

การตรวจ enterotoxigenic *Escherichia coli* ที่ก่อโรคอุจจาระร่วงในลูกสุกรด้วยวิธี  
polymerase chain reaction  
Detection of Enterotoxigenic *Escherichia coli* in Diarrheal Piglets by Polymerase  
Chain Reaction

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### บทคัดย่อ

คณะผู้วิจัยได้ทำการตรวจกลุ่มยีนบ่งชี้ก่อความรุนแรง ได้แก่ *lt* (heat labile), *stp* (heat stable in porcine) และ *sth* (heat stable in human) ของ enterotoxigenic *Escherichia coli* (ETEC) ด้วยวิธี polymerase chain reaction (PCR) โดยเก็บตัวอย่างอุจจาระจากลูกสุกรที่แสดงอาการอุจจาระร่วงจำนวน 100 ตัวอย่าง ผลการศึกษาพบ *E. coli* จำนวน 98 สายพันธุ์ที่แยกได้จาก 100 ตัวอย่าง แบ่งได้เป็น 38 สายพันธุ์ที่พบในลูกสุกรระยะดูดนมและ 60 สายพันธุ์จากลูกสุกรระยะหย่านม พบเป็น ETEC ร้อยละ 11.2 พบยีนก่อโรคชนิด *stp* อย่างเดียวร้อยละ 13.4 เฉพาะในลูกสุกรระยะหย่านม ขณะที่ยีน *lt* อย่างเดียวร้อยละ 2.6 และร้อยละ 3.3 ที่พบได้ทั้งในลูกสุกรระยะดูดนมและหย่านมตามลำดับ ข้อมูลจากการศึกษานี้จะเป็นประโยชน์อย่างยิ่งสำหรับเฝ้าระวังการระบาดของโรคอุจจาระร่วงของลูกสุกรในฟาร์มต่อไป

### ABSTRACT

We detected virulence factors namely *lt* (heat labile), *stp* (heat stable in porcine) and *sth* (heat stable in human) of the enterotoxigenic *Escherichia coli* (ETEC) by polymerase chain reaction (PCR). A total of 100 rectal swabs from diarrheal piglets were collected. As a result, 98 *E. coli* strains, 38 strains from suckling and 60 strains from weaning piglets were isolated from these samples. Furthermore, 11.2% of total *E. coli* strains were ETEC. The ETEC strains harboring *stp* (13.4%) were found only in weaning piglets, whereas the strains harboring *lt* were found in both in suckling (2.6%) and weaning (3.3%) piglets, respectively. The findings from this study are necessary for surveillance of diarrheal disease outbreaks in piglets in swine farms.

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**Keywords:** enterotoxigenic *Escherichia coli*, piglet, PCR

## INTRODUCTION

*Escherichia coli* (*E. coli*) are known to be harmless and play a role as a part of the normal flora in gastrointestinal tract. Generally, *E. coli* are a common indicator of fecal contamination in water and food samples. Some strains are documented as pathogenic *E. coli* that can cause diarrhea in animal and human (Cortes *et al.*, 2002). Diarrheagenic *E. coli* are also causing foodborne diseases in human worldwide. Enterotoxigenic *E. coli* (ETEC) is the major cause of diarrhea among travelers, as well as in infants in many developing countries with poor sanitation (Porat *et al.*, 1998).

Moreover, newborn and young domestic animals (calves, lambs, and pig) are receptive to ETEC-induced diarrhea. ETEC strains are recognized as causes of illness and death in neonatal and weaning pigs. ETEC has been a predominant cause of colibacillosis in piglets with diarrhea during suckling and weaning periods (Nagy and Fekete, 1999; Blanco *et al.*, 2006). To date, ETEC is still a causative agent causing diarrheal disease outbreaks in piglets. These diseases cause a large impact on national economic loss. ETEC carry two virulence factors i.e. fimbriae and enterotoxins specifically associated with certain host species. The common enterotoxins are heat-labile (*lt*), heat-stable in porcine (*stp*), heat-stable in human (*sth*) (Nataro and Kaper, 1998).

Recently, Polymerase Chain Reaction (PCR) has been applied for detecting the presence of a specific pathogenic organism and able to diagnose for diseases. PCR assay has been applied to detect the gene encoding virulence factors of ETEC isolated from piglets (Zajacova *et al.*, 2011). The aim of this study, virulence factors of ETEC were determined in diarrheal piglets during suckling and weaning periods in swine farms by PCR assay.

## MATERIALS AND METHODS

### 1. Sample Collection

A total of 100 rectal swab samples were collected from diarrheal piglets (40 samples from suckling piglets and 60 samples from weaning piglets) in Kanchanaburi and Ratchaburi provinces in 2012. The swabs were immediately transferred to the laboratory and processed within 24 hour.

### 2. Bacterial isolation and identification

The swab samples were directly cultured onto MacConkey agar. The suspected pink colonies were selected and subsequently identified for *E. coli* using TSI, LIM, and IMViC tests (Koneman *et al.*, 2006).

### 3. Detection of virulence genes of ETEC by single PCR

#### 3.1 Reference strains

Two reference strains of ETEC were used; ETEC RIMD0509275 (*lt+*, *stp+*), ETEC RIMD0509276 (*sth+*). The *E. coli* JM 109 were used as a negative control.

#### 3.2 Genomic DNA extraction

The tested *E. coli* strains and *E. coli* reference strains were grown into LB broth and incubated at 37 °C for 18 – 24 h. One milliliter of overnight culture was washed with TE buffer and then heated at 100°C for 10 min. The tubes were immediately placed on ice for 5 min. The gDNA was harvested by centrifugation at 10,000 x g for 2 min. The gDNA solution was kept at -20°C until further analysis.

#### 3.3 Oligonucleotide primers and single PCR condition

Nucleotide sequence of primers of *lt*, *sth*, *stp* gene are shown on Table 1. PCR mixture were performed in a total volume of 50 µl containing 1X PCR buffer, 0.2 mM dNTP, 0.5 µM of each primer (*lt*, *sth*, *stp*), 1.25 U of *Taq* DNA polymerase, and 10 µl of DNA template. PCR condition was started by initial denaturation at 95°C for 5 min, then followed by 35 cycles of denaturation at 95°C

for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 5 min. The products were analyzed by electrophoresis on 2.0% v (w/v) agarose gel. The gel was stained with ethidium bromide for 5 min and then destained for 10 min with distilled water. The amplicon sizes were visualized under ultraviolet light using gel documentation.

**RESULTS AND DISCUSSION**

A total of 100 rectal swabs were isolated and biochemically identified for *E. coli*. Only 98 samples were positive for *E. coli* which were isolated from 38 of 40 (95%) suckling and 60 of 60 (100%) weaning piglets. All these *E. coli* strains were further determined their virulence factors by PCR.

The results showed that the detection rate of ETEC was 11.2% (11/98) harboring *lt* 3.1% (3/98) and *stp* 8.1% (8/98). ETEC that positive for only *lt* was found in suckling piglets 2.6% (1/38), whereas ETEC positive for *lt* and *stp* were detected in weaning piglets 3.3% (2/60) and 13.4% (8/60), respectively. None of ETEC harboring *sth* was detected. However, virulence genes in weaning piglets 16.7% (10/60) were found higher percent than those in suckling piglets 2.6% (1/38).

The results in the present study were agreed to those of the previous study in South Korea (Lee *et al.*, 2008). However, the suckling piglets found more resistance to ETEC infection than weaning piglets, which might be related to the decreasing colostral antibody levels in weaning periods to protect the infection of pathogens (Devillers *et al.*, 2011).

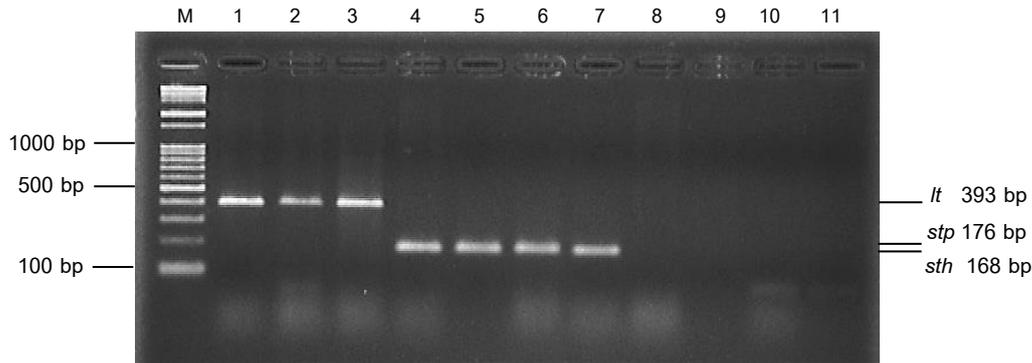
**Table 1** Primer sets used for single PCR assay

Pathotypes	Target genes	Sequences	Tm (°C)	Size (bp)	Reference
ETEC	<i>lt</i> -F	ATGACGGATATGTTTCCACTTCTC	68	393	Changkeaw, on publication
	<i>lt</i> -R	AACCTTGTGGTGCATGATGAATCC	70		
	<i>stp</i> -F	TTAATAACATCCAGCACAGGCAGG	70	176	Changkeaw, on publication
	<i>stp</i> -R	TCCCCTCTTTTAGTCAGTCAACTG	68		
	<i>sth</i> -F	TTCACCTTTCGCTCAGGATGCTA	68	168	Changkeaw, on publication
	<i>sth</i> -R	CACCCGGTACAAGCAGGATT	62		

ETEC; *lt* (heat-labile), *stp* (heat-stable in porcine) and *sth* (heat-stable in human)

**Table 2** Prevalence of virulence genes of ETEC in piglets

Diarrheal piglet	No. of <i>E. coli</i> strains	No. (%) of positive strains				Total	No. (%) of negative strains
		<i>lt</i>	<i>stp</i>	<i>sth</i>			
Suckling	38	1 (2.6%)	0	0	1 (2.6%)	37 (97.4%)	
Weaning	60	2 (3.3%)	8 (13.4%)	0	10 (16.7%)	50 (83.3%)	
Total	98	3 (3.1%)	8 (8.1%)	0	11 (11.2%)	87 (88.8%)	



**Figure 1** Detection of virulence genes of ETEC by single PCR. M; DNA ladder (size 0.1-2kbp; WakoNIPPON GENE, Japan), positive reference strains: lane 1; ETEC RIMD0509275 (*It*, *stp*<sup>+</sup>), lane 4; ETEC RIMD0509275 (*It*, *stp*<sup>+</sup>), lane 7; ETEC RIMD0509276 (*sth*<sup>+</sup>). *E. coli* tested isolates: lane 2; No.40, lane 3; No.50, lane 5; No.84, lane 6; No.85, lane 8; No.99, lane 9; No.100, lane 10; Negative control (*E. coli* JM109), lane 11; no DNA template

## CONCLUSION

In this study, ETEC carrying *It* and *stp* were found in weaning piglets, whereas those harboring only *It* were found in suckling piglets. However, more diarrheal piglets should be included to get the significant prevalence of ETEC infection in swine farms.

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