

AN ETIOLOGICAL STUDY OF BACTERIAL DIARRHEA AMONG INFANTS IN PARAGUAY IN 1990

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Abstract: An etiological study of bacterial diarrhea in infants under five years of age in Paraguay from May to July of 1990 was done. Enteropathogenic *E. coli* (EPEC) strains of known serogroups were isolated from 35.8% of the patients with diarrhea, *Salmonella* spp. were isolated from 15.1%, enterotoxigenic *E. coli* (ETEC) strains were isolated from 5.7%, and *Shigella* and *Campylobacter* spp. were isolated from 3.8% respectively. No enteroinvasive *E. coli* (EIEC) or *Vibrio* spp. was isolated. Out of 53 diarrheal patients, 34 cases (64.1%) had bacterial diarrheal diseases. The infants under one year of age with diarrhea were predominant among inpatient and outpatient children under five years of age and the total number of male diarrheal cases was greater than that of female cases. Enteropathogenic organisms were isolated from nine out of 12 water samples taken from the Paraguay River. The major serotype (O18) of the EPEC isolated from the water samples of the Paraguay River corresponded to the major serotype isolated from patients with diarrhea.

INTRODUCTION

Bacterial diarrheal disease is still one of the main public health problems with high morbidity and mortality rates, especially in the tropics and subtropics (Merson, 1982).

It is reported that diarrheal diseases accounted for 8.2% of the infant mortality rate in Paraguay in 1989, according to a report by the Ministry of Health, however the etiology of diarrhea as well as its prevalence is still unknown. Surveys and studies conducted so far on diarrhea in Paraguay have been confined mainly to traditional enteropathogens such as *Salmonella* and *Shigella*. The first four cases of diarrhea due to *Campylobacter jejuni* in Paraguay were reported by Achucarro *et al.* (1989) and this was the first report on

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campylobacter gastroenteritis in Paraguay. However, an etiological investigation including one concerning gastroenteritis due to *E. coli*, *Campylobacter* and *Vibrio* sp. has not been done.

In this report we tried to clarify the present status of diarrhea, especially the etiological agents of bacterial diarrheal diseases among children under five years of age in Paraguay. In order to treat and control diarrheal diseases, we also investigated the water of the Paraguay River which is closely associated with the life of the people. In addition, the drinking water was studied bacteriologically to clarify the degree of enteropathogen contamination resulting in diarrhea, because diarrheal diseases are recognized to be a fecal-oral infection, so that drinking water and food are considered major sources of infection.

MATERIALS AND METHODS

Rectal swabs (Carrymate media, Ono Pharmaceutical Co. Ltd., Japan) were taken from children under five years of age having diarrhea who visited or were admitted to the Department of Pediatrics, at the School of Medicine of the Asunción University and LACIMET Hospital from May to July of 1990 and were processed bacteriologically in the Bacteriology Section of the Instituto de Investigaciones en Ciencias de la Salud. The rectal swabs were inoculated directly on TCBS, SS, EMB and Skirrow media and incubated at 37°C overnight.

The specimens were, at the same time, subcultured in alkaline peptone water (pH 8.5) and selenite broth. After being incubated at 37°C overnight, three loopfuls of bacterial culture were inoculated on TCBS and SS media. A suspicious colony from each plate was examined with the following media for identification, Kligler iron, SIM, Voges-Proskauer (VP), lysine, ornithine and Simmons citrate, and was simultaneously checked for the level of cytochrome oxidase. Ten colonies of *E. coli* were inoculated on BTB media to check purity and three colonies each were checked for serotyping with diagnostic antisera for enteropathogenic *E. coli* (EPEC) (Denka Seiken, Japan) when *E. coli* was predominant on the EMB media. The serotyping for *Shigella* and *Salmonella* was done with diagnostic antisera (Denka Seiken, Japan).

Skirrow agar medium for *Campylobacter* containing 5% horse blood, trimethoprim (5 mg/ml), vancomycin (10 mg/ml) and polymyxin B (2.5 µ/ml) was incubated at 37°C for 3 days or 42°C for 2 days in a microaerobic atmosphere containing 5-10% oxygen generated by a CampyPak (BBL Microbiology System, Cockeysville, Maryland). The minimum criteria used for presumptive identification of *C. jejuni/coli* were: Gram-negative, spiral-shaped, motile, oxidase positive and growth at 42°C but not at 25°C. The sodium chloride tolerance test was also employed for further confirmation and classification of *Vibrio* sp.

Thousand milliliter samples of water were collected in sterile bottles from 12 water points in the Paraguay River and from nine water points for drinking in two areas in Asunción (Fig. 4).

Each water sample was concentrated to 10 ml with a milipore filter and 3 ml each was subcultured in double strengthened alkaline peptone water (pH 8.5), peptone water (pH 7.2) and selenite broth at 37°C overnight. A portion of the concentrated water sample was also inoculated on EMB media and incubated at 37°C overnight. The filter paper used for concentration was placed onto SS media and incubated at 37°C overnight. The bacterial culture in alkaline peptone water was streaked on TCBS and that in the peptone water and

selenite broth was both streaked on SS media.

A suspicious colony from each plate was examined with identification media after being incubated at 37°C overnight. To test the sensitivity against several antibiotics, a single disk method (Bauer *et al.*, 1966) was employed and the results were reported to the pediatric ward.

The production of LT and ST was checked by GM₁-ELISA and suckling mouse assay, respectively, as previously described (Honda *et al.*, 1984; Takeda *et al.*, 1979).

RESULTS

The isolation rate of different organisms and the percentage of all the detected agents are shown in Fig. 1. From 53 diarrheal patients, 34 cases were recognized to be bacterial diarrheal diseases. The most dominant bacterial pathogen was enteropathogenic *E. coli* (EPEC) with 19 isolates (35.8%) which was followed by *Salmonella* with eight isolates (15.1%). No *S. typhi* or *S. paratyphi-A* was isolated. Serotyping of the *Salmonella* could not be done thoroughly because there were not enough serotyping kits for diagnosis when they were isolated. Out of eight isolates identified biochemically as *Salmonella*, four isolates were serotyped, two strains were serogroup O4, and one strain each was serogroup O13 and O35. However, the remaining four isolates were not adequately serotyped because of contamination during stock. Three strains of enterotoxigenic *E. coli* (ETEC) were isolated. Heat labile enterotoxin (LT) was not detected by GM₁-ELISA from all the strains serotyped as EPEC or ETEC. Two EPEC strains (FA ratio: 0.141, 0.85) and one strain serotyped as ETEC (FA ratio: 0.096) were revealed to be positive in the suckling mouse assay. Two strains each of *Shigella flexneri* 2a and *Campylobacter jejuni/coli* were isolated. Three mixed infection cases were found, the included; EPEC O1 and 18; EPEC O86a and O157; *Salmonella* serogroup

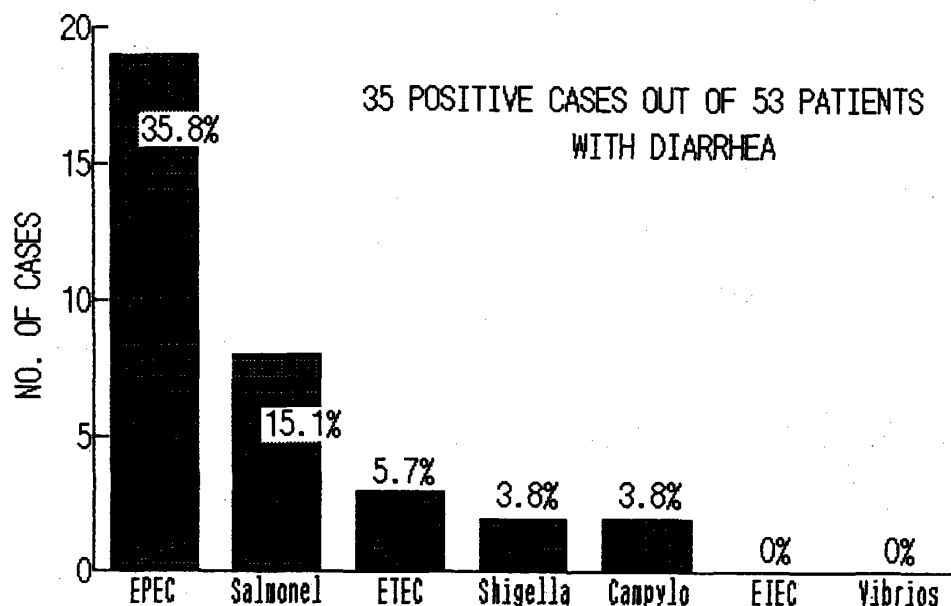


Figure 1 Pathogenic bacterial agents isolated from diarrheal patients under five years of age obtained between May and July of 1990.

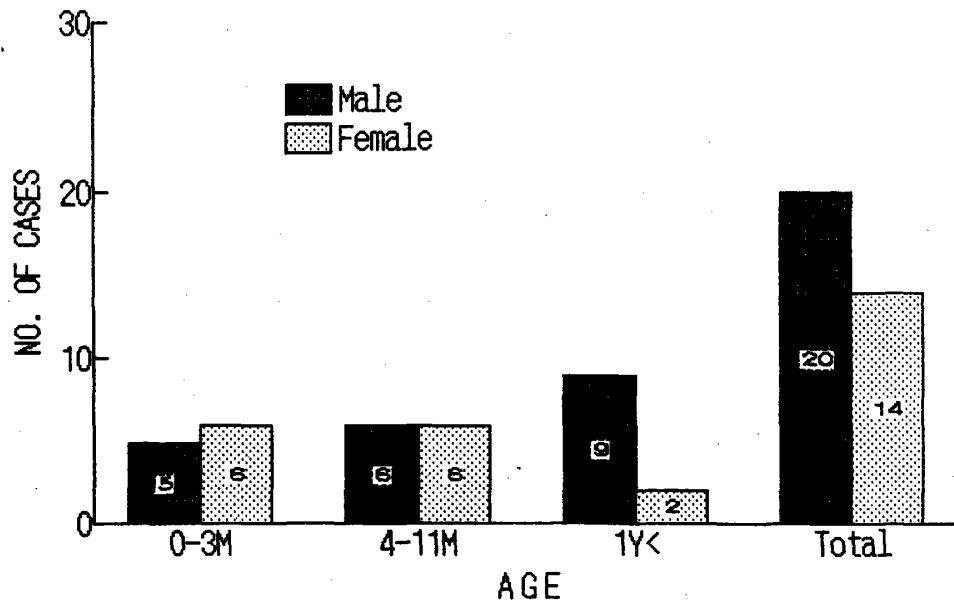


Figure 2 Age and sex distribution of 34 children with diarrhea.

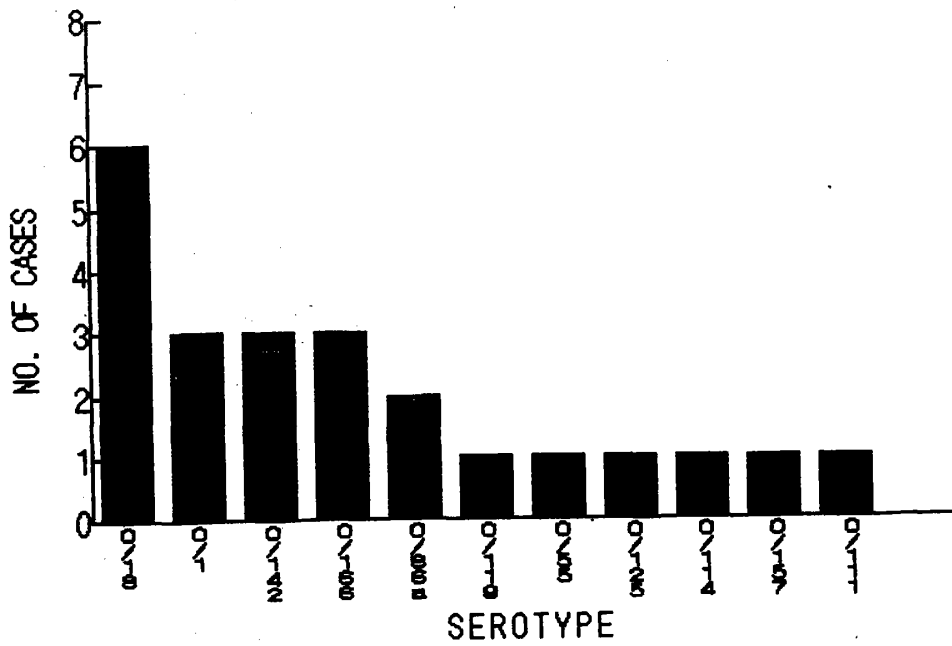


Figure 3 Distribution of serotyping of enteropathogenic *E. coli* isolated from children with diarrhea.

O4 and EPEC O86a, and the last case was thought to be a *Salmonella* infection (Fig. 1). No enteroinvasive *E. coli* or *Vibrio* sp. was isolated. A total of 41.5% of the diarrhea cases was, thus, considered to be due to *E. coli*. The infants under one year of age with diarrhea were predominant among inpatient and outpatient children under five years of age and the total number of male diarrheal cases was greater than that of female cases (Fig. 2).

Eleven kinds of EPEC serotypes were isolated and O18 in the EPEC isolates were predominant (Fig. 3). Twelve water samples from the Paraguay River were taken at the

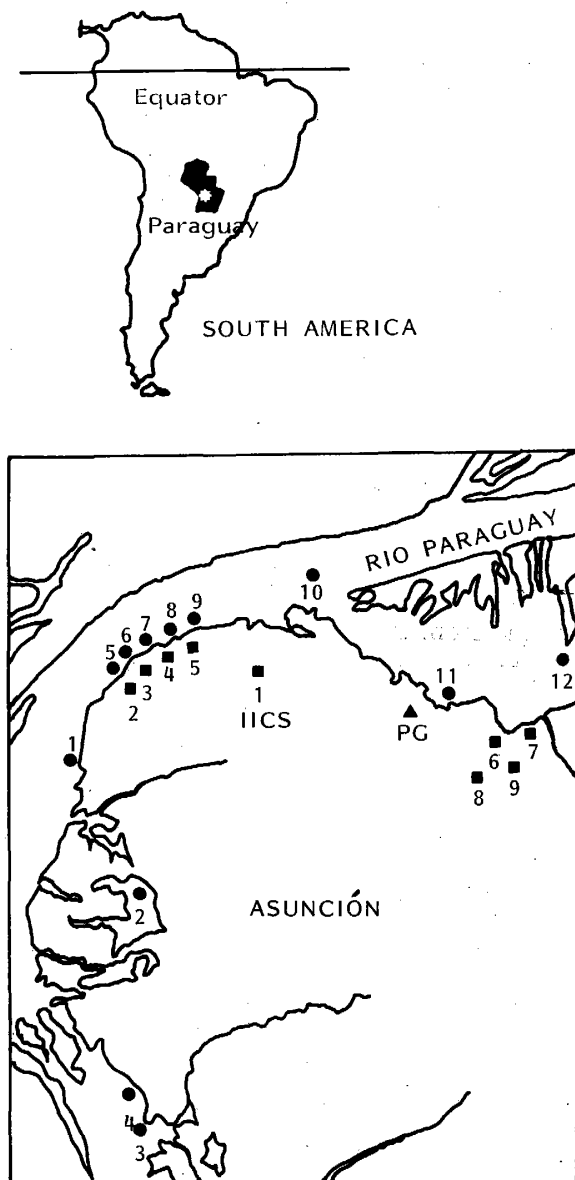


Figure 4 A map of Asunción, Paraguay. The numbers represent the points where the water samples (●) and drinking water (■) were obtained. PG: The Palace of the President of Paraguay, IICS: Instituto de Investigaciones en Ciencias de la Salud.

Table 1 The enteropathogens isolated from water of the Paraguai River

Point 2	EPEC (O18, O44)
Point 4	EPEC (O18, O26, O142)
Point 5	<i>Salmonella</i> (serogroup O13)
Point 6	<i>Salmonella</i> (serogroup O21), EPEC (O18, O146)
Point 7	<i>Salmonella</i> (ND)
Point 8	EIEC (O28a,c)
Point 9	<i>Salmonella</i> (serogroups O1, 3, 19), <i>Vibrio mimicus</i>
Point 10	<i>Salmonella</i> (ND)
Point 12	EPEC (O18, O128)

ND: The isolates were identified biochemically but serotyping of the isolates was not done.

points shown in Fig. 4. These points were selected as they were near to the areas where people in relatively low-socioeconomic conditions live. Enteropathogenic organisms were isolated from nine points out of the 12 points. Two or three EPECs were isolated from four points and the serotype O18 was isolated from all four points. *Salmonella* was isolated from five points.

Each strain of EIEC and *Vibrio mimicus* was isolated. Enteropathogenic organisms were isolated from all the points where people in low-socioeconomic conditions live. The major serotype of the EPEC isolated from the water samples of the Paraguai River corresponded to the major serotype isolated from the patients with diarrhea (Table 1).

In the analysis of the drinking water, enterobacteria such as *Salmonella*, *Enterobacter* and *Proteus* spp. were isolated from three points out of nine points tested. No enteropathogens were isolated from the tap water.

In the drug sensitivity test, six or seven kinds of discs containing ampicillin, cephaloridine, cefotaxime, ceftazidime, gentamicin, amikacin, chloramphenicol and trimethoprim were used. Four strains of EPEC showed resistant against three or four kinds of antibiotics including ampicillin and cephaloridine and the other pathogenic *E. coli* strains were relatively sensitive. One strain of *Salmonella* was resistant against five kinds of antibiotics including ampicillin and cephaloridine and the other strains were relatively sensitive to all the antibiotics except for ampicillin and cephaloridine. One strain of *Shigella* was resistant against aminoglycosides and chloramphenicol but the other was sensitive to all the antibiotics except for ampicillin. Two strains of *Campylobacter* were sensitive against aminoglycosides and tetracycline but resistant to all the other antibiotics.

DISCUSSION

This etiological study of bacterial diarrhea was performed over a short period of time and the instruments and the materials for making diagnosis were not adequate enough that we could identify the organisms in detail and thoroughly analyze characteristics such as toxin productivity. However, additional investigations over an extended period of time will provide more meaningful results.

The following findings are from the results of this study. Firstly, more than 60% of the diarrheal patients were considered to have bacterial diarrhea, although this study was conducted during the dry and cold season. Since the rotavirus is also an important organism causing diarrhea among children during the dry and cold season as previously reported (Koopman *et al.*, 1984; Brandt *et al.*, 1982; Brandt *et al.*, 1983), it is necessary to investigate viral enteropathogens as well as bacterial enteropathogens. Secondly, the dominant bacterial pathogen that caused diarrhea among inpatients and outpatients was EPEC. It is reported that EPEC is the leading cause of diarrhea in black South African children and that diarrhea in white children is largely attributable to rotavirus (Robins-Browne, 1984). Of course, it is difficult to consider it to be the overall infection rate of EPEC, because the diarrheal patients who came to the Asunción University Hospital and LACIMET Hospital were severe cases and diarrheal patients in low-socioeconomic areas who do not visit a hospital unless their condition becomes severe. Thirdly, the isolation rate of *Campylobacter jejuni/coli* was 3.8% in this study, which is almost the same as that in other countries (Chyou *et al.*, 1988; Tang *et al.*, 1984).

Shimotori *et al.* (1986) reported that the overall isolation rate of *Campylobacter jejuni* from children with diarrhea in Kenya was 12.6% but in the cold and dry season it was 6.3%. It is considered that the infection rate of *C. jejuni/coli* in Paraguay is within the ranges reported earlier for developing countries. Fourthly, the EPEC serotype of the enteropathogenic organisms isolated from nine out of 12 water points in the Paraguay River corresponded to that of the diarrheal patients. The outbreak of diarrheal diseases seems to be closely related to the usage of contaminated water from the Paraguay River. The water level of the Paraguay River was elevated because of rainfall during the spring, so a fairly large number of people who lived on the banks of the river had found refuge on a hill overlooking the river. The people had to build their houses temporarily in this area. Chlorinated tap water was, of course, supplied to the people of this area. The drinking water was kept in their houses because the water supply was not connected to individual houses, thus resulting in it becoming contaminated. The water from the Paraguay River is used for domestic use such as washing dishes and clothes and bathing. It is, therefore, believed that the establishment of a water supply to this area as well as health education is required. The last point of note is on the high mortality rate due to diarrheal diseases. Firstly, an effective and cheap drug for diarrheal patients should be chosen, with due consideration being given to the most appropriate drug for pediatric cases. Secondly, particular attention should be paid to the care of hospital patients, and to the improvement of laboratory diagnostic techniques. Thirdly, effective use of oral rehydration solution in rural areas should be promoted because of the high mortality rate. Finally, it is important to continue the etiological examination of diarrheal diseases.

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パラグアイ、アスンシオン市における幼児の細菌性下痢症

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1990年5月から7月までの3カ月間、パラグアイ・アスンシオン市の医療施設において、5歳以下の下痢症患者を対象に、下痢便の細菌学的検査を行った。53症例から34名(64.1%)に下痢原因菌を検出したが、その結果は病原性大腸菌の19株(35.8%)を筆頭に、サルモネラ8株(15.1%)、毒素原性大腸菌3株(5.7%)、赤痢菌とカンピロバクターがそれぞれ2株(3.8%)が検出され、このうち混合感染が3例あった。

診断用抗血清による凝集試験で、病原性大腸菌では11種に型別され、O18型が最も頻度が高かった。血清学的抗原構造で毒素原性大腸菌に属するものからは、易熱性毒素(LT)は検出できなかったが、乳のみマウスによる耐熱性毒素(ST)の検査で、毒素原性大腸菌から1株が陽性を示し、病原性大腸菌も2株が陽性を示した。

河川水や飲料水の細菌学的検査において、病原性大腸菌、組織侵入性大腸菌、サルモネラ、ビブリオ属などの病原菌の他に、腸内細菌科の細菌も検出された。特にパラグアイ川からは、下痢便で最優先であったものと同型の、O18型病原性大腸菌が検出されたことは、この地域住民に水系感染の危険性があることが推察された。

またディスク法によって、抗生物質7種類に対する薬剤感受性試験を行ったが、同一菌種間においても、その感受性パターンは多様性を示した。

パラグアイにおける細菌性下痢症の調査は、従来、サルモネラや赤痢菌などの古典的下痢症については行われてきた。しかしカンピロバクターについては、1989年において、初めて4症例の報告がなされた状況であり、腸管病原性大腸菌やビブリオ属の調査については、まったくなされていない。

パラグアイでの下痢症の疫学的・病因的調査は不十分な状況であるので、この調査を機会に、今後も継続して行っていく必要性を強調したい。

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