

A two-year survey of the incidence of heat-labile enterotoxin-producing *Escherichia coli* and other enteric pathogens in travellers returning to the Sheffield area

BY P. A. CHAPMAN AND DEBORAH L. MITCHELMORE¹

*Public Health Laboratory, Northern General Hospital, Herries Road,
Sheffield S5 7AU*

(Accepted 5 May 1988)

SUMMARY

A case-controlled study of the incidence of heat-labile enterotoxin-producing *Escherichia coli* (LT⁺ETEC) and other enteric pathogens in travellers returning to the Sheffield area was conducted from May 1984 to April 1986. LT⁺ETEC were found in 35 (5.8%) of 600 travellers to developed countries (mainly popular Mediterranean holiday resorts), 36 (11.3%) of 320 travellers to less-developed countries, and 11 (0.9%) of 1282 control patients whose illness was not associated with recent travel abroad. A seasonal peak of LT⁺ETEC infection was observed only in travellers to developed countries, with infections being significantly commoner in August to October. There was no significant deviation from expected age/sex distribution of LT⁺ETEC infection. Strains of LT⁺ETEC from travellers produced more toxin than strains from control patients, strains from travellers to less-developed countries producing most of all.

INTRODUCTION

Diarrhoea is the most frequent health problem of travellers from developed to less-developed countries; of an estimated 300 million international travellers in 1985, 16 million would have travelled to less-developed countries, and of these about one-third would have developed diarrhoea during, or shortly after their return from their travels (Report, 1985).

Heat-labile enterotoxin-producing *Escherichia coli* (LT⁺ETEC) are an important cause of diarrhoea in children and adults in less-developed countries, and in travellers from developed to less-developed countries (Rowe, 1979; Guerrant, 1985). Despite the importance of LT⁺ETEC, they are not recognized by many diagnostic laboratories. Little is known of the prevalence of LT⁺ETEC in the developed countries of Western Europe, or of the incidence of LT⁺ETEC as a cause of diarrhoea in travellers from England to the popular Mediterranean holiday destinations.

The aim of this study was to investigate the incidence of LT⁺ETEC and other pathogens in diarrhoea of adult travellers to developed and/or developing

* Present address: National Collection of Type Cultures, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT.

countries, and in a control group of patients whose diarrhoea was not associated with recent travel outside the United Kingdom.

MATERIALS AND METHODS

Selection of patients

Patients selected for study were adults (age ≥ 15 years) with acute, unexplained diarrhoea of presumed infectious origin, who had recently (< 1 month) returned from travel outside the UK. Faecal samples were taken from those submitted by general practitioners or the communicable diseases unit of Lodge Moor Hospital, Sheffield. They were assigned to one of two groups depending on the information on the request form accompanying the faecal sample: (1) those recently returned from Northern or Western Europe or North America (developed countries); or (2) those recently returned from other locations (less-developed countries). Control patients were selected from those whose illness was not associated with travel outside the UK. For each traveller selected for study, two control patients of the same sex, whose age, symptoms and date of onset of illness matched as closely as possible those of the traveller, were chosen.

Survey period

The survey was conducted from 1 May 1984 to 30 April 1986 inclusive.

Examination of faecal samples

All samples were examined for: (1) salmonellas using brilliant green MacConkey agar (Oxoid CM 7B with brilliant green (BDH) 0.004%), and tetrathionate broth enrichment; (2) shigellae using deoxycholate citrate agar (Oxoid CM 227); (3) campylobacters using broth enrichment (Bolton & Robertson, 1982) followed by subculture on to Skirrow's medium (Skirrow, 1977); (4) oocysts of *Cryptosporidium* using faecal smears stained by a modified Ziehl-Neelsen method (Garcia *et al.* 1983); and (5) LT⁺ETEC using a simplified cell-culture assay (Chapman & Swift, 1984).

In addition, samples from all travellers were examined for ova, cysts or mature parasites using a formalin-ether concentration method, and those from travellers to less-developed countries were also examined for vibrios using thiosulphate citrate bile sucrose medium (Oxoid CM333) and enrichment in alkaline peptone water (Furniss, Lee & Donovan, 1978), and for *Aeromonas hydrophila* and *Plesiomonas shigelloides* using prill-xylose ampicillin agar (Rogol *et al.* 1979).

Further examination of LT⁺ETEC isolates

Titre of heat-labile enterotoxin (LT) produced by isolates of LT⁺ETEC obtained during the survey was determined in triplicate. Doubling dilutions in phosphate-buffered saline, from 1 in 10 to 1 in 2560, were prepared from broth cultures and assayed for toxin as described earlier (Chapman & Swift, 1984).

Table 1. Detection rates of gastro-intestinal pathogens in the three patient groups

Pathogen	1282 control patients		600 travellers to developed countries		320 travellers to less-developed countries	
	Number* positive	Percentage* positive	Number positive	Percentage positive	Number positive	Percentage positive
LT+ETEC	11 (9)	0.9 (0.7)	35 (33)	5.8 (5.5)	36 (28)	11.3 (8.8)
Salmonellas	66 (64)	5.1 (5.0)	72 (65)	12 (10.8)	11 (8)	3.4 (2.5)
Shigellas	1 (0)	0.1 (0)	1 (1)	0.2 (0.2)	5 (5)	1.6 (1.6)
Campylobacters	202 (199)	15.8 (15.5)	90 (81)	15 (13.5)	35 (33)	10.9 (10.3)
<i>Cryptosporidium</i>	23 (20)	1.8 (1.5)	13 (11)	2.2 (1.8)	7 (6)	2.2 (1.9)
<i>G. intestinalis</i> †	6 (6)	0.5 (0.5)	4 (4)	0.7 (0.7)	15 (14)	4.7 (4.4)
<i>A. hydrophila</i>	NE		0 (0)	0 (0)	2 (1)	0.6 (0.3)
Others	12 (11)	1 (0.9)	5 (4)	0.8(0.7)	20(13)	6.3 (4.1)

* Figures in parentheses are numbers and percentages in which the organism was the sole pathogen found.

† Not all control patients were examined for *G. intestinalis*.
NE, not examined.

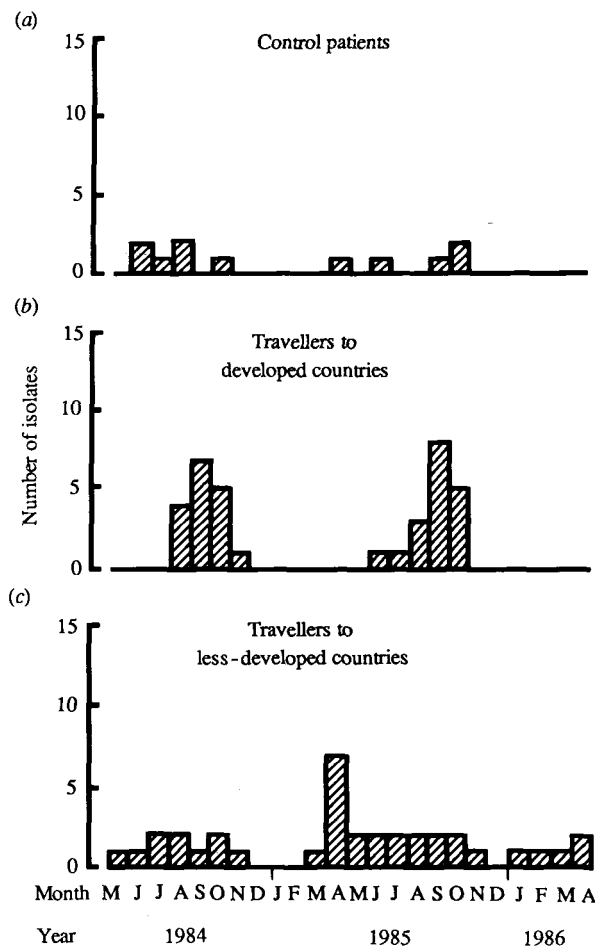
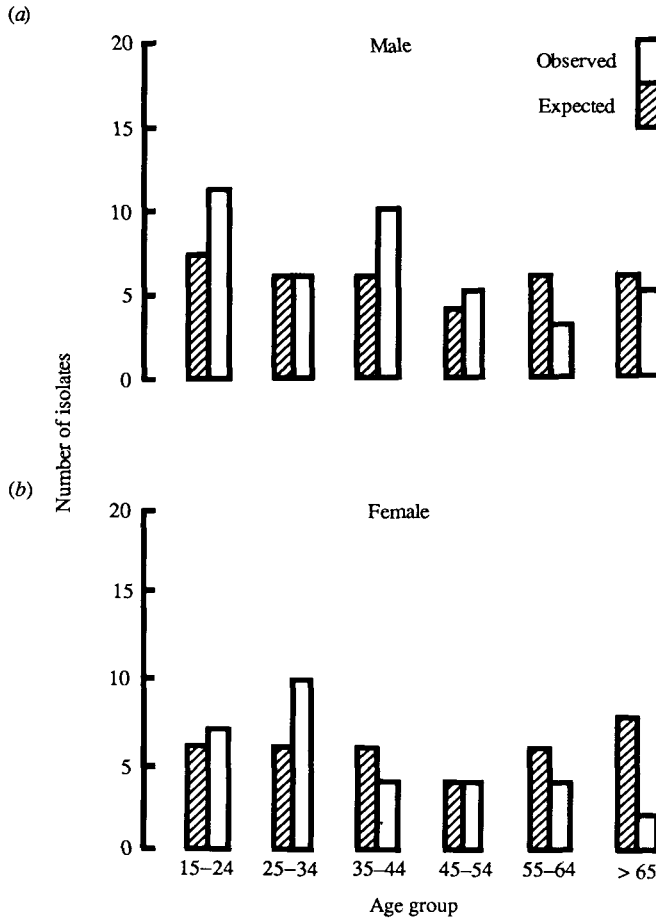


Fig. 1. Seasonal distribution of LT+ETEC infections in the three patient groups.

Table 2. *Titres of LT produced by LT⁺ETEC strains isolated from the three groups of patients*

Titre of LT	Number of strains isolated from		
	Control patients	Travellers to developed countries	Travellers to less-developed countries
10	1	5	3
20	2	1	0
40	4	2	1
80	2	0	2
160	1	6	2
320	1	6	7
640	0	8	5
1280	0	2	2
≥ 2560	0	5	14

Fig. 2. Age and sex distribution of LT⁺ETEC in travellers returning to the Sheffield area.

Statistical analysis

Incidence and age/sex distributions of the various pathogens were compared using the χ^2 test. Toxin titres produced by LT⁺EPEC isolated from the three patient groups were compared using the Mann–Whitney U test.

Results

During the 2-year period, 2833 faecal samples from 2202 patients were examined. Enteric pathogens were found in specimens from 321 (25%) of 1282 control patients, 220 (36.7%) of 600 travellers to developed countries, and 131 (40.9%) of 320 travellers to less-developed countries. Table 1 presents detailed findings in each of the three groups.

The effect of season on LT⁺EPEC infection in the three patient groups is shown in Fig. 1. In travellers to developed countries, isolation of LT⁺EPEC were made from 32 (8.6%) of 371 patients returning during August–October, and 3 (1.3%) of 229 patients returning during November–July ($P < 0.01$).

Seasonal peaks of salmonella and campylobacter infections occurred in summer in control patients and travellers to developed countries; such seasonal peaks were not observed in travellers to less-developed countries. The age and sex distribution of LT⁺EPEC infections in travellers is shown in Fig. 2.

Titres of LT produced by isolates of LT⁺EPEC are shown in Table 2. Isolates from travellers to less-developed countries produced significantly more toxin than isolates from control patients ($P < 0.001$) or travellers to developed countries ($P < 0.05$). Isolates from travellers to developed countries produced significantly more toxin than those from control patients ($P < 0.005$).

DISCUSSION

LT⁺EPEC infections were significantly commoner in travellers than in controls, and were commoner in travellers to less-developed countries than in those to developed countries ($P < 0.001$). No significant difference from expected age and sex distribution was observed for LT⁺EPEC infections in travellers ($P < 0.1$, see Fig. 2). The findings of a < 1% incidence of LT⁺EPEC in non-travellers and an 11.3% incidence in travellers to developing countries are similar to results obtained in other studies (Echeverria, Blacklow & Smith, 1975; Back, Blomberg & Wadstrom, 1977; Brunton *et al.* 1980; Abe *et al.* 1984; Kudoh & Sakai, 1985). Education and personal hygiene play a major part in preventing the spread of EPEC infection in developed countries.

It has been suggested that diarrhoea in some patients returning from the popular and more developed Mediterranean holiday resorts may be due to EPEC (Gross, 1983); previous reports, however, have done little to confirm this suggestion (Back, Blomberg & Wadstrom, 1977; Gross *et al.* 1979). The findings in this survey confirmed that LT⁺EPEC were an important cause of diarrhoea in this group of travellers; a probable reason why the findings in this survey differ from those in previous surveys is a marked seasonal variation not only in the numbers of travellers returning from Europe, but in the percentage of these travellers with

LT⁺EPEC as a probable cause of their diarrhoea. In travellers to developed countries, there was a marked increase in isolations of LT⁺EPEC in August–October (see Fig. 1). In travellers to less-developed countries, both the number of patients investigated and the percentage isolation rate of LT⁺EPEC varied little from month to month over the course of the survey.

The spectrum of illness observed with EPEC infections ranges from mild diarrhoea to severe cholera-like symptoms (Ericsson & DuPont, 1985). In studies in India, Sack *et al.* (1971, 1977) noted a strong association of EPEC with severe clinical cholera not caused by *Vibrio cholerae*; this association has also been noted in Bangladesh (Nalin *et al.* 1975; Khan & Greenough, 1985). According to Sack (1978) this severe illness is probably due to host factors such as malnutrition rather than increased bacterial virulence, although the latter was not studied. Although malnutrition, concomitant infections with other agents, and various other factors are all important in determining host response to an infection (Gordon, Chitkara & Wyon, 1963; Keusch & Scrimshaw, 1986), the results of our estimations of quantitative toxin production suggest that variations in severity of symptoms seen in diarrhoea due to LT⁺EPEC may in part be due to variations in amount of toxin produced.

Campylobacters were found with equally high frequency in control patients and travellers, supporting their importance as a major worldwide cause of diarrhoeal disease. The incidence of salmonellosis in travellers differed significantly from that expected ($P < 0.001$), being more common in those travelling to developed countries and less common those travelling to less-developed countries. This may reflect less intensive farming methods and food processing in less-developed countries, both of which perhaps increase the incidence of salmonellosis in England and Wales (Rowe & Gross, 1984). A seasonal peak of campylobacter and salmonella infections was observed in mid to late summer in both control patients and travellers to developed countries; such seasonal distribution has been recorded nationally (CDSC, 1986). Ambient temperatures in England during the summer of 1985 were lower than average (H.M.S.O., 1987); this may in part explain the low overall incidence of salmonellas in control patients. The high incidence of salmonellas in travellers to developed countries, which was at its highest (25%) in those returning from the Iberian peninsula in summer, probably reflects a combined effect of high ambient temperatures and a breakdown of kitchen hygiene in the very large catering establishments serving mass tourism. Reports of the importance of salmonellas as a cause of diarrhoea in travellers returning from less-developed countries conflict. Steele (1983) found that of 369 travellers returning from developing countries to the USA, 53 were excreting salmonellas, yet only 9 of these had a history of gastro-intestinal symptoms. Such results suggest that a finding of salmonella in a case of travellers' diarrhoea should be viewed with caution, and other possible aetiological agents should not be excluded on this basis.

Shigellae have often been associated with travellers' diarrhoea (Ericsson & DuPont, 1985). However, in this survey they were found in only 1 (0.2%) of 600 travellers to developed countries, and in only 5 (1.6%) of 320 travellers to less-developed countries.

Cryptosporidium has been reported as a cause of travellers' diarrhoea (Jokipii, Pohjola & Jokipii, 1983, 1985; Ma *et al.* 1985; Soave & Ma, 1985). In this survey the incidence of *Cryptosporidium* oocysts was low and did not differ significantly in the three patient groups ($P > 0.9$)

Enterotoxigenic *Aeromonas hydrophila* have been reported as a frequent cause of travellers' diarrhoea, and haemolysis on blood agar recommended as a presumptive indicator of toxigenicity of isolates from faecal samples (Gracey *et al.* 1984). We found *A. hydrophila* in only two (0.6%) of travellers to less-developed countries, and these isolates, despite being clearly haemolytic on blood agar, did not produce toxins detectable by cell-culture assays. In one patient, *A. hydrophila* was present in a faecal sample concomitantly with LT⁺ETEC and oocysts of *Cryptosporidium*; a second sample from this patient while still symptomatic yielded only LT⁺ETEC, and a third sample, taken after symptoms had ceased, failed to reveal any of the previously detected organisms. Our findings have done little, therefore, to support the role of *Cryptosporidium* or *A. hydrophila* as frequent causes of travellers' diarrhoea.

Giardia intestinalis is apparently commoner in travellers to less-developed countries than in the other patient groups, but this cannot be stated with certainty since sufficient data to allow statistical analysis are not available from the other patient groups. In view of the marked increase recently in giardiasis in the USA and other developed countries (Craun, 1986), the wisdom of screening non-travellers on a very selective basis must be questioned.

Other agents detected in small numbers in this survey were *Plesiomonas shigelloides* (2), *Entamoeba histolytica* (2), helminth ova (12), *Clostridium difficile* cytotoxin (15), *Clostridium perfringens* enterotoxin (3), *Vibrio parahaemolyticus* (1) and *Vibrio alginolyticus* (2). Of these the *V. alginolyticus* were unusual in producing a cytotoxin active against a wide variety of cell lines (Chapman, 1987).

The total number of international tourists and international business travellers has increased substantially over the last two decades (Business Statistics Office, 1985; World Tourism Organisation, 1985) and the rate of this increase shows no sign of stabilizing. The demand, therefore, for laboratory investigations of travel-associated illnesses is likely also to continue to increase. A screen for LT⁺ETEC should therefore be considered a valuable and necessary part of routine diagnostic procedure.

We wish to thank Dr B. W. Barton of Sheffield Public Health Laboratory, Dr D. C. Turk (formerly of Sheffield Public Health Laboratory), Professor B. I. Duerden of Sheffield University Medical School, and Dr Sylvia Scotland of the Central Public Health Laboratory, Colindale, London for their interest and constructive advice throughout this work. We also thank Dr A. Page of Sheffield City Polytechnic for guidance with statistical analysis of results.

REFERENCES

- ABE, H., ICHIKI, S., HASHIMOTO, S., NAKANO, H., SATO, K., KANDA, T., YANAI, Y., TSUKAMOTO, T., KINOSHITA, Y., ARITA, M., HONDA, T., TAKEDA, Y. & MIWOTANI, T. (1984). Isolation and characterisation of enterotoxigenic *Escherichia coli* from patients with travellers' diarrhoea in Osaka. *Journal of Diarrhoeal Diseases Research* **2**, 83–87.
- BACK, E., BLOMBERG, T. & WADSTROM, T. (1977). Enterotoxigenic *Escherichia coli* in Sweden. *Infection* **5**, 2–5.
- BOLTON, F. J. & ROBERTSON, L. (1982). A selective medium for isolating *Campylobacter jejuni/coli*. *Journal of Clinical Pathology* **35**, 462–467.
- BRUNTON, J., HINDE, D., LANGSTON, C., GROSS, R. J., ROWE, B. & GURWITH, M. (1980). Enterotoxigenic *Escherichia coli* in central Canada. *Journal of Clinical Microbiology* **11**, 343–348.
- BUSINESS STATISTICS OFFICE (1985). Business monitor annual statistics. Overseas travel and tourism. London: Her Majesty's Stationary Office.
- CHAPMAN, P. A. (1987). *Vibrio alginolyticus* and diarrhoeal disease. *Journal of Diarrhoeal Diseases Research* **5**, 40.
- CHAPMAN, P. A. & SWIFT, D. L. (1984). A simplified method for detecting the heat-labile enterotoxin of *Escherichia coli*. *Journal of Medical Microbiology* **18**, 399–403.
- COMMUNICABLE DISEASES SURVEILLANCE CENTRE (1986). Foodborne disease surveillance in England and Wales 1984. *British Medical Journal* **293**, 1424–1427.
- CRAUN, G. F. (1986). Recent statistics of waterborne disease (1981–1983). In *Waterborne Diseases in the United States* (Ed. G. F. Craun), pp. 161–170. Boca Raton, U.S.A.: CRC Press.
- ECHEVERRIA, P., BLACKLOW, R. R. & SMITH, D. H. (1975). Role of heat-labile toxigenic *Escherichia coli* and reovirus-like agent in diarrhoea in Boston children. *Lancet* **ii**, 1113–1116.
- ERICSSON, C. D. & DUPONT, H. L. (1985). Travellers' diarrhoea: recent developments. *Current Clinical Topics in Infectious Diseases* **6**, 66–84.
- FURNISS, A. L., LEE, J. V. & DONOVAN, T. J. (1978). *The Vibrios: Public Health Laboratory Service Monograph Series No. 11*. London: Public Health Laboratory Service.
- GARCIA, L. S., BREWER, T. C., BRUCKNER, D. A. & SHIMIZU, R. Y. (1983). Acid fast staining of *Cryptosporidium* from human faecal specimens. *Clinical Microbiology Newsletter* **5**, 60–61.
- GORDON, J. E., CHITKARA, I. D. & WYON, J. B. (1963). Weanling diarrhoea. *American Journal of Medical Science* **245**, 129–161.
- GRACEY, M., BURKE, V., ROBINSON, J., MASTERS, P. L., STEWART, J. & PEARMAN, J. (1984). *Aeromonas* spp in travellers' diarrhoea. *British Medical Journal* **289**, 658.
- GROSS, R. J. (1983). *Escherichia coli* diarrhoea. *Journal of Infection* **7**, 177–192.
- GROSS, R. J., SCOTLAND, S. M. & ROWE, B. (1979). Enterotoxigenic *Escherichia coli* causing diarrhoea in travellers returning to the United Kingdom. *British Medical Journal* **278**, 1463.
- GUERRANT, R. L. (1985). Microbial toxins and diarrhoeal disease: introduction and overview. In *Microbial Toxins and Diarrhoeal Disease*, Ciba Foundation Symposium No. 112 (ed. D. Evered and J. Whelan), pp. 1–27. London: Pitman Medical.
- Annual Abstract of Statistics. London: HER MAJESTY'S STATIONARY OFFICE.
- JOKIPII, L., POHJOLA, S. & JOKIPII, A. M. M. (1983). *Cryptosporidium*: a frequent finding in patients with gastrointestinal symptoms. *Lancet* **ii**, 358–361.
- JOKIPII, L., POHJOLA, S. & JOKIPII, A. M. M. (1985). Cryptosporidiosis associated with travelling and giardiasis. *Gastroenterology* **89**, 838–842.
- KEUSCH, G. T. & SCRIMSHAW, N. S. (1986). Selective primary health care: strategies for control of disease in the developing world. XXIII. Control of infection to reduce the prevalence of infantile and childhood malnutrition. *Reviews of Infectious Diseases* **8**, 273–287.
- KHAN, M. U. & GREENOUGH, W. B. (1985). Epidemiology of bacterial diarrhoeal diseases in Bangladesh. In *Bacterial Diarrhoeal Diseases* (Ed. Y. Takeda, and T. Miwatani), pp. 37–52. KTK Scientific, Tokoyo and Martinus Nijhoff, Boston.
- KUDOH, Y. & SAKAI, S. (1985). Current status of bacterial diarrhoeal diseases in Japan. In *Bacterial Diarrhoeal Diseases* (Eds. Takeda, Y., Miwatani, T.), pp. 83–93. Tokyo: KTK Scientific Boston: Martinus Nijhoff.
- MA, P., KAUFMAN, D. L., HELMICK, C. G., D'SOUZA, A. J. & NAVIN, T. R. (1985). Crypto-

- sporidiosis in tourists returning from the Caribbean. *New England Journal of Medicine* **312**, 647–648.
- NALIN, D. R., McLAUGHLIN, J. C., RAHAMAN, M., YUNUS, M. & CURLIN, G. (1975). Enterotoxigenic *Escherichia coli* and idiopathic diarrhoea in Bangladesh. *Lancet* **ii**, 1116–1119.
- REPORT (1985). Consensus conference on travellers' diarrhoea held by the National Institutes for Health, U.S.A. *Journal of the American Medical Association* **253**, 2700–2704.
- ROGOL, M., SECHTER, I., GRINBERG, L. & GERICHTER, C. B. (1979). Pril-xylose-ampicillin agar, a new selective medium for the isolation of *Aeromonas hydrophila*. *Journal of Medical Microbiology* **12**, 229–231.
- ROWE, B. (1979). The role of *Escherichia coli* in gastroenteritis. *Clinics in Gastroenterology* **8**, 625–644.
- ROWE, B. & GROSS, R. J. (1984). Salmonellosis, campylobacter enteritis and shigella dysentery. In *Microbes and Infections of the Gut* (Ed. C. S. Goodwin), pp. 47–78. London: Blackwell Scientific Publications.
- SACK, D. A., KAMINSKY, D. C., SACK, R. B., WAMOLA, I. A., ORSKOV, F., ORSKOV, I., SLACK, R. C. B., ARTHUR, R. R. & KAPIKIAN, A. Z. (1977). Enterotoxigenic *Escherichia coli* diarrhoea of travellers: a prospective study of American peace corps volunteers. *Johns Hopkins Medical Journal* **141**, 63–70.
- SACK, R. B. (1978). The epidemiology of diarrhoea due to enterotoxigenic *Escherichia coli*. *Journal of Infectious Diseases* **137**, 639–640.
- SACK, R. B., GORBACH, S. L., BANWELL, J. G., JACOBS, B., CHATTERJE, B. D. & MITRA R. C. (1971). Enterotoxigenic *Escherichia coli* isolated from patients with severe cholera-like disease. *Journal of Infectious Diseases* **123**, 378–385.
- SKIRROW, M. B. (1977). *Campylobacter* enteritis: a new disease. *British Medical Journal* **275**, 9–11.
- SOAVE, R. & MA, P. (1985). Cryptosporidiosis: travellers' diarrhoea in two families. *Archives of Internal Medicine* **145**, 70–72.
- STEELE, J. H. (1983). World epidemiology of salmonellosis. *International Journal of Zoonoses* **10**, 45–52.
- WORLD TOURISM ORGANISATION (1985). *Tourism Compendium*. Graficas FCC, SL, Madrid.