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Prevalence of Pathogenic *Escherichia coli* in a Swine Breeding Environment in Can Tho Province, Vietnam

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Abstract

To determine the presence of enterotoxigenic *Escherichia coli* (*E. coli*) (ETEC), Shiga toxin-producing *E. coli* (STEC) and attaching and effacing *E. coli* (AEEC) were collected in swine and in a water system in 17 swine farms in the Can Tho province of the Mekong delta. A total of 258 samples which were collected from swine feces (n=169), from feed (n=39) and from water (n=50) in the irrigation system were examined by PCR to determine whether they harbored the *stx*, *eae,* and enterotoxin genes. STEC was detected in 10 of 169 (6%) fecal samples, and AEEC in 5 fecal samples (3%). Both STEC and AEEC were detected in one fecal sample. AEEC was mainly detected in fecal samples from pigs that were less than 4 months old. STEC or AEEC was detected in 18 samples from 5 out of 17 (29%) farms. On the other hand, STEC and AEEC were also detected in the water and feed samples. ETEC was not detected in diarrhea samples.

Discipline: Animal health
Additional key words: AEEC, *eae*, ETEC, STEC, *stx*

Introduction

Of the pathogenic *Escherichia coli* (*E. coli*) strains found in pigs, enterotoxigenic *E. coli* (ETEC) is considered to be the most important pathogen, since ETEC causes a lethal infection involving severe water-like diarrhea in newborn animals[3]. ETEC contains the *E. coli* strains that produce at least one member of 2 defined groups of enterotoxins, LT and ST[13]. Swine edema disease is typical of the entero-toxemia caused by Shiga toxin-producing *E. coli* (STEC)[15]. STEC produces the Shiga toxin (Stx), which is also known to be the causal agent of human hemolytic uremic syndrome[14,19]. Enterohemorrhagic *E. coli* (EHEC), represented by O157:H7, is classified among the STEC which cause hemorrhagic colitis[19]. Furthermore, attachment and effacing *E. coli* (AEEC) has recently been identified[10]. Once categorized as human and animal enteropathogenic *E. coli* (EPEC), almost all the EHEC strains are presently being classified as AEEC. AEEC strains produce intimin, a kind of bacterial outer membrane protein generated by the *eae* gene, which adheres directly to the entero-epithelium cells[10]. A watery diarrhea will be observed due to the adherence of the pathogen to host cells. AEEC is considered to be related to swine postweaning diarrhea[9]. Human diarrhea due to AEEC, such as traveler's diarrhea or infant diarrhea in developing countries, has been well analyzed as an EPEC-type infection[4]. All the pathogenic *E. coli* described above except for ETEC are considered to be zoonotic organisms. Cattle and other ruminants have been investigated in relation to the prevalence of these types of *E. coli*[5,12]. Ruminants have been implicated as the principal reservoir of both STEC and AEEC[5,7,20]. Although a few reports have described the prevalence of STEC in pigs[5], there are no reports originating from developing countries to date.

In the Mekong Delta in southern Vietnam, farming

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systems employ a kind of agricultural recycling technique that combines crop production, animal husbandry, and aquaculture. Swine feces are shed directly into fish-raising ponds located in the proximal part of the systems. Indirect passage through a bio-gas digester also occurs[18]. In the current study, we investigated the prevalence of pathogenic *E. coli* in swine fecal samples, as well as in water samples originating from swine farms. The water sources included swine drinking water tanks, fish ponds, drilled wells, canals, etc. Furthermore, to evaluate the bacteriocidal effects of the bio-gas digester apparatus, the number of enterobacteria in the water samples from the input and output ends of the apparatus were counted.

Materials and methods

1. Presence of *E. coli* (including other enterobacteria) in water sources from swine farms

A total of 50 water samples were collected in 17 swine breeding farms (2 canals, 16 swine drinking water sources, 5 fish ponds, 2 swine drinking water tanks, 1 tap water source, 15 well water sources, and 9 bio-gas digester apparatuses) located in Omon province (number of sources = 2) and Tan Phu Thanh village (n=15) in January 2002. All the water samples were cooled in an ice-box and immediately transferred to the laboratory for examination. An aliquot of 0.1 mL of each water sample was spread onto a MacConkey agar (Eiken, Tokyo) plate and incubated at 37ºC for 18 h. All the colonies appearing on the plates were considered to be enterobacteria.

2. Screening for STEC, AEEC, and ETEC in a swine breeding environment

A total of 258 samples, including the 50 water samples described above, were examined for the presence of the *stx* and *eae* genes by PCR screening for STEC and AEEC, respectively. The details regarding 208 samples collected in January 2002 were as follows: 16 swine fecal samples originated from pigs less than 10 days old, 6 from pigs 11 days to 1-month old, 60 from pigs >1 month to 3 months old, 36 from pigs >3 months to 7 months old, 50 from sows, 2 from boars and the remaining 39 from swine feed samples. Fecal samples were collected from the pens. When diarrheic pig(s) were detected in their pen, up to 3 diarrheic samples were collected from individual pigs. A 10 mL water sample was mixed into the same aliquot of double-concentrated trypticase soy broth (TSB, Difco, USA) and approximately 1 g of fecal and feed samples was transferred to 10 mL TSB. These inoculums were incubated at 37ºC for 18 h. Consequently, loops of the incubated medium were each spread onto separate MacConkey plates and were incubated at 37ºC for 18 h. Thickly colonized areas on the MacConkey plates were derived from the portion of the loop and were suspended in 0.5 mL saline solution. Suspensions of 5 µL were used as PCR templates. The *LT*1, *ST*1, and *ST*2 toxin genes were targeted by PCR for ETEC detection which was restricted to the diarrheic samples from piglets (less than 1 month old)[16].

In vitro amplification by PCR for the detection of STEC, AEEC, and ETEC was carried out, with 5 µL of the samples in a reaction mixture of 50 µL containing 1.25 mM $MgCl_2$, 0.25 mM of each dNTP, 5 µL of x10 PCR buffer, 0.625 unit of *Taq* DNA polymerase (rTaq polymerase, TOYOBO, Japan), and 20 pM of each primer. The PCR cycles consisted of pre-heating mixtures at 94ºC for 2 min, denaturation at 94ºC for 45 sec, annealing at 55ºC for 1 min and extension at 72ºC for 1 min. The amplifications were performed for 30 cycles. The PCR products were analyzed by agarose gel (1.5%) electrophoresis and visualized by staining with ethidium bromide. The PCR primers used in this study are listed in Table 1. In this report, *stx*- or *eae*-PCR positive samples were defined as either STEC- or AEEC-positive.

Results and discussion

1. Bacterial contamination levels in water samples and bacteriocidal potential at the level of a bio-gas digester apparatus

Enterobacteria were detected in more than half of the water samples. Of 4 well water samples, enterobacteria were detected at identical levels with those of the adjacent canal (>10^2 to 10^4 CFU/mL). Fish ponds showed higher contamination levels, ranging from 10^3 to10^5 colony-forming units (CFU) per mL, than did the water samples from other sources (Table 2). These results were not surprising, as swine feces were shed directly from pens to ponds. As one gram of swine feces contained around 10^9 CFU of *E. coli*, about 100 L of water was needed for dilution. Therefore, contamination levels in fish ponds were considered to be lower than expected, and the majority of the pathogens was considered to be stacked at the bottom of the ponds. The bacterial contamination levels in the wells and drinking water (most of the drinking water was supplied from wells in small-scale farms) were the lowest. The contaminants seeped into wells from fish ponds, and were supplied to pigs via the drinking water sources.

As for the bio-gas digester apparatus (Table 2), no significant difference was found between the results of bacterial analysis at the input and output ends of the apparatus. As the water samples were taken from

Table 1. Nucleotide sequences of the PCR primers used for the detection of STEC, AEEC and ETEC by *in vitro* amplification

PCR primer (orientation)	Target gene(s)	Sequences (5'–3')	Amplicon size (bp)	References
VT-com-u (forward) VT-com-d (reverse)	*stx*1 and *stx*2[a)]	GAGCGAAATAATTTATATGTG TGATGATGGCAATTCAGTAT	518	21
eae-1 (forward) eae-2 (reverse)	*eae*	ACGTTGCAGCATGGGTAACTC GATCGGCAACAGTTTCACCTG	815	11
LT-1F (forward) LT-1R (reverse)	*LT*1	GGCGACAGATTATACCGTGC CCGAATTCTGTTATATATGTC	696	17
ST1-F (forward) ST1-R (reverse)	*ST*1	CCGTGAAACAACATGACG ACATCCAGCACAGGCAGGATT	240	1
ST2-F (forward) ST2-R (reverse)	*ST*2	ATCGCATTTCTTCTTGCATC GGGCGCCAAAGCATGCTCC	172	6

a): The positions of the *stx* conserved sequences of the primers for the *stx* genes are as follows: Sense primer VT-com-u, positions 280 to 300, and antisense primer VT-com-d, positions 778–797.

streams of water within the apparatus, most of the bacteria appearing in the water were considered to have passed through the apparatus within a short period of time. Although it was confirmed that the effluent of the apparatus had the highest levels of contaminants, bacterial levels in the sludge of such an apparatus should be examined in order to evaluate the bacteriocidal potential.

2. Screening of STEC, AEEC and ETEC in the swine breeding environment

Most of the pigs bred in both the Omon and the Tan Phu Thanh farms appeared to be quite healthy. No diarrheic pigs were found in any of the 15 farms in the Tan Phu Thanh village. However, a total of 17 diarrheic samples from newborn piglets were collected from 8 pens in 2 large-scale farms in the Omon province (Table 3). ETEC was not detected among these samples by PCR. STEC and/or AEEC were detected in 18 samples from 5 out of 17 (29.1%) farms (Table 3); 4 of these samples originated from feed and water supplies (Table 4). Thus,

Table 2. Number of *E. coli* (including other enterobacteria) in water sources from swine farms

Sample	No. of enterobacteria in water samples (CFU/mL)					
	0	$1–10^2$	$>10^2–10^3$	$>10^3–10^4$	$>10^4–10^5$	$>10^5$
Canal (n=2)			1	1		
Drinking water (n=16)	7	3	4	2		
Fish pond (n=5)			2	2	1	
Tank water (n=2)			1	1		
Tap water (n=1)			1			
Well water (n=15)	5	6	3	1		
Bio-gas digester #1						
Input (Not tested)						
Output						● a)
Bio-gas digester #2						
Input					○ b)	
Output						●
Bio-gas digester #3						
Input						○
Output						●
Bio-gas digester #4						
Input					○	
Output						●
Bio-gas digester #5						
Input						○
Output					●	

a): The black circles denote the location of the number of enterobacteria above written at the output end of the bio-gas digester.
b): The white circles denote the location of the number of enterobacteria above written at the input end of the bio-gas digester.

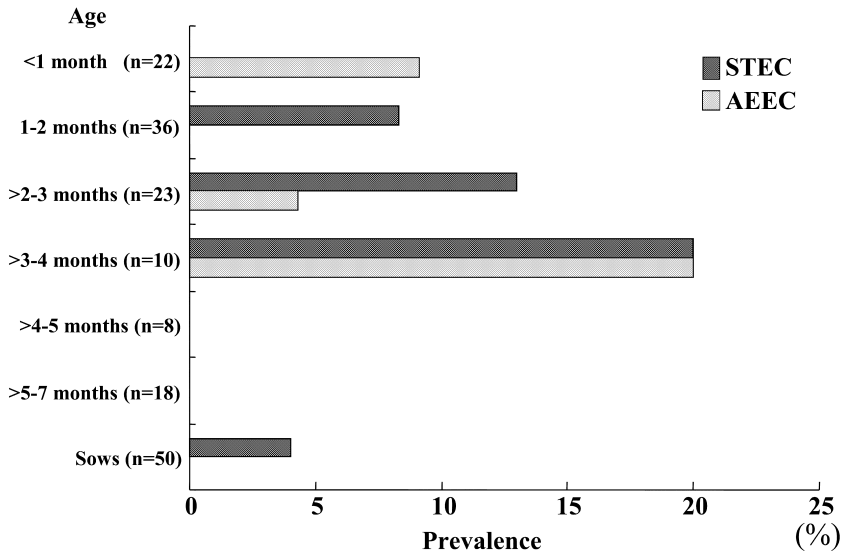

Fig. 1. Prevalence of STEC and AEEC in pigs

the bacterial pathogens might have circulated between the water system and the farm animals. On the other hand, STEC and AEEC were highly prevalent in young pigs. AEEC in particular was only detected in pigs less than 4 months old (Fig. 1). It is known that intimin has a strong antigenicity. Intimin is classified into several subtypes; α, β, γ, ε, ζ and θ and there is no immunological cross-reaction among these subtypes[2,8]. Previously, we confirmed that intimin was expressed in the *E. coli* strains frequently isolated among feces from free-range cattle. More than one subtype of intimin-positive *E. coli* strains is usually detected in animals in a herd. (unpublished study). Even in the cases when the animals are immunized with a subtype of intimin, the other subtypes of *E. coli* strains can still infect the animal. Therefore, limited subtypes of *E. coli* strains will eventually spread in swine farms. The best method of removing AEEC from farms is thus suitable processing of feces of pigs less than 4 months old.

Although a small number of samples was tested in this study, we confirmed that pathogenic *E. coli* were more prevalent among young pigs. In order to detect pathogenic *E. coli* in farms in Vietnam, PCR or detection

Table 3. STEC and AEEC from swine farms

Farm	No. of samples	STEC	AEEC
1	56(4)[a)]	0	0
2	11	0	0
3	6	0	0
4	7	0	0
5	7	0	0
6	7	0	0
7	11	0	0
8	6	0	0
9	15	0	3
10	7	2	1
11	3	0	0
12	4	1	0
13	3	0	0
14	5	0	0
15	9	0	0
16	10	3	2
17	91 (13)	6	2
Total	258 (17)	12	8

a): The numbers in parentheses represent the number of diarrheic fecal samples.

Table 4. Screening for STEC and AEEC in a swine breeding environment

Sample	STEC	AEEC	STEC+AEEC
Pig:			
<3 months old (n=82)	5	2	1
3–7 months old (n=36)	2	2	
sow (n=50)	2		
boar (n=2)			
Feed (n=39)		1	
Canal (n=2)			
Drinking water (n=16)	1	1	
Fish pond (n=5)			
Tank water (n=2)			
Tap water (n=1)			
Well water (n=15)			
Bio-gas digester (input) (n=4)			
Bio-gas digester (output) (n=5)			1

by conventional methods (e.g. cytotoxicity, serological identification, biological determination using experimental animals, etc.) will be essential. However, it should be noted that the more expensive conventional methods can not be applied in Vietnam at present. In addition, epidemiological studies will require the examination of many samples simultaneously, which cannot be performed by conventional methods. In contrast, PCR is easy to perform, enables to analyze many samples at one time, provides clear results, and can be used to detect different kinds of pathogens, simply by changing the primer pairs.

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