Identification of Enterotoxigenic *Escherichia coli* Isolated from Swine with Diarrhea in Thailand by Colony Hybridization, Using Three Enterotoxin Gene Probes

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The DNA colony hybridization assay was used to identify enterotoxigenic *Escherichia coli* among *E. coli* isolated from 803 swine with diarrhea at 10 farms in Thailand. Between 5 September and 8 December 1981, enterotoxigenic *E. coli* were identified in 40% of 58 litters of piglets under 10 days old and 17% of 29 litters between 10 and 21 days old with diarrhea at farms at four different locations in Thailand. All *E. coli* that hybridized with one or more of the three enterotoxin gene probes produced heat-labile or heat-stable toxin or both, as determined by testing culture supernatants in the Y1 adrenal and suckling mouse assays. The DNA colony hybridization technique is a specific method of identifying enterotoxigenic *E. coli* from swine and can be used to further characterize these enteric pathogens.

Enterotoxigenic *Escherichia coli* (ETEC) organisms have been implicated in diarrheal disease of swine, other species of animals, and humans (1, 12, 14, 16-18). ETEC produce a heat-labile toxin (LT), identified by testing culture supernatants of *E. coli* in the Y1 adrenal cell assay (13), and a heat-stable toxin (ST), identified by the suckling mouse assay (3). Both techniques are time consuming, and the assay for ST requires a large number of suckling mice which are frequently not available. To determine the prevalence of ETEC as an enteric pathogen in pigs in Thailand, *E. coli* isolated from swine with diarrhea at farms at four different locations in Thailand were examined for homology with three enterotoxin gene probes (9). Colonies that were positive in the DNA colony hybridization assay were tested in the more conventional assays (3, 13).

Between 5 September and 8 December 1981, 803 *E. coli* strains were isolated from swine (1 strain per animal) at 10 different pig farms at four different locations in Thailand. These *E. coli* were recovered from 87 litters of pigs between 1 and 21 days old with diarrhea. All isolates were stored on nutrient agar slants and tested within 6 months of isolation for homology with the enterotoxin gene probes. *E. coli* K-12 C600 organisms containing the following plasmids were used to prepare DNA fragments employed as enterotoxin gene probes: pWD299, a recombinant plasmid encoding LT production (2); pRIT10036, a recombinant plasmid encoding ST production (ST-P) (7); and pSLM004, a recombi-

nant plasmid producing ST (ST-H) (10). Both the ST-P and the ST-H are detected in suckling mice. Both the LT and ST-P probes were initially derived from ETEC of porcine origin (2, 7), whereas the ST-H probe was derived from an ETEC of human origin (10).

Plasmid DNA was isolated from *E. coli* K-12 C600(pWD299), (pRIT10036), and (pSLM004) and cleaved with restriction endonucleases as previously described (9). DNA fragments were isolated by polyacrylamide gel electrophoresis of digested DNA, and the appropriate fragment was removed by electroelution. The isolated DNA fragments which were used as probes for enterotoxin genes were labeled in vitro with [α -³²P]deoxynucleotide triphosphate (New England Nuclear Corp., Boston, Mass.) by nick translation (8).

Each colony to be tested was inoculated onto three different pieces of nitrocellulose paper (3 by 5 cm) (BA-85; Schleicher & Schuell Co., Keene, N.H.) layered on MacConkey agar. *E. coli* K-12 C600, containing the plasmids from which the enterotoxin gene probes were derived, LT and ST producing ETEC B2C, which hybridized with the LT and ST-H probes, and non-ETEC K-12 Xac were included as controls. After incubation at 37°C for 24 h, the papers were removed from the MacConkey agar and placed onto pieces of Whatman no. 3 paper (Whatman, Inc., Clifton, N.J.) saturated with 0.5 N NaOH. After 10 min, the nitrocellulose papers were transferred to similar Whatman paper saturated with 1.0 M ammonium acetate

and 0.02 N NaOH for 1 min. This process was repeated four times, and after the last transfer, the papers were kept on the saturated Whatman paper for 10 min. The papers were then air dried and baked at 65°C overnight. Each paper was hybridized with one of the three radioactively labeled LT, ST-H, and ST-P enterotoxin gene probes (9). The nitrocellulose papers were then exposed to X-Omat AR X-ray film (Eastman Kodak Co., Rochester, N.Y.) with a single Cronex Lightning-Plus intensification screen (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) for 48 h at -70°C.

Isolates from 28 of 87 litters hybridized with the enterotoxin gene probes. All of the 49 colonies which hybridized with the LT probe produced LT as determined by the Y1 adrenal cell assay (11), and 68 colonies which hybridized with the ST-P enterotoxin gene probes produced ST as determined by the suckling mouse assay (3). No *E. coli* isolated from piglets with diarrhea hybridized with the ST-H probe derived from an ST ETEC of human origin.

As shown in Table 1, ETEC, the majority of which produced ST only, were isolated more frequently from litters of piglets under 10 days old than from older litters of animals (32/58 versus 5/29; $P < 0.025$). LT, ST, and LT ETEC were uncommon in the piglets with diarrhea examined.

The DNA colony hybridization assay was used to determine the incidence of ETEC infection in swine at 10 pig farms in Thailand. It was possible with this new technique to screen a large number of colonies for enterotoxigenicity. This methodology would be a valuable tool in further defining the importance of this enteric pathogen in swine as well as in humans.

In this study, we did not examine all enterotoxin gene probe-negative *E. coli* in the Y1 adrenal (3) and suckling mouse assays (3). When 160 probe-negative *E. coli* were examined in the standard assays, all were negative. We have, however, found that culture supernatants of a small number of *E. coli* isolated from swine, water buffalo, and humans in villages in Thailand cause rounding of Y1 adrenal cells not inhibited by anti-LT antisera (unpublished data). One of these isolates, SA-53, has been examined by Green et al. (5). The enteropathogenicity of these organisms is presently undefined. To date we have not found any *E. coli* in either animals or humans which are positive in the suckling mouse assays (3) but do not hybridize with either the ST-H or ST-P probes.

ETEC have previously been isolated from animals living in close proximity to humans (1, 4), suggesting that ETEC may be zoonotic infection agents. The serotypes of ETEC isolated from animals are, however, different from sero-

TABLE 1. ETEC infections in 87 litters of swine with diarrhea at 10 farms in Thailand in 1981

Farm	Date (1981)	Location	No. of isolates	No. of litters with ETEC/ no. litters examined	No. of litters with type of ETEC infection ^b
A	5 Sept.	Nakorn Pathom	12	3/3	3 ST
B	5 Sept.	Nakorn Pathom	5	2/2	2 ST
C	5 Sept.	Nakorn Pathom	25	4/4	4 ST
D	5 Sept.	Nakorn Pathom	24	2/3	2 ST
E	5 Sept.	Sri Racha	133	2/17	2 LTST
F	5 Sept.	Sri Racha	144	6/15	1 LTST, 2 LT, 3 ST
G	27 Nov.	Panom Sarakam	103	2/7	2 ST
B	8 Dec.	Nakorn Pathom	95	2/7	2 ST
Subtotal ^c			541	23/58 (40%)	1 LTST, 2 LT, 20 ST
H	26 Sept.	Panom Sarakam	64	1/8	1 ST
I	2 Oct.	Rayong	72	2/5	2 ST
H	14 Oct.	Panom Sarakam	37	0/7	
I	14 Nov.	Rayong	29	0/3	
J	14 Nov.	Panat Nikom	50	2/5	1 LTST
I	8 Dec.	Rayong	10	0/1	1 ST
Subtotal ^d			262	5/29 (17%)	1 LTST, 4 ST
Total			803	28/87 (32%)	2 LTST, 4 LT, 22 ST

^a One isolate per pig with diarrhea.

^b LTST, heat-labile and heat-stable ETEC; LT, heat-labile ETEC; or ST, heat-stable ETEC.

^c Subtotals from piglets <10 days old.

^d Subtotals from piglets 10 to 21 days old.

types of ETEC commonly isolated from humans (6, 11, 18). Likewise, the majority of ST ETEC isolated from humans in Thailand hybridized with the ST-H rather than the ST-P enterotoxin gene probes (9). Although these observations are preliminary, these results suggest that the ST ETEC that usually infect humans carry plasmids coding for ST which are genetically different from those that infect swine. Further studies employing the DNA hybridization technique are in progress in a rural community in Thailand to determine the role of livestock in the epidemiology of ETEC infections in humans.

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