

Prevalence of Heat-Stable II Enterotoxigenic *Escherichia coli* in Pigs, Water, and People at Farms in Thailand as Determined by DNA Hybridization

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The DNA hybridization assay employing a 460-base-pair fragment of DNA encoding for the methanol-insoluble form of heat-stable toxin (ST-II) was used to determine the prevalence of ST-II enterotoxigenic *Escherichia coli* (ETEC) in pigs, people, and water at 57 farms in Sri Racha, Thailand. ST-II ETEC was found in 62 (3%) of 2,110 suckling, 181 (32%) of 560 weaned, and 4 (1%) of 457 adult pigs examined. Of 62 suckling pigs with ST-II ETEC infections 21% had diarrhea, but none of 185 infected older pigs had diarrhea. ST-II ETEC was found more frequently in suckling pigs with diarrhea than without diarrhea (13 of 146 versus 49 of 1,964) ($P < 0.001$). ST-II ETEC was detected in water collected from 3 of 57 clay jars containing water used to bathe at three pig farms, in 1 jar used to bathe immediately after working in the barn, and from one farmer who did not have a recent history of diarrhea. Evidence of this organism was not found in 245 other individuals living on the pig farms or in 220 inhabitants and 114 water specimens collected at tapioca farms nearby. In Thailand ST-II ETEC was found in suckling pigs with diarrhea but was infrequently found in humans.

Enterotoxigenic *Escherichia coli* (ETEC) is a common cause of diarrheal disease in swines and humans (1, 6, 10, 14, 15). ETEC produce a heat-labile toxin (LT), a heat-stable toxin (ST-I) as identified in the suckling mouse assay (3), or both. A methanol-insoluble form of heat-stable toxin, ST-II, has also been identified (2). Sterile culture supernates of ST-II ETEC cause distension of the jejunal loops of pigs but are negative in the suckling mouse assay (5). The role of ST-II ETEC in piglet diarrhea has not yet been determined, largely because studies of ST-II ETEC infections have been hampered by the difficulty of examining specimens in pig ileal loops. The genes coding for ST-II have recently been cloned and sequenced and shown to be distinct from those of ST-I (7, 13). The DNA hybridization assay employing a radiolabeled fragment of DNA encoding for ST-II was used as a probe to determine the prevalence of ST-II ETEC in swine, in water sources on pig farms, and in pig handlers in Thailand.

MATERIALS AND METHODS

Study population. Between 11 and 15 January 1982 rectal swabs were collected from pigs at 57 pig farms under contract to the C-P Pig Co., Sri Racha, Thailand. Diarrhea was determined by the presence of dried liquid stool on the hindquarters of each animal. Persons living on the farm were asked whether they had diarrhea in the preceding week. Diarrhea was defined as three watery stools in a 24-h period. Rectal swabs were collected from all persons regardless of their history of gastrointestinal disease. On the same day, neighbors living on tapioca farms who did not raise pigs were also asked about recent diarrheal disease and cultured.

Water was collected from clay jars used for bathing and drinking purposes at homes of the pig and tapioca farmers. Water in clay jars used for bathing immediately after working in the pig barns was also investigated.

Processing of specimens. Each rectal swab obtained from pigs and persons living on the farms was transported in Cary-Blair medium, and inoculated onto two different pieces of nitrocellulose paper (Schleicher & Schuell Co., Keene, N.H.) layered on MacConkey agar. *E. coli* K-12 DHI(pCHL6) and *E. coli* K-12 Xac were included on each sheet as positive and negative controls for the ST-II enterotoxin gene probe. Two 100-ml samples of water collected in open-mouthed sterile glass bottles from clay jars used to store water were passed through 0.45- μ m nitrocellulose filters and processed as previously described (4). After incubation at 37°C overnight, the DNA of the resultant bacterial growth was fixed on the nitrocellulose paper (11).

Plasmid DNA was isolated from *E. coli* K-12 DHI(pCHL6) and cleaved with *Hind*III and *Hinf*I under conditions specified by the manufacturer (Bethesda Research Laboratories, Gaithersburg, M.D.) (7). DNA fragments were isolated by polyacrylamide gel electrophoresis of digested DNA, and the 460-base-pair fragment was removed by electroelution. The isolated DNA fragment encoding for ST-II was labeled with [α -³²P]deoxynucleotide triphosphate (New England Nuclear Corp., Boston, Mass.) by nick translation (8). Filtrates of water and sheets of nitrocellulose paper containing 30 to 40 separate specimens were hybridized with the α -³²P-labeled ST-II enterotoxin gene probe. The nitrocellulose papers or filters were then exposed to X-Omat AR X-ray film (Eastman Kodak Co., Rochester, N.Y.) with a single Cronex Lightening-Plus intensification screen (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) for 48 h at -70°C. All specimens were examined in duplicate with different preparations of the ST-II enterotoxin gene probe.

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RESULTS

The ST-II enterotoxin gene probe identified ST-II ETEC in 62 of 2,110 (3%) suckling pigs, 181 of 560 (32%) weaned pigs, and 4 of 457 (1%) adult pigs. Of 62 suckling pigs with ST-II ETEC infections, 21% had diarrhea, but none of 185 older pigs with this organism had diarrhea. ST-II ETEC was isolated from 13 (9%) of 146 suckling pigs with diarrhea compared with 49 (2.5%) of 1,964 suckling pigs without diarrhea ($P < 0.001$; chi-square analysis). ST-II ETEC was isolated from 7 of 80 litters of piglets with diarrhea.

ST-II ETEC was identified in pigs in 15 of 57 farms examined. Weaned piglets at nine farms were infected with ST-II ETEC, and suckling pigs at five of these nine farms were also infected. In comparison, suckling pigs were infected with ST-II ETEC in 6 of 48 farms that did not raise weaned piglets (5 of 9 versus 6 of 48) ($P < 0.005$).

Diarrheal disease occurred in 6 of 246 individuals at the pig farms and in 11 of 220 persons at the tapioca farms. None of these individuals with diarrhea were infected with ST-II ETEC.

ST-II ETEC was found in 1 of 246 persons living at pig farms but in none of 220 individuals at tapioca farms nearby. This organism was found in one farmer, and 8 of 13 litters of suckling pigs on his farm had ST-II ETEC-associated diarrhea. This farmer had not experienced any gastrointestinal symptoms in the preceding week.

Water collected from clay jars used to store drinking and bathing water, from bathing water at the barn at 57 pig farms (171 water specimens), and from jars used to store drinking and bathing water at tapioca farms (114 specimens) was examined for ST-II ETEC. Four specimens (three in bathing water at pig farms and one in bathing water outside of a pig barn) contained ST-II ETEC.

DISCUSSION

Rectal swabs collected from pigs on farms in Thailand were examined with a DNA probe for genes encoding for ST-II. Of 146 suckling pigs with diarrhea, 9% were infected with ST-II ETEC. Samples that hybridized with the ST-II probe were found more frequently in suckling pigs with diarrhea than in those without diarrhea. Of litters of piglets with diarrhea, 9% (7 of 80) were infected with ST-II ETEC. In a previous study of LT and ST-I ETEC in piglets with diarrhea in Thailand, ETEC, of which 79% were ST-I producers, was found in 32% of litters of piglets with diarrhea (12). These specimens were collected at different times and other locations in Thailand, and a comparison between these two studies must be made with caution.

In the present study, 32% of weaned piglets were infected with ST-II ETEC; none however had diarrhea. ST-II ETEC infections were more often found in suckling pigs in barns that housed weaned pigs, suggesting that weaned pigs might act as the reservoir of infection and that their removal from the suckling pigs environment may prevent transmission of ST-II ETEC to suckling pigs.

The role of ST-II ETEC as a cause of diarrhea in pigs or humans is uncertain. ST-II *E. coli* that produce neither LT nor ST-I have been isolated from piglets with diarrhea, but these isolates did not cause disease in experimentally infected piglets (9). In this study, ST-II ETEC was found more often in suckling pigs with diarrhea than without. This organism was also found in water used to bathe at pig farms, however, in only one instance was it detected in an asymptomatic farmer. This study suggests that ST-II ETEC may be an enteric pathogen in suckling pigs in Thailand. Further

studies of diarrheal disease among pigs and pig farmers are necessary before more definite conclusions can be drawn about the enteropathogenicity of ST-II ETEC in pigs or humans.

The DNA hybridization technique with cloned genes for ST-II provides a means of examining a large number of specimens for ST-II producing ETEC. This method is less time consuming and expensive than performing ligated pig intestinal loops. The DNA hybridization that has been used successfully in identifying LT and two different forms of ST-I ETEC (11) could also be used to expand the understanding of ST-II ETEC infection in swine and possibly in humans. This study indicates that *E. coli* containing the genes for ST-II (previously cloned by Lee et al. [7]) are prevalent among pigs in Thailand and appear to be associated with diarrhea in suckling pigs.

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