HIGH LEVEL OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* AND OCCURRENCE OF *STX*-NEGATIVE *E. COLI* O157 FROM RAW MEATS: CHARACTERIZATION OF VIRULENCE PROFILE AND GENETIC RELATEDNESS

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Abstract. Raw meats, especially beef, are particularly prone to Shiga toxin-producing *Escherichia coli* (STEC)/enterohemorrhagic *E. coli* (EHEC) contamination. However, data regarding their quantity in raw meats are seldom reported in Thailand. Among four common meat types, beef possessed highest value of stx_1-producing *E. coli* (STEC1) contamination in February 2015 [> 1,100 most probable number (MPN)/g] and stx_2-producing *E. coli* (STEC2) highest MPN/g (460) in March of the same year. STEC2 was found, for the first time, in shrimp samples in March and April, 2015 with MPN/g value of 6.6 and 9.3, respectively. EHEC at 3 MPN/g was detected in only one (2%) beef sample. Even though *stx*-negative *E. coli* O157 from beef has rarely been reported in Thailand, isolation of *E. coli* O157 using immunomagnetic separation method revealed that four strains (PSVX-1, PSVX-2, PSVX-3, and PSVX-4) from three (8%) beef samples were shown to be *stx*-negative *E. coli* O157. These strains were members of phylogenetic group A and were multi-drug resistant. Genetic relatedness as determined by polytrinucleotide $(GTG)_5$-PCR and BOX-PCR showed identical DNA profiles of PSVX-2 and PSVX-4, which by BOX-PCR were 90% to a clinical isolate, O157 strain PSU120, from Hat-Yai Hospital in 2014. The presence of these environment *stx*-negative *E. coli* O157 strains with the ability to acquire additional virulence properties could pose a potential public health problem particularly in this region of Thailand.

Keywords: *Escherichia coli*, O157, beef, EHEC, immunomagnetic separation, STEC, Thailand

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) is a food-borne pathogen frequently associated with food poisoning outbreaks worldwide (Dundas *et al*, 2001; Rangel *et al*, 2005). Over 40 serotypes of STEC are reported to be involved with

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severe forms of the disease (Paton and Paton, 1996), including hemorrhagic colitis (HC) (Riley *et al*, 1983) and hemolytic uremic syndrome (HUS) (Karmali *et al*, 1983). STEC is capable of producing Shiga toxin 1 (Stx1), Shiga toxin 2 (Stx2) or both and these toxins are the principal virulence factors. Enterohemorrhagic *E. coli* (EHEC) is defined by possession of *stx* and *eae* (encoding intimin). Pathogenesis by Stx results from toxin binding to its receptor globotriaosylceramide (Gb3) (de Sablet *et al*, 2008) and subsequent internalization of the toxin into target cell, whereupon Stx causes the elimination of an adenine residue from 28S ribosomal RNA, resulting in inhibition of protein biosynthesis and cell death (Nataro and Kaper, 1998).

STEC serotype O157:H7 is the most important serotype causing food poisoning outbreaks worldwide (Themphachana *et al*, 2014). Massive outbreaks of *E. coli* O157:H7 infection in elementary schools in many districts of Sakai city, Japan in 1996 resulted in 8,355 children exhibiting one or more symptoms and 398 were hospitalized (Michino *et al*, 1999). Contaminated school lunch containing uncooked white radish sprouts was concluded to be the cause of the outbreak. A recent study in 2012 identified a novel *E. coli* O157:H7 clone, named Xuzhou21, closely related to the strain that caused the outbreak in Sakai city in 1996, responsible for an HUS outbreak in China leading to 195 people hospitalized and 177 deaths (Xiong *et al*, 2012).

Many types of food vehicles were reported to carry *E. coli* O157:H7 to humans, *eg*, raw beef, raw milk, unpasteurized apple cider, and salad (Michino *et al*, 1999; Hilborn *et al*, 2000; Guh *et al*, 2010), with raw beef considered to be the most important vehicle because cattle is the crucial reservoir host of STEC. Contamination of STEC O157:H7 from cattle intestines to meat and meat products is the most likely route of contamination occurring during the slaughtering process.

In Thailand, although the presence of STEC is important, only sporadic STEC cases were reported (Bettelheim *et al*, 1990; Leelaporn *et al*, 2003). Recently, an infection caused by a *stx*-negative *E. coli* O157 was reported from Thailand (Themphachana *et al*, 2014). This clearly suggests that *stx*-negative *E. coli* O157 is important and possesses the capability of causing illness.

In this study, not only quantification of STEC/EHEC from meats, we also report the presence of *stx*-negative *E. coli* O157 strains from beef marketed in southern Thailand. In addition, virulence and genetic relatedness to a strain from clinical source were investigated. This study provides information on the distribution and potential virulence of a previously overlooked group of bacteria that can pose a health risk in this region of the country.

MATERIALS AND METHODS

Sample collection

A total of 84 meat samples were purchased from various fresh food markets in Hat Yai City, Songkhla Province, Thailand during January to September, 2015. Forty-eight meat samples (pork, beef and chicken, $n = 12$ each) were used in STEC/EHEC quantification assay using most probable number-polymerase chain reaction (MPN-PCR) twice a month for a period of six months (January to June) and 36 beef samples were subjected to STEC/EHEC O157 isolation using an immunomagnetic separation (IMS) technique. All samples were processed within 2 hours after purchase.

MPN-PCR quantification of EHEC/STEC from raw meat

MPN-PCR was performed as described previously (Phetkhajorn *et al*, 2014). In brief, 25 g of meat were homogenized with 225 ml of tryptic soy broth (TSB) (Becton Dickinson, Sparks, MD). The supernatant was used in a three-tube MPN-PCR: 10-, 100- and 1,000-fold dilutions. One ml aliquot from each dilution was transferred into triplicate MPN tubes, and then incubated at 37ºC for 24 hours. One ml aliquot of each culture was subjected to PCR template preparation using boiling method. A 10-fold diluted (in sterile deionized water) aliquot of the boiled supernatant was used as PCR template. Oligonucleotide primers for amplification of stx_1, stx_2 and *eae*, and their annealing temperatures are shown in Table 1. PCR amplification was carried out in 25-µl reaction mixture comprising 1X Go*Taq* Flexi Green buffer, 3.0 mM $MgCl_2$, 0.1 mM dNTPs, 0.4 µM each primer pair, 0.5 U Go*Taq* DNA polymerase (Promega, Madison, WI) and 2 µl of DNA template. After 35 amplification cycles, amplicons were analyzed by 1.0% agarose gel-electrophoresis (Invitrogen, Carlsbad, CA), stained with ethidium bromide and documented using WSE-5200 Printpraph 2M gel imaging system (ATTO, Tokyo, Japan). EHEC strain EDL933 was used as positive strain for amplification of stx_1, stx_2, and *eae*. MPN values were computed using MPN calculator (VB6 version software).

IMS of *E. coli* O157 from raw meat

E. coli O157 was isolated from raw beef samples using an IMS method as previously described (Sukhumungoon *et al*, 2011). In short, 50 g of beef was mixed with 450 ml of TSB in a sterile plastic bag, homogenized for 1 minute in a Stomacher CIR-400 instrument (Seward, London, UK) and supernatant was incubated without shaking at 37ºC for 6 hours. Then 1 ml aliquot was mixed with O157-coated magnetic beads (Invitrogen, Carlsbad, CA) and incubated at ambient temperature with occasional gentle inversion for 30 minutes. Magnetic beads were washed with phosphate-buffered saline, pH 7.4 (PBS) and isolated following the manufacturer's instructions. Magnetic beads were re-suspended in 100 µl of PBS and streaked on eosin methylene blue (EMB) agar (Becton Dickinson, Sparks, MD) and on CHROMagarO157 plate (CHROMagar, Paris, France). Ten green metallic sheen colonies on EMB agar and 10 mauve colonies on CHROMagarO157 plate for each sample were selected for further identification of *E. coli* O157.

***E. coli* O157 serotyping**

Somatic O-antigen specific for *E. coli* O157 was identified by PCR as described by Sirikaew *et al* (2015). Amplification was performed in a 25-µl reaction mixture consisting of PCR components as described above except for specific oligonucleotide primers, O157-F and O157-R (Table 1). Amplicon was separated by 1.0% agarose gel-electrophoresis and documented as described above. In addition, confirmation of O157 serotype was performed by an agglutination assay using O157 antibodies (Set 1) (Denka Seiken, Tokyo, Japan). H7 (flagella)-antigen was investigated by the detection of *fliC*H7 by PCR as described above (Table 1).

Virulence genes investigation

STEC virulence genes, stx_1, stx_2, and *eae*, including *escV*, marker for type III secretion system (T3SS) located in locus of enterocyte effacement (LEE), together with detection of adherence-associated genes, *csgA lpf*, *agn43*, and *fimH*, were investigated by PCR (Table 1) and analyzed

(using 1.0% agarose gel-electrophoresis) as described above.

PCR amplification of insertion locus

PCR was performed for amplification of 5 frequently reported insertion loci using specific primers (Table 1) and analyzed (using 1.0% agarose gel-electrophoresis) as described above. DNA template preparation was prepared as described above. If PCR exhibited no amplicon, it was interpreted that a prophage occupied that particular locus (Serra-Moreno *et al*, 2007).

PCR-based phylogenetic group analysis

E. coli O157 phylogenetic group investigation was performed by PCR amplification of three specific DNA regions, namely, *chuA*, *yjaA*, and TspE4.C2 fragment (Clermont *et al*, 2000) employing primers listed in Table 1. Thermocycling conditions were as follows: 95°C for 3 minutes; followed by 35 cycles of 94°C for 50 seconds, 54°C for 50 seconds, and 72°C for 30 seconds; with a final step of 72°C for 5 minutes. Amplicons were analyzed as described above.

Antimicrobial susceptibility

Antimicrobial susceptibility of *E. coli* O157:H7 was assessed using a disk diffusion method (CLSI, 2014) with 10 antimicrobial agents: amikacin (30 μg), cephalothin (30 μg), ciprofloxacin (5 μg), cetriaxone (30 μg), fosfomycin (200 μg), gentamicin (10 μg), imipenem (10 μg), streptomycin (10 μg), tetracycline (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg) (Oxoid, Hamshire, UK).

DNA profiling

Typing of *E. coli* O157 strains was performed using two methods, namely, polytrinucleotide $(GTG)_5$-PCR and BOX-PCR (Sirikaew *et al*, 2015; Sukkua *et al*, 2015). Both methods were carried out in a 25-μl reaction mixture composed of 0.2 μM $(GTG)_5$ or BOXA1R primer (Table 1), 0.2 mM dNTPs, 1X Go*Taq* DNA polymerase buffer, 3.0 mM $MgCl_2$, 1.25 U Go*Taq* DNA polymerase (Promega, Madison, WI) and 50 ng of DNA template prepared using a glass fiber matrix spin column (Geneaid, New Taipei City, Taiwan). Thermocycling conditions were as follows: 95°C for 3 minutes; followed by 30 cycles of 94°C for 3 seconds, 92°C for 30 seconds, 40°C for 1 minute (for $(GTG)_5$-PCR) or 50°C for 1 minute (for BOX-PCR), and 65°C for 8 minutes. Amplicons were analyzed as described above. Dendrograms were constructed using unweighted pair-group method of arithmetic average (UPGMA) (BioProfile software, Waltham, MA). A *stx*-negative *E. coli* O157 strain PSU120 isolated from a diarrheal patient in a hospital in southern Thailand (Themphachana *et al*, 2014) and a *stx*-negative *E. coli* O157 strain PSU132 isolated in 2013 from beef in the same region of southern Thailand were also used as controls.

RESULTS

MPN-PCR quantification of EHEC and STEC from raw meat samples

In the course of 6 months of MPN-PCR quantification of EHEC and STEC from four common meat types (chicken, beef, pork, and shrimp), beef possessed the highest value of stx_1-producing *E. coli* (STEC1) contamination in February with > 1,100 MPN/g (Table 2). A slightly lower STEC contamination rate was found in March with MPN/g value of 1,100. In addition, in March, stx_2-producing *E. coli* (STEC2) had the highest MPN value of 460 MPN/g. Interestingly, MPN value of 6.6 and 9.3 was determined in shrimp in March and April, respectively (Table 2). Only one beef sample was shown to carry

Table 1
Oligonucleotide primers used in the study.

Primer name	Sequence (5′ to 3′)	Gene	Annealing temperature	Amplicon size (bp)	Reference
EVT-1	CAACACTGGATGATCTCAG	stx_1	55	350	Sukhumungoon *et al*, 2011
EVT-2	CCCCCTCAACTGCTAATA				
EVS-1	ATCAGTCGTCACTCACTGGT	stx_2	50	404	Sukhumungoon *et al*, 2011
EVS-2	CCAGTTATCTGACATTCTG				
AE-19	CAGGTCGTCGTGTCTGCTAAA	*eae*	55	1,087	Gannon *et al*, 1993
AE-20	TCAGCGTGGTTGGATCAACCT				
O157-F	CGTGATGATGTTGAGTTG	*rfb*O157	50	400	Maurer *et al*, 1999
O157-R	AGATTGGTTGGCATTACTG				
FlicH7-F	GCGCTGTCGAGTTCTATCGAGC	*fli*CH7	57	625	Gannon *et al*, 1997
FlicH7-R	CAACGGTGACTTTATCGCCATTCC				
escV-F	GGCTCTCTTCTTCTTTATGGCTG	*escV*	45	534	Müller *et al*, 2006
escV-R	CCTTTTACAAACTTCATCGCC				
lpfA1-F	GGTCGTTTTTGCCTTAACCGC	*lpf*	55	≈500	Torres *et al*, 2004
lpfA1-R	AGGTTGAAATCGACCTGCGC				
csgA-Fw	TGGTAACAGCGCCACTCTTG	*csgA*	55	155	Lloyd *et al*, 2012
csgA-Rv	GACGGTGGAATTAGATGCAGTC				
1-Kpn	GAACCTGTCGGTACCGATGCCCTCCC	*agn43*	67	≈900	Danese *et al*, 2000
2-Bam	CGGGATCCGTTGCCACTGTACCGGGCTTGACGACC				
fimH-F	TGCAGAACGGATAAGCCGTGG	*fimH*	55	508	Johnson and Stell, 2000
fimH-R	GCAGTCACCTGCCCTCCGGTA				
Z2577-F	AACCCCATTGATGCTCAGGCTC	Z2577	53	909	Koch *et al*, 2003
Z2577-R	TTCCCATTTTACACTTCCTCCG				
sbcB1	CATGATCTGTTGCCACTCG	*sbcB*	50	1,800	Ohnishi *et al*, 2002
sbcB2	AGGTCTGTCCGTTTCCACTC				
EC10	GCCAGCGCCGAGCAGCACAATA	*yecE*	60	400	DeGreve *et al*, 2002
EC11	GGCAGGCAGTTGCAGCCAGTAT				
*wrb*A1	ATGGCTAAAGTTCTGGTG	*wrbA*	47	600	Toth *et al*, 2003
*wrb*A2	CTCCTGTTGAAGATTAGC				
Primer A	AAGTGGCGTTGCTTTGTGAT	*yehV*	50	340	Shaikh and Tarr, 2003
Primer B	AACAGATGTGTGGTGAGTGTCTG				

chuA1	GACGAACCAACGGTCAGGAT	*chuA*	54	279	Clermont *et al*, 2000
chuA2	TGCCGCCAGTACCAAAGACA				
yjaA1	TGAAGTGTCAGGAGACGCTG	*yjaA*	54	211	Clermont *et al*, 2000
yjaA2	ATGGAGAATGCGTTCCTCAAC				
TspE4.C2-1	GAGTAATGTCGGGGCATTCA	TspE4.C2	54	152	Clermont *et al*, 2000
TspE4.C2-2	CGCGCCAACAAAGTATTACG				
$(GTG)_5$	GTGGTGGTGGTGGTG	-	40	Variable	Versalovic *et al*, 1991
BOXA1R	CTACGGCAAGGCGACGCTGACG	-	50	Variable	Mohapatra *et al*, 2007

Table 2
Most probable number/g of Shiga toxin-producing and enterohemorrhagic *E. coli* in meats from southern Thailand, January-June, 2015.

Meat type (Total, $n = 48$)	Pathotype	Month											
		January		February		March		April		May		June	
		Min[a]	Max[a]	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Pork	STEC1	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3
	STEC2	< 3	< 3	< 3	3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3
	EHEC	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3
Chicken	STEC1	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3
	STEC2	< 3	< 3	< 3	3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3
	EHEC	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3
Beef	STEC1	< 3	31	< 3	> 1,100	< 3	1,100	< 3	16	< 3	< 3	< 3	31
	STEC2	< 3	62	< 3	41	< 3	460	< 3	20	< 3	< 3	< 3	31
	EHEC	< 3	< 3	< 3	< 3	< 3	< 3	< 3	3	< 3	< 3	< 3	< 3
Shrimp	STEC1	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3
	STEC2	< 3	< 3	< 3	3	< 3	7	< 3	9	< 3	< 3	< 3	< 3
	EHEC	< 3	< 3	< 3	< 3	< 3	< 3	< 3	< 3*	< 3	< 3	< 3	< 3

[a]Minimum and maximum most probable number values of each pathotype in each month. *Only *eae* is present and categorized as *eae*-carrying *E. coli*.

Table 3
Characteristics of four *E. coli* O157 strains from beef using immunomagnetic separation method.

Number of samples	Strain	Date of isolation	Virulence gene							*rfb*O157	*flic*H7	Phylogenetic group	Antibiogram pattern[a]
			stx1	*stx2*	*eae*	*escV*	*lpf*	*csgA*	*agn43*	*fimH*				
28	PSVX-1	29 July 2015	-	-	-	-	-	-	-	+	+	-	A	KF, S, TE
29	PSVX-2	29 July 2015	-	-	-	-	-	-	-	-	+	-	A	FOS, KF, S, SXT
	PSVX-3	29 July 2015	-	-	-	-	-	-	-	+	+	-	A	FOS, KF, S, TE
35	PSVX-4	2 September 2015	-	-	-	-	-	-	-	-	+	-	A	FOS, KF, S, TE

[a]FOS, fosfomycin; KF, cephalothin; S, streptomycin; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline.

EHEC of 3 MPN/g in April. Chicken was found to carry only 3 MPN/g of STEC2 in February and < 3 MPN/g for STEC1 and EHEC (Table 2).

IMS of EHEC and STEC from raw meat samples

In view of the above results, beef was chosen to be investigated for the presence of STEC/EHEC O157:H7 by IMS method. A total of 494 isolates from 36 beef samples were selected from both EMB agar and CHROMagar O157 plates for O157 serotype identification. From EMB agar four strains (PSVX-1, PSVX-2, PSVX-3, and PSVX-4) (8%) from 3 beef samples carried O157 serotype. These four strains possessed motility but did not carry *flic*H7 and were designated as H7$^-$.

Virulence genes investigation

All 4 *E. coli* O157 in this study did not carry stx_1, stx_2 and *eae* (Table 3) and were classified as *stx*-negative *E. coli* O157. In addition they did not possess *escV*, encoding a protein component in type III secretion system and lacked adherence-associated genes, *csgA*, *lpf* and *agn43,* but two strains contained *fimH,* encoding type I fimbrial component, responsible for adherence and autoaggregation phenomena.

PCR amplification of insertion loci

Intactness of *yecE*, Z2577, *wrbA*, and *yehV* was observed in all four *E. coli* O157 strains, suggesting that they have no prophages inserted in these loci, but *sbcB* could not be amplified (Fig 1).

Antimicrobial susceptibility

The four *E. coli* O157 strains were multi-drug resistant, with resistance to ≥ 3 classes of antimicrobial agents (Table 3). PSVX-1 was resistant to cephalothin, streptomycin, and tetracycline; PSVX-2 to cephalothin, fosfomycin, streptomycin, and trimethoprim/sulfamethoxazole; and

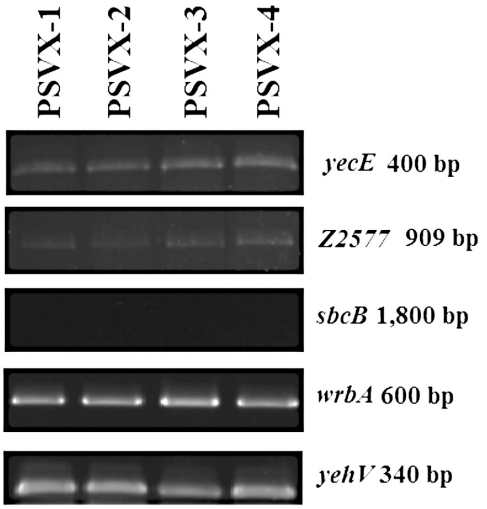

Fig 1–Insertion locus occupancy in stx_2-negative *E. coli* O157. Five bacteriophage insertion loci were PCR amplified and analyzed as described in Materials and Methods. Successful amplification indicates intactness of the target locus while the locus inserted by bacteriophage becomes too large to be amplified.

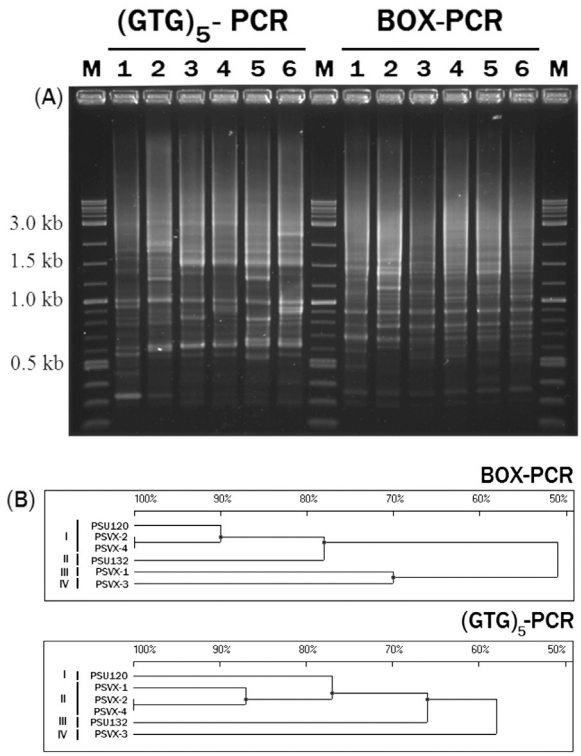

Fig 2–Genetic relatedness of four *stx*-negative *E. coli* O157 strains isolated from beef, southern Thailand. (A) DNA profiling generated by polytrinucleotide $(GTG)_5$-PCR and BOX-PCR. Amplicons were separated by 1.5% agarose gel-electrophoresis at 100V for 1 hour. Lane M, 2 log DNA ladder (NEB, USA); lane 1, PSU120; lane 2, PSU132; lane 3, PSVX-1; lane 4, PSVX-2; lane 5, PSVX-3; lane 6, PSVX-4. (B) Dendrogram generated by UPGMA method using Bioprofile software. Similarity of strains is analyzed at 80%.

PSVX-3 and PSVX-4 to cephalothin, fosfomycin, streptomycin, and tetracycline.

DNA profiling

Both $(GTG)_5$-PCR and BOX-PCR generated similar DNA profiles for the four *E. coli* O157 strains. At 80% similarity, both methods exhibited 4 distinguishable clusters (cluster I to IV) with PSVX-3 being most distantly related at 58% and 51% for $(GTG)_5$-PCR and BOX-PCR, respectively (cluster IV) (Fig 2B). PSVX-2 and PSVX-4 isolated from different samples and at different time intervals, exhibited identical DNA profile by both methods (Fig 2). Interestingly, PSVX-2 and PSVX-4 showed by BOX-PCR of 90% similarity to a clinical *E. coli* O157 strain, PSU120, isolated from Hat-Yai Hospital in 2014.

DISCUSSION

Preliminary quantification of STEC and EHEC in raw meat samples conducted in 2013 revealed very low levels of STEC1 (maximum MPN/g of 16) and STEC2 (maximum MPN/g of 27) contamination in beef (Pewleang *et al*, 2014).

Alarmingly, this study found that STEC contamination of beef has increased > 68 folds (February, 2015), the highest ever recorded in raw meat from Thailand and may be capable of reaching human colon. This suggests the possibility that at a certain time of the year, STEC may be able to contaminate beef at a high rate. In general, very low levels of STEC were detected in all four meat samples (chicken, beef, pork, and shrimp) during the 6-month period of the survey.

Regarding to the data in the same experimental area, Hat Yai City, detection rate (8%) of *E. coli* O157 from beef in this study was comparable with that (4%) in one study conducted by Vuddhakul *et al* (2000) but lower than that (30%) of a more recent report (Sukhumungoon *et al*, 2011). The difference in bacterial prevalence in meats may result from various factors, namely, season, slaughtering processes, feed composition, and/or ability of bacteria to persist in cattle bowel and in the environment (Fairbrother and Nadeau, 2006). Recently, increase of STEC shedding frequency was found to be involved with stress or negative energy balance-associated lactation, resulting in higher beef contamination (Venegas-Vargas *et al*, 2016). We suggest that *E. coli* O157 is indeed circulating in the environment and many *E. coli* O157 clones exist in southern Thailand, although at lower levels.

Detection of STEC in seafood is seldom reported because aquacultures are not specific vehicles for STEC. Gourmelon *et al* (2006) reported STEC and *E. coli* O157 in mussels and oysters in France during July 2002 to August 2004 at 27.8%. On the other hand, EL-Alfy *et al* (2013) did not find STEC in oysters, squids and bivalves in Egypt. We found STEC2 in raw shrimps from fresh markets in Hat-Yai city, southern Thailand at low levels in March and April, 2015. It remains to be determined whether STEC directly contaminated shrimp at pre- or post-harvest.

Although genotypic characteristics of the principal virulence genes of O157 strains from beef in this area of Thailand in the last two decades represented stx_1^-, stx_2^+, eae^+ profile, a clinical O157 strain from a diarrheal patient in Thailand, PSU120, exhibited stx_1^-, stx_2^-, eae^- profile (Themphachana *et al*, 2014). PSU120 strain is the first *stx*-negative *E. coli* O157 detected from a diarrheal patient in Thailand and is considered as pathogenic because it was detected as the sole bacterial species in stool sample and possesses type III secretion system component gene, *escV*. It was noted that this *E. coli* O157 clone may exist in the environment. This was borne out by the isolation from raw beef of four *stx*-negative O157 strains. Their possible virulent characteristics were manifested in their antimicrobial resistance profiles. In addition, these strains were genetically related to PSU120 as demonstrated by $(GTG)_5$-PCR and BOX-PCR.

In summary, this study shows a high level of STEC contamination in raw beef (at a certain time in the year) from markets in southern Thailand. Due to the scarcity of reports of STEC quantification in Thailand, the present study is important in revealing previously undiscovered STEC contamination in commonly consumed meat types. This is the first report of STEC contamination in raw shrimps in Thailand, albeit at low levels. The *stx*-negative *E. coli* O157 strains present in this region of Thailand showed high genetic relatedness to a clinical strain. Owing to their antimicrobial-resistant profile and presence of adherence-associated genes, including their potential for horizontal gene transfer as indicated by intactness of a number of insertion loci, these *stx*-

negative *E. coli* O157 could acquire additional virulence properties and eventually become a public health problem.

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