini dianggap sebagai strain patogen manusia yang paling lazim diantara STEC bukan O157 di seluruh dunia. Kadar pengasingan pada agar SMAC dan WSBA tidak beherti secara statistik walaupun SMAC agar memberikan kadar pengasingan yang lebih tinggi. WSBA gagal untuk mengasingkan strain STEC bukan O157 yang E-Hly yang negatif. Ini mungkin kerana tidak semua STEC menghasilkan E-Hly dan semua *E. coli* yang menghasilkan E-Hly adalah bukan STEC. Terdapat perkaitan yang kuat antara ujian DV dan m-PCR untuk mengesan kehadiran Shiga toxin. Walau bagaimanapun, isolat yang menunjukkan *stx*₂ positif pada m-PCR gagal untuk menghasilkan Stx₂ pada ujian DV. Ini

mungkin *Stx2* yang dihasilkan oleh strain tersebut adalah di bawah had pengesanan ujian DV atau strain gagal untuk menghasilkan *Stx2* walaupun mereka memiliki *stx2*. Dalam kajian ini, 93% STEC bukan O157 pada lembu dan kambing mempunyai lebih daripada satu gen virulens dengan 40% mempunya *stx2* dan *eaeA* yang dikaitkan dengan penyakit tahap teruk pada manusia, Oleh itu, penemuan ini mempunyai kepentingan kesihatan awam.

Bagi pengajian akan datang, kawasan persampelan perlu diperluaskan lagi kepada bahagian-bahagian lain di Malaysia untuk menentukan kekerapan STEC bukan O157 di seluruh negara. Bilangan serokumpulan STEC bukan O157 lazim juga perlu ditambah kerana terdapat lebih daripada 100 serotip STEC bukan O157 yang dilaporkan di seluruh dunia.

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I certify that an Examination Committee has met on 11 November, 2011 to conduct the final examination of Tay Zar Aye Cho on his degree thesis entitled "Isolation of non-O157 Shiga toxin-producing *Escherichia coli* from cattle and goats and detection of virulence genes" in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the degree of Master of Veterinary Science.

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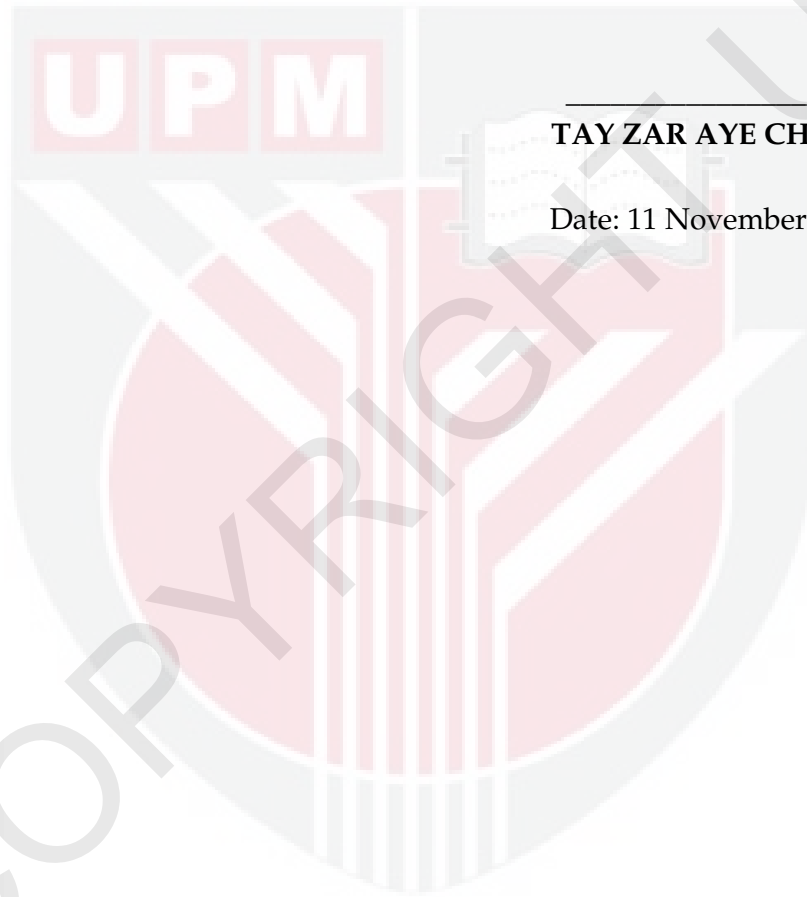
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



TAY ZAR AYE CHO

Date: 11 November 2011

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