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Two-Year Study of Endemic Enteric Pathogens Associated with Acute Diarrhea in New Caledonia

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A longitudinal study of diarrheal disease among patients of all ages with acute diarrhea was carried out in New Caledonia from January 1990 to December 1991. Stool samples from 2,088 diarrheal patients were examined for parasites, rotavirus, and bacterial pathogens. Potential sources of contamination (drinking water, seawater and bovine and porcine feces) were investigated. One or more enteric pathogens were identified in 41.8 and 40.6% of the persons with diarrhea, in 1990 and 1991, respectively. *Salmonella* spp., *Shigella* spp., HEp-2 cell adherent *Escherichia coli* (diffuse adherent and enteroaggregative), enteropathogenic *E. coli* (EPEC) (EPEC adherence factor-positive strains belonging to classical serotypes), localized adherent *E. coli* (non-EPEC), and enterotoxigenic *E. coli* were the frequently identified enteropathogenic bacteria. Other major enteropathogens were *Entamoeba histolytica* and *Giardia lamblia*. *Campylobacter jejuni*, *Clostridium difficile*, *Clostridium perfringens*, *Yersinia enterocolitica*, and rotavirus were isolated from only a few patients. No *Vibrio* spp., *Aeromonas* spp., *Plesiomonas* spp., Shiga-like-toxin-producing *E. coli*, enterohemorrhagic *E. coli*, or enteroinvasive *E. coli* were identified. Shiga-like toxin I-producing *E. coli* were present in adult bovines and calves, and heat-stable enterotoxin II-producing enterotoxigenic *E. coli* were found in pigs.

Studies on diarrhea in the South Pacific have focused on children and have not been able to identify many different types of enteric pathogens (1). Numerous bacterial enteropathogens, especially diarrheagenic *Escherichia coli*, have been implicated in regional disease episodes (28); however, their incidence appears to vary according to local geography, the epidemiological characteristics of the study site, and the resident populations involved (12). Furthermore, it is known that diarrheal diseases are an important health problem in developing countries located in the tropical zone (12). Although New Caledonia is a developed French overseas territory located in this area, diarrheal diseases continue to be a health problem. Consequently, a longitudinal survey was carried out to determine the etiology of diarrheal diseases in children and adults; and potential sources of contamination have been investigated.

MATERIALS AND METHODS

Study population. From January 1990 to December 1991, patients admitted with acute diarrheal disease to the territory's Gaston Bourret Hospital were studied to determine the etiology of their illnesses. Patients of all ages with three or more loose or watery stools per day were selected as subjects. Hospital policy emphasized treatment of diarrhea with rehydration alone until culture results were obtained, and so antibiotic treatment following admission was not a factor; however, self-treatment prior to admission cannot be completely excluded.

Isolation and identification of enteric pathogens. No transport media were used; specimens were quickly examined in the laboratory (about 1 h after collection). Stools were cultured daily for *E. coli*, *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Aeromonas* spp., *Plesiomonas* spp., *Vibrio* spp. and *Campylobacter* spp. by standard methods (28). *E. coli* isolates were screened for the following virulence factors: heat-labile (LT) (9) and heat-stable (ST) (8) enterotoxins and Shiga-like toxin (SLT) (15). Antisera to purified SLT-I were prepared in New Zealand White rabbits, to perform seroneutralization. The SLT-I preparation used was purified from *E. coli* E40705 (O157:H7) kindly provided by B. Rowe (Public Health Laboratory Service, London, England) by following exactly the procedure described by O'Brien and LaVeck (20). Individual isolates were also tested for classic enteropathogenic *E. coli* (EPEC) serogroups as described in a World Health Organization manual (27) and for HEp-2 cell adherence in an attempt to detect localized adherent (LA), diffuse adherent, or aggregative adherent strains (17, 27). Fibroblast cell monolayer cultures were used to detect the cytopathic toxins of *Clostridium difficile* in stools by the method of Kim et al. (13). The specificity of the toxin was determined by demonstrating neutralization of the cytopathic effect of positive specimens by using antiserum to *C. difficile* toxin kindly provided by M. Popoff (Service des Anaérobies, Institut Pasteur, Paris, France). The detection of *C. perfringens* enterotoxin in stool specimens was used for the diagnosis of *C. perfringens* enteritis (18). Rotavirus was identified with a commercial latex agglutination test (Diagnostics Pasteur). Fresh stool specimens were treated with saline and iodine preparations and examined for intestinal parasites; furthermore, formalin-ether concentrates and smears stained with Merthiolate-iodine-formaldehyde solution were also prepared from each of the specimens and examined microscopically.

Full serotypes of EPEC and enterotoxigenic *E. coli* (ETEC) strains were determined with O- and H-specific antisera (from collections in the Pasteur Institutes in New Caledonia and in France) (22). When O- and H-specific antisera were not available from the Pasteur Institute, O and H antigens were identified at the International Center of *Escherichia* and *Klebsiella*, Statens Seruminstitut, Copenhagen, Denmark.

All *E. coli* strains were blotted onto separate pieces of nitrocellulose filters (BA85; Schleicher & Schuell) in order to be hybridized (2) with the following DNA probes: SLT-I (4),

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TABLE 1. Incidence and distribution by area of enteric pathogens, in 1990 and 1991, in patients with acute diarrhea in New Caledonia[a]

Pathogen	No. of cases (%) with identified pathogens	No. (%) of cases in:	
		Urban areas	Rural areas
Salmonella spp.	128 (6.1)	28 (2.2)	100 (12.4)
Shigella spp.	62 (3)	20 (1.6)	42 (5.2)
EPEC	24 (1.1)	19 (1.5)	5 (0.62)
ETEC	37 (1.8)	25 (1.95)	12 (1.5)
EAEC	333 (15.9)	268 (20.9)	65 (8.04)
Campylobacter jejuni	2 (0.1)	2 (0.16)	0
Clostridium difficile	2 (0.1)	2 (0.16)	0
Clostridium perfringens	2 (0.1)	2 (0.16)	0
Yersinia enterocolitica	1 (0.5)	1 (0.08)	0
Rotavirus	16 (0.76)	7 (0.55)	9 (1.1)
Entamoeba histolytica	73 (3.5)	42 (3.3)	31 (3.8)
Giardia lamblia	164 (7.8)	96 (7.5)	68 (8.4)
Multiple	17 (0.8)	14 (1)	3 (0.4)
Total[b]	844 (40.4)	512 (40)	332 (41.1)

[a] There was a total of 2,088 patients (1,280 from urban areas and 808 from rural areas).
[b] Multiple pathogens were excluded from the calculation.

TABLE 2. Detection rates of enteropathogenic agents from persons with diarrhea in New Caledonia in 1990 and 1991[a]

Pathogen	No. (%) of cases in:		
	Children under 15 yr	Adults	Total
Salmonella spp.	24 (1.4)	104 (24.7)	128 (6.1)
Shigella spp.	18 (0.9)	44 (10.4)	62 (2.9)
EAEC	317 (19)	16 (3.8)	333 (15.9)
LA (non-EPEC)	137 (8.2)	3 (0.7)	140 (6.7)
Diffuse adherence	140 (8.4)	9 (2.1)	149 (7.1)
Aggregative adherence	40 (2.4)	4 (0.9)	44 (2.1)
ETEC	37 (2.2)	0	37 (1.8)
EPEC	23 (1.4)	1 (1.02)	24 (1.1)
Campylobacter jejuni	0	2 (0.5)	2 (0.09)
Clostridium difficile	2 (0.1)	0	2 (0.09)
Clostridium perfringens	2 (0.1)	0	2 (0.09)
Yersinia enterocolitica	1 (0.05)	0	1 (0.05)
Rotavirus	16 (0.96)	0	16 (0.76)
Entamoeba histolytica	65 (0.48)	8 (1.9)	73 (3.5)
Giardia lamblia	151 (9.1)	13 (3.1)	164 (7.8)
Multiple	14 (0.8)	3 (0.7)	17 (0.8)
Total[b]	656 (39.3)	188 (44.7)	844 (40.4)

[a] There was a total of 2,088 patients (1,668 children less than 15 years old and 420 adults).
[b] Multiple pathogens were excluded from the calculation.

SLT-II (26), LTh (6), STh and STp (24), EPEC adherence factor (EAF) (19), enterohemorrhagic *E. coli* (16), and enteroinvasive *E. coli* (3); colonies that hybridized with the enteroinvasive *E. coli* probe were tested by the Sereny test.

Environmental sources of diarrheagenic *E. coli*. In 1991, drinking water (257 samples) and surface seawater (67 samples) were analyzed weekly. Drinking water was collected from the city water supply in Noumea, New Caledonia, and from several municipal piped systems in rural areas. Seawater was collected from beaches bordering Noumea. All samples were examined in accordance with European standards (11). *E. coli* from each sample were studied in order to identify potential diarrheagenic strains as described above. For both years of the study, *E. coli* strains isolated from bovine and porcine diarrhea samples were studied in the same way.

Statistical analysis. Numeric data were analyzed by using the chi-square test and Yates' correction as appropriate.

RESULTS

One or more pathogens were identified in 41.8 and 40.6% of the persons with diarrhea investigated in 1990 (1,064 patients) and 1991 (1,024 patients), respectively.

Enteropathogenic bacteria. Results for enteropathogenic bacteria are summarized in Table 1. Diarrheagenic *E. coli*, *Salmonella* spp., and *Shigella* spp. were the predominant pathogenic bacteria; no *Plesiomonas* spp., *Aeromonas* spp., or *Vibrio* spp. were identified; except for *Shigella* spp. ($\chi^2 = 3.8$; $P > 0.3$), no significant difference was observed for enteropathogenic bacteria between 1990 and 1991. The term enteropathogenic *E. coli* (EPEC) included *E. coli* strains with traditional EPEC O:H serotypes that possessed EAF and belonged to the LA group. *E. coli* strains that adhered to HEp-2 cells with diffuse adherent or aggregative adherent patterns were termed diffuse adherent *E. coli* (DAEC) or enteroaggregative *E. coli*, respectively. Tests for enterotoxigenicity showed that 13 LT, 21 ST (11 STp and 10 STh), and 3 LT-ST (2 LT-STh and 1 LT-STp STh) ETEC strains were isolated, as determined by biological assays and hybridization procedures. The 13 strains with positive Y1 adrenal cell test results hybridized with the LTh probe. The following EPEC serotypes were identified: O119:H6 (14 strains), O127:NM (nonmotile) (5 strains), and O142:H6 (5 strains). Of the 140 LA-positive strains, 121 (86.4%) were EAF positive. Nine (6%) DAEC strains belonged to traditional EPEC O serogroups (four were O55, three were O124, and two were O125). Four enteroaggregative *E. coli* strains (9%), belonged to traditional EPEC O serogroups (three were O125 and one was O128). No enterohemorrhagic or SLT-producing *E. coli* and no enteroinvasive *E. coli* were identified from samples from patients.

Incidence of enteropathogenic bacteria in relation to area, age, season, and sex. The incidence of enteropathogenic bacteria in relation to area is summarized in Table 1. The isolation frequencies of diarrheagenic *E. coli* were significantly greater for patients from urban areas than for patients from rural areas ($P < 0.001$). *Salmonella* spp. and *Shigella* spp. were significantly predominant in rural areas ($P < 0.01$). Of 62 *Shigella* strains isolated, only two species were identified: *Shigella flexneri* (6 strains in 1990 and 17 in 1991) and *S. sonnei* (1 strain in 1990 and 38 in 1991); there was no predominant species in relation to area. Of 128 *Salmonella* strains serotyped, *Salmonella typhimurium* constituted 51.5%, and other strains identified included *S. brandenburg* (9.4%), *S. virchow* (6.25%), *S. hadar* and *S. weltevreden* (4.7% each), *S. newport*, *S. lexington*, and *S. agona* (3.1% each); other serotypes (*S. enteritidis*, *S. infantis*, *S. rissen*, *S. oranienburg*, and *S. schwarzengrund*) constituted less than 5% of the strains. *S. typhimurium* and *S. brandenburg* accounted for a greater proportion of the *Salmonella* spp. isolated from rural areas (48 *S. typhimurium* and 12 *S. brandenburg* strains) than from urban areas (18 *S. typhimurium* strains); furthermore, serotype *S. brandenburg* was identified only in 1991. Other enteropathogenic bacteria (*Campylobacter jejuni*, *Yersinia enterocolitica*, *C. difficile*, and *C. perfringens*) were rarely isolated, and no significant incidence in relation to area was observed.

The incidence of enteropathogenic bacteria in relation to patient age is summarized in Table 2. Isolation rates for *Salmonella* spp., *Shigella* spp., and diarrheagenic *E. coli* were significantly different by age. *Shigella* and *Salmonella* strains were rarely identified in children but were predominantly

identified in samples from adults ($P < 0.001$). Diarrheagenic *E. coli* was rarely identified from adults but was an important pathogen in children ($P < 0.001$). Interestingly, only 24 of the 86 typed *E. coli* strains were confirmed as belonging to EPEC O serogroups when full O:H serotyping was performed, and 49 of these O-grouped but non-true EPEC strains were nonadherent. EPEC serotypes and their incidence rates in children under 15 years old were 3.7% for O119:H6 (14 strains), 1.3% for O127:NM (5 strains), and 1.1% for O142:H6 (4 strains); and an EPEC O142:H6 strain was isolated from a 16-year-old child. ETEC serotypes were as follows: O1:NM (one strain), O2:H7 (one strain), O6:H16 (eight strains), O25:NM (four strains), O27:H7 (three strains), O28ab:H9 (one strain), O52:H10 (one strain), O64:H5 (four strains), O78:H12 (five strains), O88:H25 (two strains), O99:H6 (one strain), O101:NM (one strain), O126:H12 (three strains), O126:NM (one strain), and O166:H30 (one strain).

Infections with *Salmonella* spp., *Shigella* spp. and ETEC were decidedly seasonal, with a peak occurrence in March and April (the end of the rainy season). The highest incidence of diarrheal disease occurred during these months. In contrast, infections with EPEC or enteroadherent *E. coli* (EAEC) had no seasonal peak. Males younger than 15 years were more frequently infected with diarrheagenic *E. coli* than females were, with incidence rates of 20.8% (177 cases) and 19.8% (162 cases) in 1990 and 1991, respectively, among males and 2.4% (21 cases) and 4.1% (34 cases) in 1990 and 1991, respectively, among females.

Parasites, rotavirus, and mixed infections. Incidence rates of parasites and rotavirus are shown in Table 1. For parasites, no significant difference between 1990 and 1991 was observed. Identification rates of parasites were significantly different by age (Table 2), and parasites were primarily identified in stool samples from children ($P < 0.001$). Rotavirus was identified only in samples from infants under 3 years old (age-specific detection rates are 1.99 and 1.76%, for years 1 and 2, respectively). No seasonal pattern or incidence in relation to area or sex could be determined for infections with parasites or rotavirus.

Infection with more than one pathogen occurred only with bacteria and parasites. Of 551 cases of bacterial infectious diarrhea, 12 patients (2.1%) were infected with two types of bacteria (1 patient with *Salmonella* spp. and DAEC O125, 5 patients with *Salmonella* spp. and DAEC, 1 patient with *Salmonella* spp. and *Shigella flexneri*, 1 patient with *Salmonella* spp. and *C. jejuni*, 1 patient with *Shigella* spp. and DAEC O55, 2 patients with *Shigella* spp. and enteroaggregative *E. coli*, and 1 patient with EPEC O119:H6 and *E. coli* with an LA phenotype), and 5 patients (0.8%) were infected with a bacterial strain and *Entamoeba histolytica* or *Giardia lamblia* (2 patients with *G. lamblia* and *Shigella* spp., 1 patient with *E. histolytica* and DAEC, and 2 patients with *G. lamblia* and *Salmonella* spp.). The frequencies of mixed infection by age in diarrheal patients were not significantly different between urban and rural areas. No coinfection with rotavirus was detected.

DISCUSSION

Acute diarrheal diseases represent an important health problem in New Caledonia, as previously described in other tropical countries, for infants under 3 years old (1, 12) but also a serious one in older children. Interestingly, on this island located in the subtropical zone, no *Vibrio cholerae*, *Plesiomonas* spp., or *Aeromonas* spp. were isolated. This is in contrast to studies performed in South Pacific countries (1, 21). As far as we know, cholera has never been reported to occur on this island; this is presumably because travellers and food imports from countries where cholera is endemic are not important and strict sanitary controls are exercised at the points of entry. Our results showed that diarrheagenic *E. coli*, the major enteropathogenic bacteria in the group aged 0 to 15 years, were prevalent in the large urban area of Noumea, presumably because 63.4% of the population under 15 years old is located in this area. Observation of the incidence of diarrheagenic *E. coli* in relation to sex during the study shows that the isolation rates of groups are significantly different, but why this is so is not known. Why *Salmonella* spp. and *Shigella* spp. are predominant in rural areas is unclear; however, sanitation practices in the large urban area of Noumea prevent most bacterial contaminations, while in rural areas they do not (5). There were over 10 different serotypes of *Salmonella* (excluding *Salmonella typhi* and *Salmonella paratyphi* isolated from blood a few times during the study), with *S. typhimurium* being the most common of the isolates. New Caledonia depends on imports for most of its supply of meat and dairy products, and this may have contributed to the large variety of *Salmonella* species on the island. While *Shigella* spp. were not isolated frequently in the first year of the study, the isolation rate was high in the second year. The higher rate in 1991 was due to an outbreak of *S. sonnei* linked to Asian homemade food that occurred in a student population in Noumea.

This work increases our understanding of the microbiologic features of EPEC and EAEC expressing localized adherence. This phenotype is under plasmid control, known to be associated with somatic EPEC serogroups of class I (O111, O119, O127, and O142), and demonstrated in volunteers to be associated with enteropathogenicity (14, 23). However, EAF was identified in EAEC strains that do not belong to EPEC serogroups; the pathogenic potential of these EAEC strains needs to be investigated in case-control studies, since these *E. coli* strains were identified in the stools of a great proportion of young patients. A small number of EAEC LA strains were EAF negative; however, hybridizations were performed by using the colony blot technique, which is less sensitive than the DNA dot blot, and only strong positive signals were interpreted as positive. Consequently, several false-negative results may exist. In the South Pacific, epidemiological surveys of diarrhea-causing *E. coli* were conducted mainly on ETEC, EPEC, and enteroinvasive *E. coli*, and incidence rates may differ from place to place (1, 12). On the basis of our data, true EPEC is not a major cause of illness in children in New Caledonia. The results obtained from the study of incidence of EPEC and EAEC according to age suggest that children between 0 and 1 year and between 3 and 6 years old (data not shown) are at the greatest risk of contracting gastroenteritis due to EPEC or EAEC and that EAEC is a significant enteropathogen in children. Seasonal variation was not observed for EPEC and EAEC infections; this is consistent with what is known about food-borne pathogens in tropical climates, where ambient temperatures support the growth of bacteria year-round. *Shigella* spp., *Salmonella* spp., and ETEC, pathogens for which foodborne transmission is important, undergo a marked seasonal variation, with high rates at the end of the rainy season. This suggests that the modes of transmission may be similar for these pathogens and that in the environment, water may be an important factor in the transmission of these bacteria. In comparison with other epidemiological surveys on diarrhea-causing *E. coli*, ETEC was encountered rarely. The ETEC isolates were diverse: few ETEC strains possessed similar plasmid profiles, and there were a variety of serotypes. Of the 37 strains, 30 belonged to estab-

lished ETEC O serogroups (i.e., O6, O25, O27, O64, O78, O88, and O126) and 7 were of O1, O2, O28ab, O52, O99, O101, or O166, serogroups not frequently found among ETEC (7).

Drinking water was free of known pathogens (data not shown); effective public health measures and improvement in the water supply have reduced waterborne infection to a minimum in Noumea. ETEC was identified in briny water, a finding that suggests that in this area, the disposal of polluted water is a previously unknown potential source of waterborne infections. ETEC that produced ST-II was identified in several cases of porcine diarrhea, where sows and weaned pigs seem to be the reservoir of infection, but the role of ST-II ETEC as a cause of diarrhea in humans is uncertain (10). Surprisingly, while no SLT-producing *E. coli* was identified in samples from humans (no patient was hospitalized for hemorrhagic colitis or hemolytic uremic syndrome), our results show that bovine livestock are a reservoir of SLT-producing *E. coli* (not of *E. coli* serotype O157). The consumption of animal food products, especially inadequately cooked products used to make minced-meat pies, and person-to-person transmission are known to be the major sources of human SLT-producing *E. coli* infection (25). In New Caledonia, the absence of fast-food chain restaurants, the sanitary supervision of commercial meat distribution, and the rare consumption of uncooked meat probably account in part for the lack of transmission of SLT-producing *E. coli* from animals to humans.

In contrast to other work performed in tropical countries, the present study shows that rotavirus is not a common enteric pathogen in New Caledonia. A previous study in 1986 (5) showed that the frequencies of isolation of rotavirus were almost the same for sick children and controls; furthermore, echovirus, coxsackievirus, and adenovirus were responsible for 14% of diarrhea cases and were rarely identified in matched controls. The failure to find pathogens for 57% of the patients in the current study may be due, in part, to the relative insensitivity of some of the techniques used. In contrast to the findings from recent studies in five countries (12), *G. lamblia* and *E. histolytica* seem to be important enteric pathogens in New Caledonia; furthermore, mixed infections commonly occurred in many countries but were a minor problem in New Caledonia.

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REFERENCES

1. **Adkins, H. J., J. Escamilla, L. T. Santiago, C. Rañoa, P. Echeverria, and J. H. Cross.** 1987. Two-year survey of etiologic agents of diarrheal disease at San Lazaro Hospital, Manila, Republic of the Philippines. J. Clin. Microbiol. **25:**1143–1147.
2. **Begaud, E., and Y. Germani.** 1992. Detection of enterotoxigenic *Escherichia coli* in fecal specimens by acetylaminofluorene-labelled DNA probes. Res. Microbiol. **143:**315–325.
3. **Boileau, C. R., H. M. d'Hauteville, and P. J. Sansonetti.** 1984. DNA hybridization to detect *Shigella* species and enteroinvasive *Escherichia coli*. J. Clin. Microbiol. **20:**959–961.
4. **Bonhert, M. G., H. M. D'Hauteville, and P. J. Sansonetti.** 1988. Detection of enteric pathotypes of *Escherichia coli* by hybridization using six DNA probes. Ann. Inst. Pasteur/Microbiol. **139:**189–202.
5. **Brethes, B., J. P. Moreau, G. Panon, Y. Germani, G. Legonidec, J. J. Floch, B. Dassy, F. Amat, D. Broc, H. Plassart, and P. Leborgne.** 1986. Etude épidémiologique des gastro-entérites infantiles aiguës en Nouvelle-Calédonie. Bull. Soc. Pathol. Exot. **79:**114–122.
6. **Dallas, W. S., D. M. Gill, and S. Falkow.** 1979. Cistrons encoding *Escherichia coli* heat-labile toxin. J. Bacteriol. **139:**850–858.
7. **Danbara, H., K. Komase, H. Arita, H. Abe, and M. Yoshikawa.** 1988. Molecular analysis of enterotoxin plasmids of enterotoxigenic *Escherichia coli* of 14 different O serotypes. Infect. Immun. **56:**1513–1517.
8. **Dean, A. G., Y. C. Ching, R. G. Williams, and L. B. Harden.** 1972. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. J. Infect. Dis. **125:**407–411.
9. **Donta, S. T., H. W. Moon, and S. C. Whipp.** 1974. Detection of heat-labile *Escherichia coli* enterotoxin with the use of adrenal cells in tissue culture. Science **183:**334–336.
10. **Echeverria, P., J. Seriwatana, U. Patamaroj, S. L. Moseley, A. McFarland, O. Chityothin, and W. Chaicumpa.** 1984. Prevalence of heat-stable II enterotoxigenic *Escherichia coli* in pigs, water, and people at farms in Thailand as determined by DNA hybridization. J. Clin. Microbiol. **19:**489–491.
11. **European Commission.** 1980. Council directive of 15 July 1980 relating to the quality of drinking water intended for human consumption (80/778/EEC). Off. J. Eur. Communities **L229:**11–28.
12. **Huilan, S., L. G. Zhen, M. M. Mathan, M. M. Mathew, J. Olarte, R. Espejo, K. Maung, M. A. Ghafoor, M. A. Khan, Z. Sami, and R. G. Sutton.** 1991. Etiology of acute diarrhoea among children in developing countries: a multicentre study in five countries. Bull. W. H. O. **65:**549–555.
13. **Kim, K. H., R. Fekety, D. G. Batts, D. Brown, M. Cudmore, J. Silva, Jr., and D. Waters.** 1981. Isolation of *Clostridium difficile* from the environment and contacts of patients with antibiotic-associated colitis. J. Infect. Dis. **143:**42–50.
14. **Knutton, S., M. M. Baldini, J. B. Kaper, and A. S. McNeish.** 1987. Role of plasmid-encoded adherence factors in adhesion of enteropathogenic *Escherichia coli* to HEp-2 cells. Infect. Immun. **55:** 78–85.
15. **Konowalchuk, J., J. I. Speirs, and S. Stavric.** 1977. Vero response to a cytotoxin of *Escherichia coli*. Infect. Immun. **18:**775–779.
16. **Levine, M. M., J. G. Xu, J. B. Kaper, H. Lior, V. Prado, B. Tall, J. Nataro, H. Karch, and K. Wachsmuth.** 1987. A DNA probe to identify enterohemorrhagic *Escherichia coli* of O157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. J. Infect. Dis. **156:**175–181.
17. **Mathewson, J. J., P. C. Johnson, H. L. Dupont, D. R. Morgan, S. A. Thornton, L. V. Wood, and C. D. Ericsson.** 1985. A newly recognized cause of travelers' diarrhea: enteroadherent *Escherichia coli*. J. Infect. Dis. **151:**471–475.
18. **McClane, B. A., and R. J. Strouse.** 1984. Rapid detection of *Clostridium perfringens* type A enterotoxin by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **19:**112–115.
19. **Nataro, J. P., I. C. A. Scaletsky, J. B. Kaper, M. M. Levine, and L. R. Trabulsi.** 1985. Plasmid-mediated factors conferring diffuse and localized adherence of enteropathogenic *Escherichia coli*. Infect. Immun. **48:**378–383.
20. **O'Brien, A. D., and G. D. LaVeck.** 1983. Purification and characterization of a *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. Infect. Immun. **40:**675–683.
21. **Olakovski, T., P. Bennett, and M. Rao.** 1981. Surveillance of communicable disease in the South Pacific. Ninth regional conference of permanent heads of health services, working paper 12 (SPC/9CDH/WP12). South Pacific Commission, Nouméa, New Caledonia.
22. **Orskov, F., and I. Orskov.** 1984. Serotyping of *Escherichia coli*. Methods Microbiol. **14:**43–112.
23. **Scaletsky, I. C. A., M. L. M. Silva, M. R. F. Toledo, B. R. Davis, P. A. Blake, and L. R. Trabulsi.** 1985. Correlation between adherence to HeLa cells and serogroups, serotypes, and bioserotypes of *Escherichia coli*. Infect. Immun. **49:**528–532.
24. **Sommerfelt, H., H. M. S. Grewal, and M. K. Bhan.** 1990. Simplified and accurate nonradioactive polynucleotide gene probe assay

for identification of enterotoxigenic *Escherichia coli*. J. Clin. Microbiol. **28:**49–54.

25. **Wells, J. G., L. D. Shipman, K. D. Greene, F. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. F. Potter, R. V. Tauxe, and I. K. Wachsmuth.** 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. **29:**985–989.
26. **Willshaw, G. A., A. R. Smith, S. M. Scotland, A. M. Field, and B. Rowe.** 1987. Heterogeneity of *Escherichia coli* phages encoding Vero cytotoxins. Comparison of cloned sequences determining VT1 and VT2 and development of specific gene probes. J. Gen. Microbiol. **133:**1309–1317.
27. **World Health Organization.** 1987. Manual for laboratory investigations of acute enteric infections. Program for control of diarrheal diseases, CDD/83.3, Rev. 1. World Health Organization, Geneva.
28. **Yam, W. C., M. L. Lung, C. Y. Yeung, J. S. Tam, and M. H. Ng.** 1987. *Escherichia coli* associated with childhood diarrheas. J. Clin. Microbiol. **25:**2145–2149.