

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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OBJECTIVE : To define the prevalence of heat-labile II enterotoxigenic *Escherichia coli* in pigs, water, and people at farms in Thailand as determined by DNA hybridization.

BACKGROUND : The DNA hybridization assay employing a 460 base pair fragment of DNA encoding for ST-II enterotoxin was used to determine the prevalence of heat-stable II enterotoxigenic *Escherichia coli* (ETEC) in pigs, people, and water at 57 farms in Sri Racha, Thailand. ST-II ETEC infections were found in 248 (12%) of 2145 suckling pigs, none of 560 weanling, or 455 adult pigs examined. Evidence of ST-II ETEC was found in pigs at 13 of 32 (41%) farms with suckling pigs with diarrhea and at three of 25 (12%) farms with pigs of the same age without diarrhea ($p < 0.025$). ST-II ETEC was detected in water collected from three of 57 clay jars used to store bathing water at three pig farms, in one jar outside of the barn, and from one asymptomatic farmer at a pig farm. Evidence of this organism was not found in 245 other individuals living at the pig farms, or in 220 inhabitants and 114 water specimens at tapioca farms nearby. ST-II ETEC was associated with young pigs with diarrhea in Thailand, but was infrequent in man.

METHODS :

Study population : Between January 11 and 15, 1982 rectal swabs were collected from 2855 pigs at 57 separate pig farms under contract to the C-P swine company in Sri Racha, Thailand. Diarrhea was determined by the presence of dried liquid stool on the hind quarters of each animal. Inhabitants of the farms were asked whether they had developed diarrhea in the preceding week as defined as three watery stools in a 24 hour period. Rectal swabs were collected from all inhabitants regardless of their history of preceding gastrointestinal disease. On the same day, neighbors living in tapioca farms nearby which did not raise pigs were also asked about recent diarrheal disease and cultured. Water was collected from clay jars used to store bathing and drinking water at homes of both the pig and the neighboring farms. Water in clay jars used to bath after working in the pig barn was also investigated.

Processing of specimens : A rectal swab was obtained from each pig or inhabitant, inoculated into Cary-Blair media, and processed within four hours of collection. The swab was inoculated onto two different pieces of nitrocellulose paper (Schleicher and Schuell, Keene, NH) layered on MacConkey agar. *E. coli* K12 DHI (pCHL6) and *E. coli* K12 Xac were included on each sheet as positive and negative control for the ST-II enterotoxin gene probe. Two 100 ml aliquots of water collected in open mouthed sterile glass bottles from clay jars used to store water were passed through nitrocellulose 0.45 μ 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* K12 DHI (pCHL6) and cleaved with Hind III and Hinf I under conditions specified by the manufacturer (Bethesda Research Laboratories Inc., Gaithersburg, MD) (7,12, C.H. Lee et al. manuscript submitted for publication). 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 labelled *E. coli* with α -³²P deoxynucleotide triphosphate (New England and Nuclear, Boston, Mass.) by nick translation (8). Filtrates of water and sheets of nitrocellulose paper containing 30-40 separate specimens were hybridized with the α -³²P labelled ST-II enterotoxin gene probe. The nitrocellulose paper or filters were then exposed to X-Omat AR X-ray film (Eastman Kodak, Rochester, NY) with a single Cronex Lightning-Plus intensification screen (DuPont de Nemours, Wilmington, Del.) for 48 hours at -70°C. All specimens were examined in duplicate with different preparations of the ST-II enterotoxin gene probe.

The ST-II enterotoxin gene probe identified ST-II ETEC in 248 (12%) of 2145 suckling pigs, but was not found in 1015 older pigs examined. More than two piglets had physical evidence of diarrhea (dried liquid stool on their hind quarters) in 16 of 57 farms studied. ST-II ETEC infections in pigs, as determined by the DNA hybridization assay, was found in 13 of the 32 (41%) "diarrheal" farms, but in only three of 24 farms (12%) without diarrheal disease ($p < 0.025$).

Water collected from clay jars used to store drinking and bathing water, as well as bathing water at the barn at 57 pig farms and from jars at matched control farms (total 342 water specimens) were examined for ST-II ETEC. Four specimens (three bathing water at pig farms and one in bathing water outside of a pig barn) contained ST-II ETEC.

Evidence of ST-II ETEC was not found in 245 other individuals living at pig farms or in 220 inhabitants of tapioca farms nearby. Diarrheal disease occurred in six of 246 inhabitants at the pig farms and in 11 of 220 individuals at the tapioca farms. None of these individuals were infected with ST-II ETEC. Evidence of ST-II ETEC was found in one farmer, three of whose eight litters of suckling pigs had ST-II ETEC associated diarrhea. This individual had not experience any gastrointestinal disease in the preceding week.

In a previous survey of diarrheal disease in pigs in Thailand 40 percent of 58 litters of piglets under ten and 17 percent of 30 litters of pigs between ten and 21 days of age were infected with ETEC (Patamaroj, V, J Seriwatana, P Echeverria. Identification of enterotoxigenic *Escherichia coli* isolated from swine with diarrhea in Thailand by colony hybridization using three enterotoxin gene probes (submitted for publication). In that survey, however, *E. coli* were only examined for LT and ST-I ETEC. Our experience in Sri Racha in January 1982 indicated that ST-II ETEC was also frequently associated with diarrheal disease in young pigs in Thailand. Evidence of this organism was also found in bathing water at the pig farms however spread to man was unusual. In the one instance it did occur the infected individual did not develop diarrhea. Further studies of diarrheal disease among pig farmers are necessary before more definite conclusions can be drawn about the enteropathogenicity of ST-II ETEC in man.

The DNA hybridization technique with cloned genes for ST-II enterotoxin provided a means of examining a large number of specimens for this organism. The time and expense of performing such a study with ligated pig intestinal loops would have been considerable. This novel application of molecular genetic technology could be used to expand the understanding of ST-II ETEC infections in swine and possibly man.

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