Epidem. Inf. (1989), **103**, 497–511
Printed in Great Britain

Enterotoxigenic *Escherichia coli* in the domestic environment of a Malaysian village

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(*Accepted 12 June 1989*)

SUMMARY

The membrane-filter assay, GM1-ELISA, and DNA–DNA hybridization assay, were used to detect enterotoxigenic *Escherichia coli* (ETEC) in samples of water, weaning food, food preparation surface swabs, fingerprints of mothers, and the fingerprints and stools of children under 5 years of age, in 20 households in a Malaysian village. Weaning food and environmental samples were frequently contaminated by faecal coliforms, including ETEC. The membrane-filter assay detected and enumerated faecal coliforms and LT-ETEC in all types of water and weaning food samples. Highest concentrations of faecal coliforms and LT-ETEC were found in weaning food, followed by well-water, stored water and stored drinking water. The GM1-ELISA detected LT-ETEC in weaning food, food preparation surfaces, fingerprints and stool samples. The DNA–DNA hybridization assay detected a larger proportion of STa2-ETEC than the other toxotypes, either singly or in combination. All the assays in combination detected the presence of ETEC in all types of samples on at least one occasion in each household. It was not possible to classify households as consistently more or less contaminated with ETEC. On individual occasions it was possible to show a significant association of the presence of LT-ETEC between the fingerprints of children and their stools, fingerprints of mothers and children, and weaning food and the stools of the child consuming the food.

INTRODUCTION

In Peninsular Malaysia it has been estimated that 65% of the population have access to piped, treated water (1). Of the remainder, 80% are served by wells or hand-pumps and 19% by rivers and canals. There is also some use of rain-water and drain-water. Sanitation is available to about 82% of the total population, of

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which 37% use the pour-flush system, and 22% use bucket latrines or pit latrines. The remaining population use other forms of excreta disposal such as over-hang latrines or indiscriminate defecation.

Diarrhoeal diseases in Malaysia account for 3–5% of all admissions into government hospitals, with a case-fatality rate amongst those admitted of 2% (2). Diarrhoea is ranked third amongst the causes of death in children below 12 years of age (3).

It is now evident that enterotoxigenic *Escherichia coli* (ETEC) is an important cause of diarrhoeal disease in many parts of the world. It has been identified as one of the most common causes of diarrhoea in travellers to areas with poor standards of hygiene, being responsible for 50–70% of cases. In the developing world, with underlying malnutrition amongst the under 5 year olds, ETEC may be responsible for up to 25% of diarrhoeal cases, some of which result in death (1–2%). In addition, ETEC has also been identified as a cause of common-source outbreaks due to faecally contaminated water and food.

In this study we report the identification of environmental sources of ETEC in a Malaysian village using selected ETEC assays. The assays were selected for their superior sensitivity and specificity as revealed in a large-scale laboratory evaluation (4). This investigation enabled us to evaluate the suitability of these assays to screen for ETEC in specific samples under field conditions. Additionally, the relative ease of performing these assays, and problems associated with assay procedures, were assessed so that modifications could be introduced to optimize the performance of the assays under field conditions.

MATERIALS AND METHODS

Selection of study village

A number of villages, at distances between 10 km and 60 km from the laboratories of the Department of Medical Microbiology, University of Malaya, were visited for the selection of a suitable village. The selection of a study village was based on the following criteria:

(i) Defined as a slum or squatter village.
(ii) No treated water supply or sewerage.
(iii) No introduction of a health education or nutritional programme.

The urban squatter village, Kampung Kenangan, Sungei Penchala, Selangor located about 10 km from the laboratory was selected. This comprised 75 houses with a total population of about 400. All houses had a supply of electricity.

Treated water was not available in the village. The village was served primarily by dug wells, some of them fitted with electric pumps. There was only one hand pump. Most of the householders had dug their own wells which were usually located in the washing/bathing area of their own houses or formed part of an enclosed communal washing/bathing area shared by four to five families. In more affluent households wells were fitted with an electric pump and well water was not stored. Others, who used a bucket-system to obtain water from the wells, stored water in plastic buckets in the washing/bathing area of their houses. Most households had pour-flush latrines. These drained into surface drains, a stream or pits. There were also a few overhang latrines in the village. Most houses had their

own latrines but some were shared amongst an average of four families. A bucket of water was usually kept in the latrine for washing after defecation and flushing faecal matter. The general sanitary condition of the village was good but differed considerably between individual households.

Selection of study cohort

The criterion used for selection of study households was the presence of at least one child under 5 years of age. Every fourth name on the village register was selected and the headman was asked if there was a child under 5 years of age present in that household. When the list was exhausted, the second name on the register was taken and from then on every fourth name was selected. This was continued until 32 households had been selected. From these 32 households, 20 were randomly selected. The remaining 12 households were kept as reserves, and one was subsequently included because of the unwillingness of one household to participate. The 20 households thus selected became the 'study households'. All children (38) under 5 years of age and their mothers (20) became the study cohort.

Samples for microbiological investigation

Each study household was visited on the same morning each week for 12 weeks. Visits commenced at 9.00 a.m. and were completed by noon. The morning was selected in order that freshly collected water samples (the earliest water collection time was dawn) and recently prepared weaning foods could be collected and processed in the laboratory on the same day. During the study, a strict collection regime was followed to eliminate variation in the collection procedure amongst households. All samples, except stools, were collected in sterile containers and transported to the laboratory in ice-boxes. The stool samples of children were collected by their mothers. All samples were processed in the laboratory 4–6 h after collection. The types of samples collected are described below.

Water

Three types of water samples were collected: well-water, stored water and stored drinking water.

Well-water. Water was collected from the well used by each household. This was the main source of water available for all purposes such as preparation of food, cooking, washing and bathing.

Stored water. All households, with the exception of those with electric pumps, stored well-water and/or rainwater in large drums or plastic buckets. Storage containers were covered or uncovered and kept within either the kitchen or bathing area.

Stored drinking water. This included well-water, filtered well-water, or rainwater. Water used for drinking was said to be boiled and cooled before being dispensed into a special container and stored. These containers were then replenished with freshly boiled water when they became half or almost empty.

Weaning food

Only infant formulae and weaning food (as distinct from adult food) was collected in this study. Of the 20 study households, 4 included infants receiving

infant formulae, 1 included an infant receiving infant formula and a commercial cereal, and 1 included an infant fed on home prepared rice-broth. Foods prepared for these 6 infants only were collected weekly for 12 weeks.

Food preparatory surface swabs

In all study households, food was washed and prepared for cooking on the floor of the washing/bathing area (which was usually the apron of the well) or in kitchen sinks, for those who had such a facility. A swab of a small area of the apron or sink was taken from all study households and transported, in Stuarts' transport medium (Oxoid, Basingstoke), to the laboratory.

Fingerprints

The tips of the fingers and thumbs of both hands of mothers and children from the study households were printed directly on to MacConkey agar (Oxoid, Basingstoke) plates and taken to the laboratory for incubation.

Stools

Stools were collected only from the study children of the study households. Stool cups were distributed one day before collection and mothers were instructed to collect the next day's stool. A field assistant visited the village again later in the day to collect additional stool samples that were not available during the morning.

Microbiological methods

The methods used for processing each sample were kept constant throughout the study and are described below.

Water. All water samples were enumerated for faecal coliforms by the membrane filtration technique (5). The membranes were then used to screen for LT-ETEC by the membrane filter assay (6). It was necessary to use appropriate volumes of water to obtain under 300 colonies per membrane so that the membranes could be used in the membrane-filter assay. Membranes used in the assay were only those that contained individually distinct colonies. Those that had a confluent matt of growth or numerous pin-point colonies were not used. In addition, membranes that had growth towards the outer edge were not used as these would have contaminated the Biken agar (7) in the second stage of the assay. The DNA–DNA hybridization assay was also used to detect LT, STa1 and STa2 ETEC (8, 9).

Weaning food. Weaning food samples were processed as described by Barrell & Rowland (10) based on the method of Thatcher & Clark (11). 1 ml of milk or 0·1–0·2 g of cereal or rice-broth was added to 1% peptone water (Gibco, UK) to give a 1 in 10 suspension and homogenized. Cereal and rice broth homogenates were serially diluted to obtain log dilutions of 10^{-2}, 10^{-3} and 10^{-4}. These dilutions were then treated in two different assays to enumerate faecal coliforms and ETEC in 1 ml of sample.

Membrane filtration technique. 1 ml sample at each dilution was filtered and cultured to obtain faecal coliform counts. Membranes were then used in the membrane-filter assay (6).

Miles and Misra counts. 20 μl of each dilution was dropped in duplicate onto MacConkey agar (Oxoid, Basingstoke) using a 50-dropper Pasteur pipette (12).

The agar plates were incubated at 44 °C. Following overnight incubation pink, lactose-fermenting, non-mucoid colonies characteristic of *E. coli* were counted and recorded. From each plate containing all three dilutions, 1 to 10 colonies were picked, pooled, and stored on fresh MacConkey agar to prepare culture supernatants for use in the GM1-ELISA (13, 14). In addition the pooled colonies were used for the DNA–DNA hybridization (8, 9).

Food preparation surface swabs, fingerprints and stools. Each of these samples was cultured onto MacConkey agar (Oxoid, Basingstoke) and incubated at 44 °C. Following overnight incubation, between 1 and 10 pink, lactose-fermenting, non-mucoid colonies, characteristic of *E. coli* were picked, pooled and stored on MacConkey agar at 4 °C. These were used to prepare culture supernatants for use in the GM1-ELISA (13, 14). In addition the pooled colonies were used for the DNA–DNA hybridization assay (8, 9).

Detection methods for ETEC

Preparation of culture supernatants. Cell-free bacterial culture supernatants were prepared for use in the GM1-ELISA, according to WHO recommendations (15), with the exception that only 3 ml of tryptone soy broth (Oxoid, Basingstoke) containing 1·2% yeast extract (Difco, UK) was used. The supernatants were harvested and stored at −2 °C and used within 3 days of preparation.

Antisera. The following antisera were used: unpurified anti-cholera toxin (anti-CT, University of Surrey; titre 256000) and affinity purified anti-LT (World Health Organization, 1211 Geneva 27, Switzerland; titre 600). Titres were determined by ELISA titration against CT at a concentration of 1000 ng/ml of phosphate buffered saline.

Membrane-filter assay. Membranes obtained from water and food samples were screened for LT-producing ETEC by the membrane-filter assay (6).

GM1-ELISA. Isolates pooled from weaning food, food preparation surface swabs, fingerprints, and stool samples were screened for LT-producing ETEC by this assay. In addition, *E. coli* strain B8, from the Biken collection (16), and cholera toxin (Sigma Chemicals, UK) at concentrations of 1–1000 ng/ml were used as the positive controls. *E. coli* strain WF5 (University of Surrey) was used as the negative control.

The assay was performed as described by Miller and colleagues (14), as modified from the method of Sack and colleagues (13). However, the substrate used by Miller and colleagues (14) reacted very poorly in the field and the colorimetric changes between negative and positive control wells were indistinguishable. A commercially produced orthophenylene diamine dihydrochloride (OPD) tablet and buffer preparation (Abbott Labs, USA) was therefore used instead. This reagent halved the reaction time from 20 min, as per Miller's protocol, to 10 min, at 37 °C and distinct colorimetric changes were obtained between negative and positive control wells. The optical density (OD) values of the samples were read by a Dynatech MR600 ELISA Reader at a wavelength of 492 nm. The positive-negative value was calculated according to Vadivelu and colleagues (4).

DNA–DNA Colony hybridization assay. The assay was performed as described by Moseley and colleagues (9) modified from the method of Grunstein & Hogness (8). This assay was used to screen for the genes coding for LT, STa1 and STa2 toxins

in isolates obtained from all samples. The preparation of 'isolate DNA' on nylon filters was carried out in Kuala Lumpur and the hybridization assay using radiolabelled DNA probes was carried out in London.

The LT probe, derived from an LT recombinant plasmid pAT153.H6, consists of a 0·8 kilobase pair *Hin* dIII generated fragment coding for LT (o 17). The STa1 probe derived from STa1 recombinant plasmid pR1T10.130 consists of an 157 bp, *Hin* fI generated fragment coding for STa1 (9, 18, 19). The STa2 probe derived from an STa2 recombinant plasmid pR1T10.250 consists of a 600 bp *Hae* III generated fragment coding for Sta2 (20, 21). The LT, STa1 and STa2 coding fragments were radiolabelled *in vitro* with ^{32}P using the random oligonucleotide-primed DNA radiolabelling method (22). The specific activities of the DNA probes were: (*a*) LT probe – $9{\cdot}0 \times 10^7$ cpm/μg DNA, (*b*) STa1 probe – $7{\cdot}3 \times 10^7$ cpm/μg DNA, (*c*) STa2 probe – $8{\cdot}4 \times 10^7$ cpm/μg DNA.

RESULTS

Detection of LT-ETEC by membrane-filter assay

The membrane-filter assay was used to screen for LT-ETEC in water and weaning food samples. The distribution of concentrations of faecal coliforms per 100 ml of well water, stored water, stored drinking water, and per 100 ml or per 100 g of weaning food samples are shown in Fig. 1. No faecal coliforms were detected in 8% of well water samples, 10% of stored water samples, 17% of stored drinking water samples and 6% of weaning food samples. The remaining samples had contamination levels per 100 ml of between 100 and 10 million for well (mean 3×10^4) and 100 and one million for stored water (mean 3×10^4), 10 and 100000 for stored drinking water (mean 3×10^3) and for weaning food between 100 and 10^9 per 100 ml or 100 g.

Table 1 summarizes, for each type of sample, the numbers of samples tested, the proportions positive and the range of concentrations per 100 ml or 100 g for both faecal coliforms and LT-ETEC. The upper end of the range of faecal coliform concentrations in each type of sample is related to the cut-off value for numbers of colonies present on a membrane-filter (300 per filter) that was suitable for use in the membrane-filter assay. Analysis of the relationship between the concentration of faecal coliforms and the LT-ETEC positivity rates is shown in Table 2. The results strongly suggest that there is no relationship between faecal coliform concentration and percentage of LT-ETEC positive samples detected.

Detection of LT-ETEC by GM1-ELISA

The GM1-ELISA was used to detect LT-ETEC in samples of weaning food, food preparation surface swabs, fingerprints of mothers and children, and stools of children. Table 3 summarizes for each type of sample the numbers of samples tested and the percentage of samples positive for each of faecal coliforms and LT-ETEC. The percentage of samples that had no faecal contamination were 17% for weaning food samples, 3% for food preparation surface swabs, 21% for mother's fingerprints, and 31% for children's fingerprints. The percentage of samples positive for LT-ETEC was between 19% for children's fingerprints and 32% for food preparation surfaces.

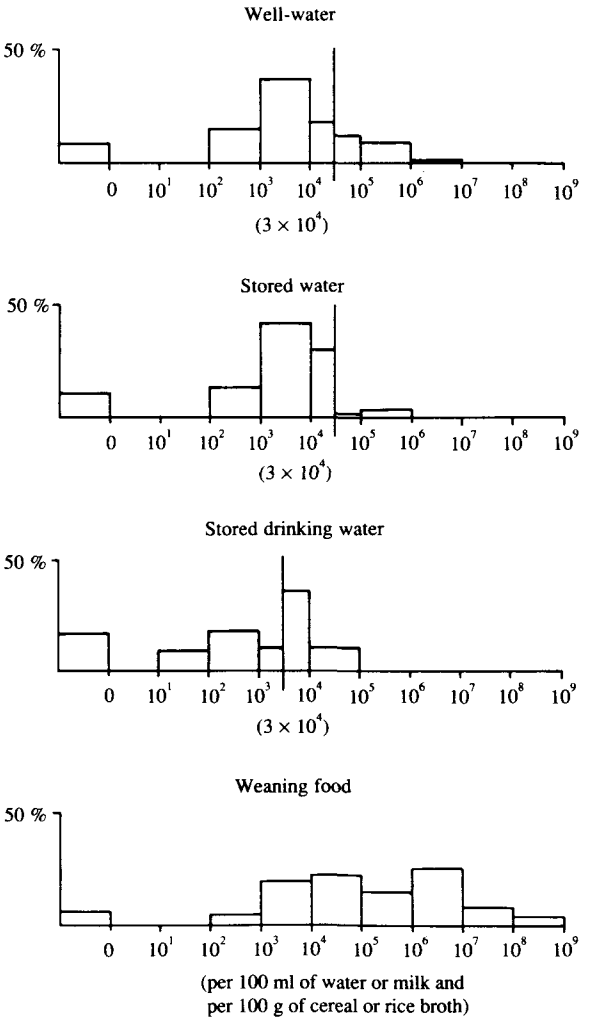

Fig. 1. The numbers of faecal coliforms in samples of various waters and of weaning foods including milk, cereal broth and rice broth.

Table 1. *Faecal coliform and LT-ETEC isolation by membrane-filter assay*

Type of sample	No. of samples satisfactorily tested for both faecal coliforms and LT-ETEC	Faecal coliforms		LT-ETEC	
		No (%) samples +ve	Range per 100 ml/100 g	No (%) samples +ve	Range per 100 ml/100 g
Well-water	104	86 (83)	0–20000	23 (22)	0–5300
Stored water	83	66 (80)	0–19800	11 (13)	0–1400
Stored drinking water	77	42 (55)	0–10000	14 (19)	0–200
Weaning food	31	26 (83)	0–10^8	5 (16)	0–200000
Commercial milk	22	22 (100)	0–10^7	3 (14)	0–200000
Commercial cereal	4	3 (75)	0–10^8	2 (66)	0–200000
Rice-broth	5	1 (20)	0–10^7	0 (0)	0

https://doi.org/10.1017/S0950268800030909 Published online by Cambridge University Press

Table 2. *Relationship between faecal coliform counts and detection of ETEC in water samples*

Faecal coliform concentration per 100 ml	No. of samples	No. (%) ETEC+ve
1–99	13	3 (23)
100–999	51	14 (27)
1000–9999	106	27 (25)
10000–20000	19	4 (21)

Table 3. *Faecal coliform and LT-ETEC isolation by GM1-ELISA*

Type of sample	No. of samples satisfactorily tested	Faecal coliform. No. (%) samples +ve	LT-ETEC. No. (%) samples +ve
Weaning food	58	48 (83)	12 (21)
Food preparation surfaces	206	199 (97)	65 (32)
Fingerprints (mother)	205	162 (79)	40 (20)
Fingerprints (children)	389	267 (69)	74 (19)
Stools	273	273 (100)	77 (28)

Table 4. *Detection of LT, STa* and LT/STa* ETEC by the DNA–DNA hybridization assay*

Type of samples	No. of samples tested	ETEC. No. (%) samples +ve	LT-ETEC. No. (%) samples +ve	STa-ETEC. No. (%) samples +ve	LT/STa-ETEC. No. (%) samples +ve
Well-water	38	16 (42)	0 (0)	16 (42)	0 (0)
Stored water	36	18 (50)	0 (0)	18 (50)	0 (0)
Stored drinking water	16	12 (75)	0 (0)	8 (50)	4 (25)
Weaning food	52	38 (73)	0 (0)	36 (69)	2 (4)
Food preparation surface swabs	192	107 (56)	3 (2)	101 (53)	3 (2)
Fingerprints (mothers)	162	29 (18)	1 (1)	28 (17)	0 (0)
Fingerprints (children)	254	42 (16)	2 (1)	40 (15)	0 (0)
Stools	*256*	*84 (33)*	*18 (7)*	*62 (24)*	*4 (2)*

* STa includes both STa1- and STa2-ETEC.

Detection of LT and STa-ETEC by DNA–DNA hybridization assays

Isolates from all samples tested by the membrane-filter assay or GM1-ELISA were stored on MacConkey agar at +4 °C, prior to screening for the presence of LT, STa1 and STa2 genes by the DNA–DNA hybridization assay. These results are presented in Tables 4 and 5. There was considerable loss of viability during storage, especially of isolates from the membrane-filter assay. This is the reason for the difference between the number of samples tested by DNA–DNA hybridization assay (Table 4) and the numbers tested by the other two assays (Tables 1 and 3). STa2 was the dominant toxotype detected.

Table 5. *Toxotypes of ETEC as identified by the DNA–DNA hybridization assay*

Type of samples	No. of samples tested	LT only	STa1 only	STa2 only	LT/STa1	LT/STa2	STa1/STa2	LT/STa1/STA2
Well-water	38	0	0	16	0	0	0	0
Stored water	36	0	0	17	0	0	1	0
Drinking water	16	0	0	8	0	2	0	2
Weaning food	52	0	0	26	0	1	10	1
Food preparation surface	192	3	3	95	0	3	3	0
Fingerprints (mothers)	162	1	1	24	0	0	3	0
Fingerprints (children)	254	2	2	33	0	0	5	0
Stools	256	18	3	52	1	2	7	1

https://doi.org/10.1017/S0950268800030909 Published online by Cambridge University Press

Table 6. *ETEC positive samples as detected by all assays in all study households*

House no.	Well-water	Stored water	Drinking water	Weaning food	Surface	Fingerprints (mothers)	Fingerprints (children)	Stools
1	3/8 (37)	4/7 (57)	1/3 (33)	N/A	6/11 (54)	0/11 (0)	5/22 (23)	5/13 (38)
2	3/7 (43)	1/6 (17)	1/2 (50)	N/A	7/11 (64)	1/9 (11)	2/10 (20)	3/7 (43)
5	1/4 (25)	3/7 (43)	1/3 (33)	N/A	5/11 (45)	5/11 (45)	2/11 (18)	3/5 (60)
6	5/9 (55)	1/5 (20)	0/2 (0)	5/8 (62)	6/10 (60)	2/10 (20)	5/20 (25)	7/14 (50)
7	0/6 (0)	0/4 (0)	0/4 (0)	7/19 (39)	9/11 (82)	3/10 (30)	6/21 (28)	11/19 (58)
9	0/2 (0)	3/5 (60)	2/6 (33)	8/9 (89)	8/10 (80)	2/10 (20)	3/21 (14)	6/12 (50)
13	0/5 (0)	1/6 (17)	1/9 (11)	N/A	4/10 (40)	2/8 (25)	9/21 (43)	8/16 (50)
14	— —	— —	2/3 (67)	N/A	7/11 (64)	2/11 (18)	4/22 (18)	9/17 (53)
15	0/5 (0)	1/8 (12)	0/5 (0)	N/A	10/11 (91)	3/11 (27)	6/21 (28)	4/9 (44)
16	0/6 (0)	1/3 (33)	0/3 (0)	6/11 (54)	4/10 (40)	5/11 (45)	9/22 (41)	12/20 (60)
20	2/6 (33)	4/9 (44)	0/3 (0)	N/A	8/11 (73)	4/11 (36)	4/11 (36)	4/5 (80)
21	3/5 (60)	3/6 (50)	8/11 (73)	8/11 (73)	6/11 (54)	4/10 (54)	4/30 (13)	7/23 (30)
22	2/6 (33)	N/A	1/4 (25)	N/A	6/10 (60)	4/10 (40)	4/19 (21)	3/19 (16)
24	3/4 (75)	0/4 (0)	0/3 (0)	N/A	5/8 (62)	1/8 (12)	3/14 (21)	8/10 (80)
25	3/3 (100)	1/2 (50)	— —	N/A	8/10 (80)	3/10 (30)	4/21 (19)	2/5 (40)
26	0/5 (0)	0/7 (0)	1/5 (20)	N/A	6/11 (54)	6/11 (54)	8/22 (36)	4/15 (27)
27	1/3 (33)	1/6 (17)	— —	N/A	7/11 (64)	4/11 (36)	7/33 (21)	13/27 (48)
28	0/7 (0)	N/A	4/7 (57)	N/A	8/11 (73)	3/11 (27)	5/22 (23)	12/17 (70)
29	3/4 (75)	N/A	1/5 (20)	0/8 (0)	6/11 (54)	2/11 (18)	2/11 (18)	4/9 (44)
30	1/7 (14)	N/A	1/6 (16)	N/A	7/11 (64)	4/10 (40)	2/10 (40)	3/6 (50)

N/A, Sample not available from household; —, Samples not tested for ETEC; ( ), Numbers in parentheses are the percentages of positive samples.

ETEC contamination considered by household

The proportions of samples that were positive for ETEC as detected by any of the three assays are shown in Table 6. In order to investigate the possibility of consistently more and less contaminated households, households were ranked by contamination of food preparation surfaces, fingerprints of mothers and children, and stools of children, and the ranking compared by Spearman's rank correlation test. There was no significant correlation between the rankings from any two types of samples and contamination by ETEC was found not to be consistently associated with particular households.

Although an individual household does not tend to be either consistently contaminated or uncontaminated through time, it is possible that on any one day of sampling, there may be a relationship between the chances of a sample being positive and the chances of other samples from the same household also being positive. Thus, if a child's stool is positive for ETEC on a particular day, it may be that the fingerprints from the same child on the same day are also likely to be positive. Such relationships were investigated by constructing a series of 2×2 tables (not shown). This was done for LT-ETEC and STa-ETEC separately and for the following comparisons: child's stool *vs.* child's fingerprints; child's stool *vs.* mother's fingerprints; child's stool *vs.* drinking water; child's stool *vs.* weaning food; child's stool *vs.* food preparation surface; mother's fingerprints *vs.* weaning food; mother's fingerprints *vs.* food preparation surface; mother's fingerprints *vs.* child's fingerprints.

These analyses show a significant relationship between LT-ETEC in child's stools and child's fingerprints, child's stools and weaning food, and child's fingerprints and mother's fingerprints ($P < 0{\cdot}05$, χ^2 test). No significant associations were found for the other comparisons or for STa-ETEC.

DISCUSSION

Although the importance of ETEC, as a major cause of diarrhoea, has long been established, the environmental vehicles of ETEC transmission have been poorly defined. Studies have been carried out to investigate the importance of water and food in the transmission of ETEC (23–25). In these studies, toxin assays which included tissue-culture and animal models were used for the detection of LT and STa. More recently DNA–DNA hybridization assays have been used for these purposes (26, 27). The problems associated with using these assays to investigate ETEC transmission in epidemiological studies are highlighted by the small number of such studies undertaken and the small number of samples screened in each of these studies. Although, to date, many assays have been developed for ETEC, most of them are not suitable for field laboratories because of their complexity and cost.

In this study the performance under field conditions of selected ETEC assays to identify potential environmental vehicles of ETEC transmission was investigated. The ETEC assays, which include the membrane-filter and GM1-ELISA assays for the LT and the DNA–DNA hybridization assay for the detection of the genes encoding for LT, STa1 and STa2, were selected on the basis of a previous large-scale laboratory evaluation (4).

Using the membrane filter assay, the distribution of concentrations of faecal coliform per 100 ml of water samples, and per 100 ml or 100 g of weaning food samples, was highest for weaning food, followed by well water, stored water and stored drinking water (Fig. 1). Fewer samples could be tested for the presence of LT-ETEC because of the unsuitability of membranes which either had a matt growth of colonies or growth towards the outer edge of the membrane. Of the samples tested for LT-ETEC, the range of per 100 ml of water sample, or per 100 ml/100 g of weaning food sample, is shown in Table 1. The LT-ETEC contamination levels in the various samples occurred in the same rank order as that of faecal coliform concentrations. Thus, a higher range of LT-ETEC concentration was detected in weaning food samples which contained a high faecal coliform range. The lowest ranges for both were detected in the stored drinking water samples.

The high concentrations of faecal coliforms and LT-ETEC in weaning food samples may have resulted from water that was used to prepare food. Although prepared with 'hot' water, the weaning food is usually topped up with stored drinking water. This has been shown to contain faecal coliforms and LT-ETEC. Weaning foods form a good substrate for the multiplication of organisms between preparation of food and collection of the sample, which ranged from approximately 30 min to 2 h. The temperature at which they were kept (30 and 35 °C) is ideal for bacterial proliferation. Such temperature-related proliferation, combined with faecal contamination of water used, may account for the higher counts in weaning food than water samples (23). The detection of LT-ETEC in water and weaning food samples was independent of faecal coliform concentration (Table 2).

Using the GM1-ELISA, 21% of weaning food samples tested were positive for LT-ETEC and these were prepared using commercial formulae. Rice broth (home-prepared) did not contain any faecal coliforms. This probably reflects the temperatures reached during boiling of rice to prepare broth. When comparing the results obtained for weaning food by the membrane-filter assay and GM1-ELISA, the GM1-ELISA detected a higher positive proportion. This may be attributed to the higher level of sensitivity of the GM1-ELISA (4). LT-ETEC were present in similar proportions on fingerprints of both mothers and children; 20% for mothers and 19% for children. 28% of stools of children contained LT-ETEC. The highest positivity rate for LT-ETEC was on food preparation surfaces (32%), which were usually the apron of the well.

The DNA–DNA hybridization assay was carried out to detect the presence of the genes encoding for LT, STa1 and STa2 isolates. However, fewer samples were tested due to the considerable loss of viability of isolates during storage. STa-ETEC appear to have occurred in higher proportions than LT or LT/STa-ETEC (Table 4). Further toxin typing of these isolates indicates that STa2-ETEC occurred more commonly than the other toxotypes (LT and STa1), singly or in combination with LT or STa1. This suggests the widespread presence of STa2-ETEC in this environment.

The presence of ETEC in households as detected by all assays indicate that ETEC contamination occurred on at least one occasion in any one type of sample. Spearman's ranking correlation test performed on food preparation surfaces, fingerprints of mothers and children, and stools of children, indicated that there

was no significant correlation for particular households between their ranking on ETEC contamination of any two types of samples. Thus ETEC contamination was not found to be consistently associated with any particular household over time.

However, it was possible to illustrate on any one occasion the association of LT-ETEC between two types of samples. Significant associations were shown between the presence of LT-ETEC on a child's fingers and in his/her stool; in weaning food and in the stool of the child that consumed the food; and on a mother's fingerprints and on that of her children. Although STa-ETEC were found in similar proportions to LT-ETEC there were no similar associations between any two types of samples.

STa2-toxigenic *E. coli* occurred in greater abundance than STa1. Most of the strains that were not STa2-toxigenic only, had either LT and or STa1 in combination with STa2. Approximately 10% of toxigenic strains (34/346) were not STa2 toxigenic. These were either LT, LT/STa1 or STa1 (Table 4). Unlike the LT plasmid, which occurs in single copy number, and is non-conjugative and easily lost on subculture, the STa2 occurs in multiple copy numbers and is less affected by subculture. This may be the reason that in many studies of ETEC, STa-ETEC have occurred in higher numbers than LT-ETEC (28–31).

Although 28·2% of stools of children were positive for ETEC, only one case of diarrhoea was reported during the entire study. This suggests that asymptomatic carriage of ETEC was occurring in this community.

Finally, this study has indicated the problems of selecting assays and carrying out assay procedures under field conditions. As these are environmental isolates, an assay selected should require minimal subcultures so that toxigenic *E. coli* are not underestimated due to loss of the LT coding plasmid. Subculturing of isolates and long term storage on selective medium may have caused the loss of the LT plasmid. Additionally the viability of many isolates was also lost during long-term storage on selective medium.

The assay enabled the screening of LT-ETEC amongst all faecal coliform colonies isolated. Using conventional assays the chances of missing small numbers of ETEC would have been great. The GM1-ELISA was useful for detecting LT producing ETEC in fingerprints, food preparation surfaces and stool samples, where enumeration of ETEC was not necessary. With the DNA–DNA hybridization assay, introduction of replica plating of isolates directly from the primary culture onto nylon fibres could have preserved LT plasmids and viability.

ACKNOWLEDGEMENTS

This study was supported by a Medical Research Council Grant, G8324074/T.

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