

## Etiological Study of Diarrheal Patients in Vientiane, Lao People's Democratic Republic

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The etiologic agents of diarrhea in Vientiane, Lao People's Democratic Republic (Lao PDR), were studied in the period from October 1996 to August 1997. A total of 880 patients with diarrhea visiting medical facilities were examined for *Shigella*, *Salmonella*, diarrheagenic *Escherichia coli*, *Vibrio*, *Aeromonas*, *Campylobacter*, and rotavirus. *Shigella* spp., heat-stable enterotoxin (ST)-producing *E. coli*, and serogroup-based enteropathogenic *E. coli* were found to be the main organisms causing diarrhea in Vientiane, with frequencies of 16.8% (148 of 880), 17.2% (111 of 645), and 11.0% (97 of 880), respectively. Relatively low incidences were observed in the cases of *Salmonella* spp., (0.6%; 5 of 880), *Campylobacter* spp. (4.4%; 39 of 880), and rotavirus (6.1%; 9 of 148), and no isolates of *V. cholerae* O1 or O139 or *Aeromonas* were recovered. An analysis of the incidences of enteropathogens with respect to age and seasonal variations demonstrated that the frequencies of isolation of *Shigella* spp. and heat-labile enterotoxin-producing *E. coli* were significantly higher in those aged 1 to 5 years than in those younger than 1 year of age and those older than 5 years of age ( $P < 0.0001$  and  $P < 0.05$ , respectively) and that the frequencies of isolation of *Shigella* spp. and ST-producing *E. coli* were significantly higher in the rainy season than in the dry season ( $P < 0.005$  and  $P < 0.001$ , respectively). Almost all strains of *Shigella* spp. tested were resistant to ampicillin, tetracycline, and erythromycin and were susceptible to cefdinir and ofloxacin. This is the first intensive and longitudinal study to define the etiologic agents of diarrheal diseases in Lao PDR.

The Lao People's Democratic Republic (Lao PDR) is a landlocked country located in the center of the Indochina peninsula. The Mekong River flows along the west end of the country, forming a large part of the national border with Thailand. Diarrheal diseases are one of the main causes of life-threatening diseases in this country, especially in younger children, as is true of some other developing countries in tropical zones (21).

The rate of mortality from diarrheal diseases in the world has decreased markedly in the past decade, mainly because of interventions that promote sanitary conditions and that educate inhabitants to encourage them to take part in primary health care activities (5, 13, 21). An epidemiological study of an infectious disease in a community is an initial step toward the introduction of the proper interventions for controlling the disease, because the features of the disease vary from place to place depending on the local meteorology, geography, and socioeconomic elements. In Lao PDR, an intensive survey of diarrheal diseases has not yet been performed, and the local characteristics of these diseases have thus been undefined.

The present study is the first intensive and longitudinal survey based on the analysis of stools from patients visiting medical facilities to determine the etiologic agents of diarrheal diseases among the inhabitants of Vientiane, the capital city of Lao PDR.

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### MATERIALS AND METHODS

**Specimens.** Stools were collected from patients presenting with diarrhea at three public hospitals and a private clinic in Vientiane, Lao PDR. The specimens that were collected were transported in plastic containers or on sterile cotton swabs with the Amies transport medium with charcoal (rectal swab). They were transported to the Bacteriology Laboratory Service in the National Institute of Hygiene and Epidemiology Vientiane, Lao PDR, and were examined there on the same day. The survey was carried out over a period of approximately 11 months, from the middle of October 1996 to the end of August 1997. In general, November through April is the cool and dry season, while May through October is the hot and rainy season in the area of the Vientiane municipality. The possibility that the patients had treated themselves with antibiotics prior to consultations at the medical facilities could not be precluded.

**Microbiological examinations.** Almost all of the specimens were investigated for *Shigella* spp., *Salmonella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Aeromonas* spp., *Campylobacter* spp., enteropathogenic *Escherichia coli* (EPEC), enteroinvasive *E. coli* (EIEC), and heat-labile enterotoxin (LT)-producing *E. coli*. A total of 191 strains of *E. coli* that agglutinated with antisera to the EPEC and EIEC serogroups and to O serogroups O1, O146, O151, O157, O158, and O166 were tested for verotoxin productivity as enterohemorrhagic *E. coli* (EHEC). The test for the detection of heat-stable enterotoxin (ST)-producing *E. coli* was not performed with samples collected after 4 July 1997. Specimens collected in plastic containers were examined for the presence of rotavirus with a latex agglutination kit (Rotalex; Denkaseiken Co., Tokyo, Japan). Specimens in plastic containers were diluted serially with sterile normal saline, and 25  $\mu$ l of each of 10-, 100-, and 1,000-fold dilutions was inoculated onto thiosulfate-citrate-bile salts-sucrose agar medium, salmonella-shigella agar medium, and modified Drigalsky agar medium (BTB Agar; Eiken Co., Tokyo, Japan). The inoculum was spread on half of the plate and was streaked on the other half of the plate. This method definitely yielded colonies. Rectal swab specimens were directly inoculated onto each agar plate by routine techniques. The colonies that grew on each medium were examined for *Salmonella* spp., *Shigella* spp., and *Vibrio* spp. by standard microbiological methods and with commercial antisera if necessary. Presumptive *Aeromonas* colonies were picked and tested for oxidase, and all oxidase-positive colonies were further identified by standard methods. To examine the specimens for diarrheagenic *E. coli*, four presumptive colonies were picked from each specimen; and somatic O-antigen serotyping for EPEC and

EIEC with diarrheagenic *E. coli* diagnostic antisera (Denkaseiken Co.), two types of toxin detection tests for LT with a reversed-passive latex agglutination kit (VET-RPLA; Denkaseiken Co.), and tests for ST with an enzyme immunoassay kit (COLIST; Denkaseiken Co.) were performed. The productivity of verotoxin was examined by using reversed-passive latex agglutination with a commercial detection kit (Escherichia Verotoxin Detection kit; Denkaseiken Co.) according to the instructions in the manual included with the kit. *Campylobacter* spp. were isolated on Campylobacter blood-free selective medium (Oxoid, Hampshire, England) by incubation in a plastic jar with GasPak (BBL, Cockeysville, Md.) at 42°C for 48 h and were identified by confirming the morphology by Gram staining followed by an assay for hippurate hydrolysis.

**Drug susceptibility test.** One hundred twenty-eight *Shigella* spp. were examined for their susceptibilities to ampicillin, tetracycline, cefdinir, ofloxacin, and erythromycin. The MIC of each drug was examined by a plate dilution technique. Heart infusion agar plates containing the drug at a serial twofold concentration of from 0.025 to 100 µg/ml were prepared. The organisms were cultured overnight in heart infusion broth at 37°C and were diluted 10-fold with sterile normal saline. The diluted culture fluid containing the organisms at about 10<sup>7</sup> CFU/ml was inoculated with a microplanter (MIT 00257; Sakuma Co., Tokyo, Japan), and the MIC of each drug for the organisms was evaluated after incubation at 37°C for 24 h.

**Statistical analysis.** Associations were determined by a two-tailed chi-square distribution analysis with Yates' correction at the 5% level of significance.

## RESULTS

Eight hundred eighty diarrheal specimens were collected and analyzed for enteropathogens over the 11-month study period. The age distribution of the patients from whom diarrheal specimens were obtained was as follows: 226 patients were younger than 1 year of age, 446 patients were aged 1 to 5 years, and 208 patients were older than 5 years of age. Among the patients, the ratio of males to females was 1:0.95. Of all specimens investigated, 151 specimens were collected in plastic containers and the remainder were collected on rectal swabs.

The incidence of enteropathogens isolated from the collected specimens is presented in Tables 1 and 2. One or more enteropathogens were isolated from 379 specimens (43.1%). *Shigella* spp., ST-producing *E. coli*, and serogroup-based EPEC were predominantly isolated. *Vibrio cholerae* O1 and O139 and *Aeromonas* were not isolated throughout the study period. The subgroups of the 148 *Shigella* strains isolated comprised 82 *S. flexneri*, 60 *S. sonnei*, and 4 *S. boydii* isolates, 1 *S. dysenteriae* isolate (not type 1), and 1 untypeable isolate. *Salmonella* spp. were isolated from five specimens, and all belonged to O-antigen group 4. We defined the EPEC strains by classical EPEC serogroup. These strains were classified into two groups basically by referring to the O-serogroup categorization suggested by Levine (16), as follows: class I EPEC comprised serogroups O26, O55, O86, O111, O119, O125, O126, O127, O128ac, and O142, and class II EPEC comprised O18, O44, O112, and O114. The prevalence of class I and class II isolates was 64 strains (7.3%) and 33 strains (3.8%), respectively. A total of 841 specimens were examined for LT production, and 645 specimens were examined for ST production. Among these specimens, LT-producing *E. coli* was detected in 23 specimens, ST-producing *E. coli* was detected in 111 specimens, and *E. coli* strains producing both toxins were detected in 3 specimens. The frequency of isolation of LT-producing *E. coli* was 3.1% (23 + 3 of 841), and that of ST-producing *E. coli* was 17.7% (111 + 3 of 645). Thirty-seven isolates belonged to EIEC serogroups (4.2%). An *E. coli* strain from the class I EPEC group (serogroup O111) was detected as EHEC. *Vibrio* spp. were rarely found in the specimens examined; two isolates of *V. parahaemolyticus*, one isolate of *V. hollisae*, and two isolates of *V. cholerae* non-O1, non-O139 were found. The frequency of isolation of *Campylobacter jejuni* was 2.4% (21 of 880), and that of *Campylobacter coli* was 2.0% (18 of 880).

The frequency of isolation of enteropathogens for those aged 1 to 5 years was significantly higher than that for those in

TABLE 1. Incidence and age distribution of enteropathogens isolated from patients presenting at hospitals or clinics in Vientiane, Lao PDR

Pathogen	No. (%) of isolates in patients of the following ages:			
	<1 yr (n = 226)	1-5 yr (n = 446)	>5 yr (n = 208)	Total (n = 880)
<i>Shigella</i> spp.	21 (9.3)	105 (23.5)	22 (10.6)	148 (16.8)
<i>Salmonella</i> spp.	0	2 (0.4)	3 (1.4)	5 (0.6)
EPEC class I	16 (7.1)	36 (8.1)	12 (5.8)	64 (7.3)
EPEC class II	9 (4.0)	19 (4.3)	5 (2.4)	33 (3.8)
ETEC				
LT producing (n = 841) <sup>a</sup>	3 (1.4)	18 (4.2)	2 (1.0)	23 (2.7)
ST producing (n = 645) <sup>b</sup>	21 (13.4)	65 (20.1)	25 (15.2)	111 (17.2)
LT and ST producing (n = 645) <sup>b</sup>	0	1 (0.3)	2 (1.2)	3 (0.5)
EIEC	5 (2.2)	24 (5.4)	8 (3.8)	37 (4.2)
EHEC	1 (0.4)	0	0	1 (0.1)
<i>V. parahaemolyticus</i>	0	0	2 (1.0)	2 (0.2)
Other <i>Vibrio</i> spp.	0	3 (0.7)	0	3 (0.3)
<i>C. jejuni</i>	10 (4.4)	9 (2.0)	2 (1.0)	21 (2.4)
<i>C. coli</i>	6 (2.7)	9 (2.0)	3 (1.4)	18 (2.0)
Total <sup>c</sup>	79 (35.0)	223 (50.0)	77 (37.0)	379 (43.1)

<sup>a</sup> A total of 841 specimens were tested for LT; 211 specimens were from those younger than 1 year of age, 429 were from those aged 1 to 5 years, and 201 were from those older than 5 years of age.

<sup>b</sup> A total of 645 specimens were tested for ST; 157 specimens were from those younger than 1 year of age, 324 were from those aged 1 to 5 years, and 164 were from those older than 5 years of age.

<sup>c</sup> Multiple pathogens isolated from a specimen were counted as one. Of 148 specimens examined, 9 specimens were positive for rotavirus: 3 of 31 from those younger than 1 year of age, 4 of 49 from those 1 to 5 years of age, and 2 of 68 from those older than 5 years of age.

the other two age groups ( $P < 0.0005$ ) (Table 1). This variation by age was typically seen in the frequency of isolation of *Shigella* spp., the highest of which was seen in those ages 1 to 5 years. Among the *Shigella* spp., two subgroups of *S. flexneri* and *S. sonnei* occurred in those aged 1 to 5 years at higher frequencies (55 and 50 strains, respectively) than in those younger than 1 year of age (11 and 5 strains, respectively) and those older than 5 years of age (16 and 5 strains, respectively) ( $P < 0.005$  and  $P < 0.0001$ , respectively). The frequencies of isolation of EPEC in the three age groups were similar. A total of 148 stool specimens were tested for rotavirus, and 9 of them were positive: 3 of 31 specimens from patients younger than 1 year of age, 4 of 49 specimens from those aged 1 to 5 years, and 2 of 68 specimens from those older than 5 years of age.

The seasonal variation in the occurrence of the pathogens is summarized in Table 2. The total frequency of isolation of the pathogens in the rainy season (45.6%) was significantly higher than that in the dry season (36.6%) ( $P < 0.05$ ). Nevertheless, the frequency of isolation of *C. jejuni* was higher in the dry season than in the rainy season (4.5 and 1.5%, respectively;  $P < 0.01$ ).

Eighty-seven specimens contained mixed pathogens (mixed

TABLE 2. Incidence and seasonal variation of enteropathogens isolated from patients presenting at hospitals or clinics in Vientiane, Lao PDR

Pathogen	No. (%) of isolates in the following season:		
	Dry (n = 265)	Rainy (n = 615)	Total (n = 880)
<i>Shigella</i> spp.	31 (11.7)	117 (19.0)	148 (16.8)
<i>Salmonella</i> spp.	4 (1.5)	1 (0.2)	5 (0.6)
EPEC class I	12 (4.5)	52 (8.5)	64 (7.3)
EPEC class II	11 (4.2)	22 (3.6)	33 (3.8)
ETEC			
LT producing (n = 841) <sup>a</sup>	8 (3.4)	15 (2.5)	23 (2.7)
ST producing (n = 645) <sup>b</sup>	24 (10.3)	87 (21.1)	111 (17.2)
LT and ST producing (n = 645) <sup>b</sup>	1 (0.4)	2 (0.5)	3 (0.5)
EIEC	9 (3.4)	28 (4.6)	37 (4.2)
EHEC	0	1 (0.2)	1 (0.1)
<i>V. parahaemolyticus</i>	2 (0.8)	0	2 (0.2)
Other <i>Vibrio</i> spp.	0	3 (0.5)	3 (0.3)
<i>C. jejuni</i>	12 (4.5)	9 (1.5)	21 (2.4)
<i>C. coli</i>	3 (1.1)	15 (2.4)	18 (2.0)
Total <sup>c</sup>	97 (36.6)	282 (45.6)	379 (43.1)

<sup>a</sup> A total of 841 specimens were tested for LT; 233 were tested in the dry season and 608 were tested in the rainy season.

<sup>b</sup> A total of 645 specimens were tested for ST; 233 were tested in the dry season and 412 were tested in the rainy season.

<sup>c</sup> Multiple pathogens isolated from a specimen were counted as one. Of 148 specimens examined, 9 specimens were positive for rotavirus; dry season, 7 of 93 specimens; rainy season, 2 of 55 specimens.

infection) (Table 3). The incidence of mixed infection among the specimens from which enteropathogens were isolated was significantly higher for patients aged 1 to 5 years (27.1%; 62 of 223) than for patients younger than 1 year of age (16.5%; 13 of 39) and patients older than 5 years of age (15.6%; 12 of 77) ( $P < 0.05$ ). The most frequent pattern of mixed infection was the combination of *Shigella* spp. with ETEC (18 patients) (Table 3). In total, 38 specimens had mixed infections associated with *Shigella* spp., 39 specimens had mixed infections associated with EPEC, and 55 specimens had mixed infections associated with ETEC.

The drug susceptibility patterns of 128 isolates of *Shigella* spp. were examined (Table 4). Two peaks of MICs were found for ampicillin: 3.13 and 100 µg/ml or greater. Almost all isolates were highly resistant to tetracycline and erythromycin and were highly susceptible to ofloxacin. The MICs of cefdinir were distributed in a narrow range, from 0.1 to 0.4 µg/ml.

## DISCUSSION

Diarrheal diseases are one of the main social problems in Lao PDR, as in other developing countries in tropical zones (1, 2, 6, 18). Clarification of the enteropathogens associated with diarrheal diseases in the country is an essential step toward the implementation of effective primary health care activities against the diseases. The present etiological study is also mean-

TABLE 3. Patients with multiple pathogens isolated from a single specimen in Vientiane, Lao PDR

Pathogens <sup>a</sup>	No. of patients in the following age group:			
	<1 yr	1-5 yr	>5 yr	Total
Shig. plus the following:				
EPEC	1	5	3	9
ETEC	1	15	2	18
EIEC	0	1	0	1
Camp.	0	2	1	3
Shig. <sup>b</sup> + ETEC	0	1	0	1
EPEC + EPEC <sup>c</sup>	0	1	0	1
EPEC + ETEC	0	2	0	2
ETEC + EIEC	1	2	0	3
EPEC plus the following:				
EPEC <sup>c</sup>	2	1	0	3
ETEC	0	7	2	9
EIEC	3	2	0	5
Camp.	2	2	0	4
EPEC <sup>c</sup> + EIEC	0	1	0	1
EPEC <sup>c</sup> + Camp.	0	1	0	1
ETEC + Camp.	0	2	0	2
ETEC + Rota.	0	1	0	1
Camp. + Rota.	0	1	0	1
ETEC plus the following:				
ETEC <sup>d</sup>	0	2	1	3
EIEC	0	3	0	3
Vib.	0	1	0	1
Camp.	3	5	1	9
EIEC	0	1	0	1
EIEC + EIEC <sup>c</sup>	0	0	1	1
Sal. + Rota.	0	1	0	1
Sal. + Rota.	0	0	1	1
EIEC + EIEC <sup>c</sup>	0	2	0	2
Total	13	62	12	87

<sup>a</sup> Shig., *Shigella* spp.; Camp., *Campylobacter* spp.; Rota., rotavirus; Vib., *Vibrio* spp.; Sal., *Salmonella* spp.

<sup>b</sup> *S. flexneri* and *S. sonnei* were isolated.

<sup>c</sup> EPEC or EIEC strains with different O serogroups were isolated.

<sup>d</sup> ETEC strains producing different toxins were isolated.

ingful in that it provides information on the prevalence of enteropathogens in a distinctive area, the central region of the Indochina peninsula. The specimens analyzed there were taken from patients who had diarrhea severe enough to bring them to a medical facility. Although the specimens collected may not reflect the genuine spectrum of diarrheal diseases in the community, the survey is still significant because, to our knowledge, this is the first intensive and longitudinal survey to define the etiological agents of diarrheal diseases in Lao PDR.

*Shigella* spp. were revealed to play an important role in the diarrhea in all age groups examined in Vientiane. The frequency of isolation of *Shigella* spp. was 16.7%, on average, which is markedly higher than that reported in other tropical countries (3, 8, 9, 11, 18). In those studies, *Shigella* spp. were closely associated with diarrhea in children in a relatively older age group (6, 7) and in adults (9), and the isolation frequency increased progressively with age (8, 11). In contrast to these data, in the present study the highest frequency of isolation of *Shigella* spp. (23.5%) was seen in patients aged 1 to 5 years. We divided the group consisting of patients of less than 1 year of age into two subgroups, those 5 months of age or younger and

TABLE 4. Drug susceptibility patterns of *Shigella* spp. isolated from patients presenting at hospitals or clinics in Vientiane, Lao PDR<sup>a</sup>

MIC ( $\mu$ g/ml)	No. of strains				
	ABPC	TC	EM	CFDN	OFLX
$\leq 0.025$	0	0	0	0	0
0.05	0	0	0	0	59
0.1	0	0	0	3	65
0.2	0	0	0	70	3
0.4	0	0	0	55	1
0.8	0	4	0	0	0
1.6	3	2	0	0	0
3.1	38	1	0	0	0
6.3	12	0	6	0	0
12.5	4	0	1	0	0
25	0	0	23	0	0
50	0	45	37	0	0
$\geq 100$	71	76	61	0	0

<sup>a</sup> A total of 128 strains were tested. ABPC, ampicillin; TC, tetracycline; EM, erythromycin; CFDN, cefdinir; OFLX, ofloxacin.

those aged 6 to 11 months, and compared the frequencies of isolation of *Shigella* spp. for the two subgroups. The comparison revealed that the frequency of isolation of *Shigella* spp. was relatively high for those aged 6 to 11 months (12.2%; 19 of 156), whereas that for those in the younger subgroup remained low (2.9%; 2 of 70). Thus, it is possible that for children in Vientiane the initial exposure to *Shigella* spp. occurs at the ages of 6 to 11 months, which is younger than the age of initial exposure for children in other countries (6, 8). In general, *S. sonnei* is reported to cause the majority of cases of shigellosis in industrialized countries, and in developing countries where hygiene is poor, the incidences of other *Shigella* species such as *S. dysenteriae*, *S. boydii*, and *S. flexneri* are relatively high (22). In the present study, *S. flexneri* and *S. sonnei* accounted for 55.4 and 40.5% of all *Shigella* isolates, respectively. Considering these frequencies, the pattern of prevalence of each *Shigella* species in Vientiane can be considered the same as that in a developing society.

Both *Shigella* spp. and enterotoxigenic *E. coli* (ETEC) were incriminated as major causes of diarrheal diseases in Vientiane. Among the ETEC isolates, ST-producing *E. coli* accounted for 81% (111 of 137), which is markedly higher than that reported in other countries (2, 6, 8, 19). ETEC, particularly LT-producing *E. coli*, was significantly associated with diarrhea in the patients aged 1 to 5 years in Vientiane ( $P < 0.05$ ).

EPEC has been identified serologically by the detection of particular O antigens which are associated with certain diarrheagenic *E. coli* strains. On the basis of rational pathogenesis, Levine (16) subdivided the EPEC category into the following two classes according to serological studies: class I, which usually possesses EPEC adherence factor (EAF), and class II, which usually does not possess EAF but which definitely causes diarrhea. Identification of a strain as an EPEC strain by detection of EAF and/or the *eae* gene or by the confirmation of localized adherence to HEp-2 cells is indisputable but requires specialized equipment. The reliability of the serological identification of EPEC has been reevaluated, and serological identification is recognized as useful (10–12), particularly in the setting of a laboratory with limited facilities in a developing country. In the present survey, the frequency of isolation of the EPEC serogroup was found to be 11.0% (97 of 880) when the isolates of the two classes were combined, which was intermediate between the high frequencies found in Chile (38.3%)

(17) and São Paulo, Brazil (34.0%) (11), and the low frequencies found in Somalia (4.0%) (6) and Thailand (5.5%) (7). EPEC has been incriminated as a significant cause of infantile diarrhea in several studies (2, 7, 12, 20); in the present study, however, there was no significant difference in the frequency of isolation of EPEC among patients in three age groups, a result which may have been obtained because (i) EPEC may not be a significant etiologic agent of infantile diarrhea or (ii) EPEC strains which possess O antigens other than those of the classical serogroup may have been substantially involved as the causative organisms of infantile diarrhea, and consequently, the incidence of infantile diarrhea caused by EPEC was apparently low in Vientiane. Unlike the findings of other studies (2, 12, 17), in the present study serogroups O128 and O126 accounted for about half of the class I EPEC isolates and serogroups O18 and O44 were predominant among the class II EPEC isolates. The specimens tested in the present study were not examined for enteroaggregative *E. coli*, which has recently been recognized as a causative organism of persistent diarrhea in children (4, 15).

The incidence of diarrhea associated with *Salmonella* spp. in Vientiane was lower than expected. A majority of species of *Salmonella* are likely to adapt to domesticated cattle and all varieties of fowl, as well as human beings. The reservoir of infection in animals constitutes the principal source of gastroenteritis caused by *Salmonella* spp. (14). Not a few inhabitants of Lao PDR, even in Vientiane, keep these reservoirs around their environments in their daily lives, and we therefore expected a higher frequency of isolation of *Salmonella* spp. as a cause of diarrhea. The situation is the same in the case of *Campylobacter*, which is also an example of a zoonotic organism and which was isolated at a relatively low frequency from patients with diarrhea in the present survey. The reasons for these results are not yet known.

About 17% of the collected specimens were tested for rotavirus because a large proportion of specimens were taken as rectal swabs. The incidence of rotavirus in Vientiane was found to be quite low (6%) compared to those in Thailand (18% [7] and 19.7% [8]), a neighboring country, and those in other tropical countries (6, 20).

Two strains of *V. parahaemolyticus*, which is an important food-borne enteropathogen in coastal areas, were isolated from diarrheal specimens in Vientiane, a landlocked city. Lao PDR has increasingly been involved in international trade, and as a consequence, daily foodstuffs including seafood have been imported from adjacent countries. Although the incidence of *V. parahaemolyticus*-related diarrhea was not high in the present survey, attention should be paid to the treatment of seafood, which is frequently incriminated as a reservoir of marine bacteria.

No *V. cholerae* O1 was isolated in the present study. In the period from 1994 to 1995, there were a series of cholera epidemics in Lao PDR, including the Vientiane municipality (personal communication). Although the samples studied were collected only in hospitals and might not be appropriate for the evaluation of cholera status in that year, our results suggest that a large cholera epidemic which brings patients to medical facilities has not yet broken out or that infection episodes occurred at such low levels as to be undetectable in the setting.

Similar to the results of other studies performed in tropical countries, *Shigella* spp. (1, 9) and ETEC (9) were isolated more frequently in the rainy season than in the dry season, which contributed to the higher rate of detection of enteropathogens from specimens in the rainy season than in the dry season. Significant seasonal variation in the frequencies of isolation of *S. sonnei* and ST-producing *E. coli* was observed; i.e., 86.7%

(52 of 60) and 78.4% (87 of 111) of these strains, respectively, were isolated in the rainy season.

The dominant patterns of combinations of enteropathogens associated with mixed infection in each age group consisted of combinations of the enteropathogens most frequently isolated from patients in each age group. Among patients younger than 1 year of age, however, there were no cases of mixed infection with ETEC and EPEC, both of which were the enteropathogens most frequently isolated from those in this age group. The incidence of mixed infection for the specimens from which an enteropathogen was isolated in each season (24.7% [24 of 97] in the dry season; 22.3% [63 of 282] in the rainy season) and the incidence of their occurrence in combination were similar.

Drug susceptibility was examined for *Shigella* spp. Almost all strains tested were resistant to ampicillin, tetracycline, and erythromycin, which is attributable to the ready availability of these antibiotics and the use of these drugs without appropriate prescriptions. The introduction of integrated guidelines for the appropriate use of antibiotics is urgently needed. All *Shigella* spp. were susceptible to an oral cephem (cefдинир), a new quinolone (ofloxacin), and nalidixic acid, an antecedent of ofloxacin, as examined by the disc diffusion method (data not shown). Nalidixic acid is recommended as the drug of choice for the treatment of adult shigellosis, because in Vientiane the drug is more readily available than the new quinolone. However, in the case of children with shigellosis, which is one of the main causes of diarrhea in 1- to 5-year-old children, cefдинир is the drug of choice.

No etiologic agent was identified in 58% of the patients with diarrhea examined in the present study, which is possibly explained by the fact that examinations for the detection of parasites or diarrhea-related viruses other than rotavirus were not performed in this setting and individuals who treated themselves with antibiotics were not precluded from participation in the study.

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