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Prevalence of Shiga Toxin-producing *Escherichia coli* in Feces and Raw Milk of Domestic Cattle and Sheep

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ABSTRACT

A total of 1060 samples of raw milk and fecal from cattle and sheep were collected from farms in the Taiwan area between September 1997 and June 1998. Shiga toxin-producing *E. coli* (STEC) strains were isolated based on the demonstration of Vitek automicrobic system and shiga toxin-PCR after enrichment and selective procedures. The results showed that 0.4% of 231 raw milk samples were contaminated with STEC. Among the 829 fecal samples of cattle and sheep, 9% tested positive for STEC. A total of 121 STEC strains were selected and further analyzed for biochemical tests, serotype and pathogenic genes of *eaeA* (attaching and effacing) as well as *hlyA* (enterohemolysin). Results revealed that only 3% of strains lacked β-D-glucuronidase activity and 4% of strains were unable to ferment sorbitol. Thirteen percent of strains were typed as O6, O8, O15, O78, O112ac, O128, O157 and O159 with 43 O-antisera. The remaining strains were non-typeable with O-antisera. Thirty one percent of strains were determined as H2, H7, H10, H16, H19, H21, H42, H45 and H51 with 22 H-antisera. Three types of shiga toxin genes were detected with different rates, namely *slt*1, 39%; *slt*2, 33% and *slt*1+*slt*2, 28%. Seventy percent of STEC strains harbored *hlyA* gene and 1.6% possessed *hlyA* and *eaeA* genes. In addition, one *E. coli* O157:H7 strain, isolated from feces of domestic sheep, carried *slt*2, *hlyA* and *eaeA* genes. This study indicates that the feces of cattle and sheep were most likely the source of STEC in the Taiwan area.

Key words: Shiga toxin-producing *E. coli*, cattle, sheep, Taiwan

INTRODUCTION

Enterohemorrhagic *E. coli* (EHEC) belongs to one of 6 subgroups of Enterovirulent *E. coli* (EEC)[1-3]. It is named as a verotoxin-producing *E. coli* (VTEC) since it is capable of producing verotoxin, an *E. coli* Shiga-like toxin (SLT) which could be neutralized by Shiga toxin of rabbit serum. The term VTEC is replaced by Shiga toxin-producing *E. coli* (STEC). The pathogenic factors of STEC are so far very controversial. Those factors, in most cases, are related to production of verotoxin, enterohaemolysin and a mechanism of attachment and effacement[4]. STEC can generate many toxins[4-9], including Shiga-like toxin 1 (SLT1 or VT1) and Shiga-like toxin 2 (SLT2 or VT2). SLT1 is toxic to both HeLa and Vero cells. It can be neutralized by shiga toxin-antiserum[10] and is composed of one big molecule A and five small molecule B. The molecule B binds to a receptor of cell, while the molecule A penetrates through cell and combines with ribonucleus to inhibit protein synthesis that could terminate cell life. SLT2 is also cytotoxic to HeLa and Vero cells. In contrast to the antigenicity of SLT1, it can not be neutralized by shiga toxin-antiserum[10]. VT2e can only be toxic to Vero cell. Its antigenicity is similar to SLT2[10]. *E. coli* O157: H7 possesses 65 mdal of plasmid (a H-antigen expression), which allows the strain to attach to small intestines cells[11]. The enterohaemolysin gene of *E. coli* O157: H7 is located at a big plasmid. The relationship between the enterohaemolysin gene and the pathogenicity of *E. coli* O157: H7 needs to be further studied[12].

The above strains can cause diarrhea (not including white blood cell), convulsions, abdominal pain, bloody stools, hemorrhagic colitis (HC), fever, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). Children and old people are susceptible to infections[4].

A few serotypes of STEC including O157: H7, O157: H^-, O26: H11, O104: H21, O111: H8, O111: NM, O48: H21, and O48: H^- have been known to be closely related to food poisoning[4]. Food poisoning outbreaks caused by *E. coli* O157: H7 have been widespread in Japan, USA, Canada, UK, Scotland, Welsh, and Africa since 1982. Among those outbreaks, the occurrence in Japan that occurred from May to November, 1996 is the largest in scope[13]. In October 1996, the apple juice produced by a company in the USA was also declared to be contaminated with *E. coli* O157: H7. The infection dosage of *E. coli* O157: H7 is in the range of 10-100 CFU[4]. Unlike other *E. coli* strains, *E. coli* O157: H7 lacks β-D-glucuronidase activity and is not capable of fermenting sorbitol[5]. However, some variants possess the above abilities. Many non-H7 serotypes of *E. coli* O157 including O157:H3, O157:H12, O157:H16, O157:H38, O157:H43, O157:H45 are often detected in food[14]. They are not capable of producing toxin. Some food poisoning outbreaks in Taiwan caused by bacteria was also isolated with some *E. coli* O157 strains. Those strains were found to be non-H7 serotype and did not carry the Shiga toxin gene[15]. *E. coli* O157: H8 belongs to EPEC and can be isolated from diarrhea patients[16].

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The major route of STEC infection is via consuming undercooked ground beef[4, 17]. According to a report from the USA, the ratios of STEC contamination in raw beef and mutton were up to 63% and 48%, respectively[4]. Among the feces samples of health animals (cattle, sheep, goats, chickens, dogs, cats, and pigs), sheep feces were found to be the top one in STEC detection rates (66.6%), followed by goat feces (56.1%) and cattle feces (21.1%). Sixty percent of the above samples were detected to be the same serotypes as the STEC's isolated from humans[18]. Several reports have pointed out that various serotypes of STEC can be isolated from raw beef, raw milk, and patients[19]. The report from Kudva, 1996 showed that 31% sheep feces were found to be contaminated with *E. coli* O157: H7[20]. Furthermore, *E. coli* O157: H7 was detected from the 4.9-5.3% of cattle less than 4-month-old in US dairy farms, and 22-50% of the above tested US dairy farms were shown to be contaminated with this strain[21]. This shows that STEC contamination sources are mostly from animal products. During slaughtering, this strain could contaminate beef and be mixed into ground beef as the contaminated beef was ground. People who consume undercooked ground beef may be thus infected. Some outbreaks which have occurred in the US and Canada were due to consuming undercooked ground beef[4].

The investigation data showed that livestock products in Taiwan could possibly be contaminated with STEC[22]. According to the World Health Organization report, the food poisoning prevention focused on 4 kinds of bacteria: STEC, *Yersinia*, *Listeria*, and *Campylobacter*. A food train from farms, animal slaughtering, and transportation to marketing was investigated to search for the sources of contamination. It was found that the above 4 kinds of bacteria were the pathogens that can be transmitted between humans and animals[19]. The outbreak prevention should focus on how to block the sources of contamination. Many studies have shown that *E. coli* O157: H7 has caught the world's attention. The purpose of our study was to investigate the STEC distribution in the feces of domestic cattle and sheep in Taiwan in order to monitor the hygiene safety and to establish the local STEC-related data. In this study, milk and feces of cattle and sheep were collected and STEC was isolated. The pathogenic genes of STEC were also tested.

MATERIALS AND METHODS

I. *Materials*

(I) *Chemicals*

Novobiocin was purchased from Sigma (St. Louis, Missouri, USA). Glycerol was obtained from Merck (Darmstadt, Germany).

(II) *Instruments*

A microorganism auto-analyzer (model: Vitek Auto Microbic System) was purchased from bioMerieux Vitek, Inc. (Hazelwood, Missouri, USA). A PCR reactor with Programmable Thermal Controller, PTC-100 was made by MJ Research (Water Town, Massachusetts, USA).

(III) *PCR Primer and Reagents*

Five primers specific to *slt*1, *slt*2, *hlyA*, and *eaeA* genes of EHEC (Table 1) were synthesized by TIB Molbiol (Berlin, Germany). DynaZyme DNA Polymerase kit was purchased from Finnzyme (Espoo, Finland).

(IV) *Pathogenic E. coli Serum and Gram Negative Identification Card*

Forty-three pathogenic *E. coli* O-antiserums and 22 H-antiserums were obtained from Denka Seiken (Tokyo, Japan). Gram negative identification card was supplied by Vitek Systems (Hazelwood, Missouri, USA).

(V) *Culture Media and Agar*

The following culture media and agar were used in this study: EC Broth, EMB Agar, Tryptic Soy Agar (TSA) (Difco, Detroit, Michigan, USA), Sorbitol MacConkey Agar (Oxoid, Hampshire, England), Fluorocult *E. coli* O157:H7 Agar (Merck, Darmstadt, Germany), and Agarose (Amresco, Solon, Ohio, USA).

(VI) *Test Strains*

E. coli CCRC 14824, which possesses *eaeA* and *hlyA* genes and is capable of producing SLT1 and SLT2, was used as reference strain of EHEC. It was obtained from the Culture Collection and Research Center at Food Industry Research & Development Institute.

(VII) *Test Samples*

In total, 1060 test samples of feces and raw milk were collected from 273 cattle or sheep farmers located in 15 geological areas of Taiwan (Taipei, Taoyuan, Hsinchu, Miaoli, Taichung, Changhua, Nantou, Yunlin, Chiayi, Tainan, Kaohsiung, Pingtung, Taitung, Hualien, and Ilan) and Penghu island from September 1997 to June 1998. Three feces and one raw milk samples were collected from every each farm. Ten farms for each cattle and sheep collection were selected from each region. The collected samples were immediately stored in a refrigerator and shipped to our laboratory waiting for analysis.

II. *Methods*

(I) *Isolation and Identification of STEC*

The test methods described in FDA Bacteriological Analytical Manual[1] were followed. Test samples (10 g) were transferred into a 90 mL TSB broth containing 20 mg/L

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novobiocin and then incubated at 42˚C for 12-18 hrs. After enrichment, 0.5 mL of culture broth was transferred into a 10-mL TSB enrichment broth and incubated at 35˚C overnight. The second TSB enrichment cultures (0.5 mL) were then tested by PCR reaction (including *slt*1, and *slt*2 genes). The second enrichment cultures (0.5 mL) for PCR reaction were centrifuged and the suspension was removed. The precipitate was washed with sterile water twice and re-suspended in 300 μL of sterile water. Twenty μL of which was then tested by PCR reaction. The test samples, which showed positive result as tested by a toxin gene-PCR analysis, were further streak cultured on EMB media at 35˚C overnight. Twelve strain colonies with typical characteristics were then transferred to TSA culture medium and incubated at 35˚C overnight for further performing a toxin gene-PCR analysis. The strains showing *slt*1 or *slt*2 toxin gene were selected for biochemical and serotyping identification.

(II) *Confirmation of Pathogenic Gene in STEC*

A PCR method[23-26] was carried out for pathogenic gene confirmation. The primers listed in Table 1 were used in this study. PCR reagent was prepared by mixing 63.8 μL of water with DynaZyme DNA Polymerase kit, which was composed of 10 μL of 10-folds buffer solution containing 1.5 mM Mg^{2+}, 1.5 μL of dNTPs (200 μM), DNA polymerase (0.5 unit), and 1 μL of each primer (100 μM). PCR reaction was performed by transferring one loop of colony to a micro-centrifugation tube, which contained 300 μL of sterile water, placed in boiling water for 10 min. After cooling, 20 μL of resulting solution was transferred to another centrifugation tube where the PCR reagent and one drop of mineral oil were added. The centrifugation tube was then incubated in a PCR thermocycler under the following program: 94˚C for 4 min followed by 94˚C for the another 1 min, 60˚C for 2 min, and finally 72˚C for 2 min (in total, 35 cycles of above program was performed). The PCR products were analyzed using a 2% agarose gel electrophoresis and the DNA-bands were checked. The above conditions were followed for the PCR reaction of other primers used in this study.

(III) *Biochemical and Serotype Tests*

The test strains identified to be *E. coli*, as analyzed by using Vitek microbial auto-analyzer, were sero-typed using 43 O- and 22 H-antiserums of pathogenic *E. coli*.

A biochemical test was carried out by streak culturing the strains on a sorbitol MacConkey Agar at 37˚C for 18-24 hrs. The typical *E. coli* strain shows pink in color. The strains, which are not capable of fermenting sorbitol show light gray in color. The strains with β-D-glucuronidase activity perform florescence blue in color under UV at 365nm after streak culturing on a Fluorocult *E. coli* O157: H7 Agar at 37˚C for 18-24 hrs.

(IV) *Culture Preservation*

The cultures, which were confirmed to be *E. coli* strains, were preserved at -70˚C. Prior to preservation, the colonies in TSA culture medium were transferred into a TSB broth vial containing 20% glycerol.

RESULTS AND DISCUSSION

The first *E. coli* O157: H7 outbreak occurred in the US, 1982. In 1995, a food poisoning outbreak took place in Australia is caused by *E. coli* O111. The outbreak that occurred in Japan during May to November, 1996 is the largest in scope[13]. The food poisoning caused by STEC is received great attention worldwide and is becoming a focus of prevention. STEC includes various pathogenic serotypes. Therefore, it is unwise to single out O157 serotype as the only target for disease prevention and inspection strategy. The inspection of all STEC is therefore necessary.

In this study, cattle and sheep feces and raw milk samples were collected from 15 areas of Taiwan and Penghu for investigation of STEC contamination. Originally, feces or raw milk samples were to sampled from 10 farmers in every area, however, this intention was not achieved. Yunlin and Chiayi lacked cattle feces and raw milk samples, while Taoyuan area was short of raw milk samples. The Hualien area could only provide sheep feces and raw milk samples. In consideration of the difficulty in sampling and transportation, the raw milk samples were not collected in the Penghu area.

I. *Isolation of STEC from Raw Milk or Feces Samples of Cattle and Sheep in the Taiwan Area*

Table 1. Primers used in PCR of EHEC

Primer	Sequence 5'-3'	Specificity	Amplicon (bp)	Reference
LP30	CAG TTA ATG TGG TGG CGA AGG	*slt*1	348	23
LP31	CAC CAG ACA ATG TAA CCG CTG			
LP43	ATC CTA TTC CCG GGA GTT TAC G	*slt*2	584	23
LP44	GCG TCA TCG TAT ACA CAG GAG C			
hlyAR	AAT AGC CAA GCT GGT TAA GCT	70-603 of *hlyA*	534	24
hlyAF	GCA TCA TCA AGC GTA CGT TCC			
eaeAF	GAC CCG GCA CAA GCA TAA GC	27-410 of *eaeA*	384	25
eaeAR	CCA CCT GCA GCA ACA AGA GG			
AE19	CAG GTC GTC GTG TCT GCT AAA	1959-3047 of *eaeA*	1087	26
AE20	TCA GCG TGG TTG GAT CAA CCT			

In total, 1060 test samples including 122 cattle and 109 sheep raw milk samples and 407 cattle and 422 sheep feces samples, were collected from 273 farmers in the Taiwan area and Penghu area. All raw milk samples of cattle showed negative results on STEC detection and only 1 (1%) out of 109 raw milk samples of sheep was detected to be positive (Table 2). In terms of feces detection, 8 (2%) out of 407 cattle feces and 69(16%) out of 422 sheep feces samples showed positive results (Table 2). With respect to STEC distribution, 8(6%) out of 131 farmers from Miaoli, Kaohsiung, Taitung, Hualien, and Penghu areas were detected to be positive in cattle feces, and 56 (39%) out of 143 farmers from all selected areas, except for Hualien where sheep feces were not collected, showed positive on STEC detection in sheep feces.

Most of the cattle dairy farms in Taiwan are equipped with one or two stainless steel tanks with refrigeration capability. However, the equipment for sheep milk production in Taiwan tends to be small-scale. Some farms even pack the sheep milk with large sterile bags instead of stainless steel tanks. The milk samples used in this study were directly obtained from collecting tanks and stored in a refrigerator after sampling. In total, 231 raw milk samples were collected from 220 dairy farms and 0.4% samples were detected to be positives. A Canadian investigation report revealed that contamination rates of *Listeria monocytogens*, *Salmonella* spp, *Campylobacter* spp. and STEC in 1720 raw milk samples of cattle were 2.73%, 0.17%, 0.47%, and 0.87%, respectively[27]. In general, the major route of milk contamination is via the milking process. It was confirmed by STEC detection of cattle and sheep feces that milk could be contaminated with STEC if hygiene for milking is not given attention. The feces of domestic cattle showed a lower STEC contamination rate (2%) than that of domestic sheep. STEC was not found in cattle milk. It was found that only one raw milk sample of sheep was contaminated with STEC. However, the sheep feces sampled from the farm, which was detected to be contaminated with STEC in the sheep milk sample, showed negative in STEC detection. The sampling technique could lead to a different result. The results of the domestic and Canadian cases as described above indicate that raw milk is likely to be contaminated with microorganisms that may lead to food poisoning. Therefore, a pasteurization process is necessary for raw milk treatment before sending it to market.

The STEC contamination rate (2%) of cattle feces is much lower than that (16%) of sheep feces. A report from Germany showed that STEC contamination rates of sheep and goat feces were 66.6% and 56.1%, respectively[18], and 21.1% and 10.8% feces of healthy cattle were found to be contaminated with STEC[18, 28]. A report from the US revealed that *E. coli* O157: H7 could be isolated from 31% sheep feces[20]. In 1995, 4914 cattle feces samples in Japan were tested for STEC using O26, O111, O128, O143, and O157 antiseren and Verotoxin-PCR methods. Results showed that 0.2% test samples were found to contain the above serotypes of STEC[29]. Some cattle feces samples were even found to contain 2 to 3 serotypes of STEC[18]. In Australia, 1.8%, 14%, and 19% feces samples from 279, cattle, calves and sheep were detected to carry STEC[30]. In Canada, 46% and 0.6% cattle feces samples were detected to be positive in STEC and *E. coli*. O157: H7[31]. The above data show that STEC contamination in Taiwan is far less than that in other countries. This explains why there has no been an *E. coli*. O157: H7 outbreak in Taiwan. To prevent domestic animal products from being contaminated with imported products, smuggling prevention should be enforced.

The investigation data in STEC contamination could vary among countries. The following factors may result in these differences: animal age, species, farm scale (eq. breeding method or farm system), and sampling methods. Besides, the health condition of animal, growing zones, and detection method may lead to different detection rates[31]. In 1996, 3 STEC strains were isolated from domestic frozen sheep products in our laboratory. A cross-contamination from sheep feces was proposed[22]. Our study suggested that STEC contamination of domestic sheep products may result from sheep feces.

Principally, the statistical comparison was not carried out because the sample sizes of cattle or sheep feces from different areas of Taiwan are different. In this study, the feces collected from all areas of Taiwan were milk cattle feces except for the Penghu area. In Taiwan, the milk sheep and meat sheep are not well differentiated when it comes to feces sample collection. The cattle feces collected from the Penghu all belonged to farm cattle because no milk cattle were bred in that area. The STEC detection rates of cattle feces in the Penghu area were higher than those in the Taiwan area. This result might be due to cattle species, breeding area, or breeding way differences but the actual reason is unclear. Three different breeding ways were investigated for comparison of the *E. coli*. O157: H7 detection rate. They were in-house hay breeding and put out to pasture (including 30 days grass-unfertilized ahead of pasturing and grass-fertilization before

Table 2. Occurrence of shiga toxin-producing *E. coli* isolated from raw milk and fecal samples of sheep and cattle in Taiwan

Sample source		STEC			
		Positive of farm/No. of tested farm	%	Positive of sample/No. of tested sample	%
Raw milk	Cattle	0/111	0	0/122	0
	Sheep	1/109	1	1/109	1
Total		1/220	0.5	1/231	0.4
Feces	Cattle	8/131	6	8/407	2
	Sheep	56/143	39	69/422	16
Total		64/273	23	77/829	9

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pasturing). Results showed that no significant difference in *E. coli*. O157: H7 detection among those samples with different breeding ways[32]. With respect to STEC detection in sheep feces, STEC were found to exist in sheep feces wherever collected from the Taiwan or Penghu areas.

II. *The Characteristics of Biochemistry, Serotypes, and Pathogenic Gene of Domestic STEC Strains*

STEC (250 strains) were isolated from 1060 tested samples collected from 273 farmers in the Taiwan and Penghu areas. The test samples might be repeatedly collected; therefore, only 121 strains of STEC were selected for biochemical, serotyping, and pathogenic gene testing. There are some controversial in STEC pathogenic factors by now. In general, those factors might be related to Verotoxin, enterohaemolysin and attachment and effacement mechanisms[4]. The pathogenic genes, *slt*, *hlyA*, and *eaeA*, which were considered to relate to above pathogenic factors, were tested in this study.

Among the 121 STEC, 4 (3%) of them lacked β-D-glucuronidase activity and 5 (4%) strains were not capable of fermenting sorbitol. This result is similar to that of a report, which showed 4% *E. coli* to be tested lacking β-D-glucuronidase activity and 5% *E. coli* not capable of fermenting sorbitol. The only strain O157: H7 isolated in this study also showed lacking β-D-glucuronidase and unable to ferment sorbitol, the same character as *E. coli* O157: H7 [5].

Three serotypes or toxin types of 4 STEC isolated from domestic sheep milk were determined to be O?: H?/*slt*1, O?: NM/*slt*1, and O?: H?/*slt*1+*slt*2. The STEC (106 strains) isolated from sheep feces were determined to contain 3 toxin types (*slt*1, *slt*2, and *slt*1+*slt*2), 8 serotypes O (O6, O8, O15, O78, O112ac, O128, O157, and O159), and 9 serotypes H (NM, H?, H2, H7, H10, H16, H19, H42, and H45). The unidentified serotype O is designated as O?. A combination of the above serotypes O and H, 17 serotypes of STEC from domestic sheep feces as follows were obtained: O?:NM, O?:H?, O?:H2, O?:H16, O?:H19, O?:H42, O?:H45, O6:H10, O8:H?, O8:H19, O15:H?, O15:H16, O78:H?, O112ac:H?, O128:NM, O157:H7, and O159:H? as listed in Table 3. It has been shown that STEC strains consist of more than 100 serotypes[4]. In this study, we found that 3 types of STEC could be isolated from the same sheep feces sample. The similar result was reported by Beutin *et al*. that 2 to 4 serotypes of STEC could be found in same sheep feces[18].

STEC (11 strains) isolated from cattle feces were also belonging to the above three toxin types. O128 was the only serotype O found in STEC from cattle feces, which however contained 7 serotypes H including NM, H?, H7, H16, H19, H21, and H51. Combination of serotypes O and H gave 8 types of STEC from domestic cattle feces. They were O?:NM, O?:H?, O?:H7, O?:H16, O?:H19, O?:H21, O?:H51, and O128:NM (Table 3). A report from Germany showed that 58% of isolated STEC (57 strains) from cattle feces were tested to be O116:H21, O82:H8, O113:H21, and O136:H12. One of the O?:H16 serotype found in this study was also isolated according to above report[28]. Two different types of STEC strain could also be found in the same cattle feces sample[18]. Our study revealed that 2 types of STEC, O?:NM and O?:H16, were found in the feces of the same cattle in Penghu area.

Table 3. Serotype, shiga-toxin types, and other pathogenic genes of STEC isolated from raw milk and fecal samples of cattle and sheep in Taiwan

Serotype	Source	No. of strain (%)	Type of *slt*			*hlyA*	*eaeA*
			slt 1	*slt* 2	*slt* 1+ *slt* 2		
O? [a]:NM	M[c], S[c], C[c]	24 (20)	12	4	8	22	1
O?:H? [b]	M, S, C	48 (40)	18	13	17	41	
O?:H2	S	13 (11)	7	5	1	5	
O?:H7	C	1 (1)		1			
O?:H16	S, C	5 (4)		4	1	1	
O?:H19	S, C	9 (7)	5	4		7	
O?:H21	C	1 (1)		1			
O?:H42	S	1 (1)		1			
O?:H45	S	2 (2)	2			2	
O?:H51	C	1 (1)			1		
O6:H10	S	1 (1)			1	1	
O8:H?	S	3 (2)		3		1	
O8:H19	S	1 (1)		1			
O15:H?	S	1 (1)	1			1	
O15:H16	S	1 (1)	1			1	
O78:H?	S	1 (1)		1			
O112ac:H?	S	1 (1)		1			
O128:NM	S, C	5 (4)			5	1	
O157:H7	S	1 (1)		1		1	1
O159:H?	S	1 (1)	1			1	
Total		121	47	40	34	85	2

[a] O?: non-typable with 43 O-antisera.

[b] H?: non-typable with 22 H-antisera.

[c] M (raw milk of sheep), S (feces of sheep), C (feces of cattle).

Among the 121 domestic STEC strains to be tested, only 16 (13%) strains could be identified using 43 commercial O-antiserum kits and 37 (31%) strains were capable of being identified using 22 H-antiserum kits. Thus, 20 serotypes of O and H as follows could be obtained: O?:NM, O?:H?, O?:H2, O?:H7, O?:H16, O?:H19, O?:H21, O?:H42, O?:H45, O?:H51, O6:H10, O8:H?, O8:H19, O15:H?, O15:H16, O78:H?, O112ac:H?, O128:NM, O157:H7, and O159:H?. Pathogenic *E. coli*. has been known involving 173 serotypes O and 60 serotypes H. However, there are only 43 O-antiserum and 22 H-antiserum kits commercially available. O? and H? represent 2 meanings: the O or H serotypes other than the types to be tested in this study, or new O or H serotypes to be found. They could be the strains without flagella (or H-) or non-motile (NM).

Three types of the SLT toxins, *slt*1 (47 strains, 39%), *slt*2 (40 strains, 33%), and *slt*1+*slt*2 (34 strains, 28%) from 121 STEC strains were isolated in this study. STEC can yield 8 types of toxins. However, not all the STEC strains can produce all toxins. Their toxin producing ability is strain dependent[4-9].

Further, the investigation of other important pathogenic genes showed that 85 (70%) strains of STEC possessed *hlyA* gene. According to a German report, 62.8%, 90%, and 57.6% of STEC isolated from sheep, goats, and cattle feces, respectively, carried *hlyA* gene after detecting 7 animal feces samples. The STEC isolated from feces of dogs, pigs, chickens, and cats carried much less *hlyA* gene[18]. Pierand *et al*. found that only 21 (31%) out of 67 STEC strains isolated from raw meat samples possessed the haemolysin gene[33].

Two STEC strains, *E. coli* O?:NM/*slt*2 and *E. coli* O157:H7/*slt*2 was found to carry the *eaeA* gene. A PCR analysis revealed that the *eaeA* gene sequences of the above 2 strains at 3'-terminal were inconsist. The *eaeA* gene sequence at 5'-terminal was more conserved but there was a significant difference at 3'-terminal[26]. In this study, the primers AE19 and AE20, which sequences located at 3'-terminal, were used. These primers are highly specific to the 3'-terminal of *eaeA* gene in *E. coli* O157: H7. The *eaeA* gene in *E. coli* O?:NM was not detected by using above primers. However, the above 2 strains could be detected to contain *eaeA* gene when eaeAF and eaeAR primers, which was near by 5'-terminal, were used[26]. It has been reported that 3% of STEC strains isolated from beef or mutton could carry *eaeA* gene[33]. The pathogenic mechanism of *eaeA* gene is unclear. Some *eae*A-free serotypes, such as O22: H8 and O153: H25, was still capable of inducing some complications like HC or HUS[34].

In this study, one *E. coli* O157: H7 strain was found to contain pathogenic genes, *slt*2, *eaeA*, and *hlyA*. This strain was lack of β-D-glucuronidase activity, nor possessed sorbitol-fermenting ability. It showed a negative result in *E. coli* O157: H7 detection when the same farm originally checked with positive was re-examined. The results of our study showed that the domestic STEC detection rate was significantly lower than other countries since only one *E. coli* O157: H7 strain was found. This indicates that a good hygiene environment is well maintained in Taiwan. Therefore, the STEC contamination from abroad should be avoided to keep the health of residents in Taiwan.

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本土牛、羊糞便或其生乳中Shiga毒素產生性大腸桿菌之分佈

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摘　要

本研究係自1997年9月至1999年6月，從台灣地區之273戶牛羊養殖場採集1060件牛羊生乳或其糞便檢體，經增菌培養、分離後，使用Vitek微生物自動鑑定儀、大腸桿菌Shiga毒素基因—PCR反應，進行Shiga毒素產生性大腸桿菌（shiga toxin-producing *E. coli*, STEC）之檢測。結果顯示，於231件牛羊生乳檢體檢出0.4%污染STEC；829件牛羊糞便檢體中，9%分離出STEC。選取121株STEC，進一步測試其生化特性、血清型及附著（*eaeA*）與溶血（*hlyA*）等致病基因。測試結果為僅有3%菌株缺乏β-葡萄糖苷酵素（β-D-glucuronidase）活性，4%菌株不能醱酵山梨糖醇（sorbitol），以43種O型血清鑑別出之血清型為O6、O8、O15、O78、O112ac、O128、O157及O159，但僅有13%菌株屬於前述血清型，其餘則未鑑別出。H血清型別以22種H型血清測試，計有31%菌株鑑別出，分別為H2、H7、H10、H16、H19、H21、H42、H45及H51。Shiga毒素型別共計三種，檢出率為*slt1*，39%；*slt2*，33%；*slt1*+*slt2*，28%。70%STEC則具有*hlyA*基因，其中1.6%菌株則同時帶有*hlyA*及*eaeA*基因。另外，從本土羊糞分離出一株*E. coli* O157:H7，其同時帶有*slt2*、*eaeA*及*hlyA*等致病基因。綜合前述，瞭解台灣地區之牛羊糞便可能為STEC之污染源。

關鍵詞：Shiga毒素產生性大腸桿菌，牛羊，台灣