

# THE SEASONAL VARIATION IN THE MICROBIAL AGENTS IMPLICATED IN THE ETIOLOGY OF DIARRHEAL DISEASES AMONG CHILDREN IN LAO PEOPLE'S DEMOCRATIC REPUBLIC

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**Abstract.** During 1994-1995, the etiological structure of children's diarrhea was monitored in outpatients at Mahosot Hospital, Vientiane, Lao PDR. Of the 191 children studied, 42% had stool specimens positive for enteropathogens; 22% had rotavirus, 21.5% enteropathogenic *E. coli*, 4.7% *Shigella flexneri*, 2.9% *Campylobacter jejuni*, 2.1% *Shigella sonnei*, and 0.5% each of *Giardia intestinalis* and *Entamoeba histolytica*. No *Vibrio cholerae* and *Salmonella* spp. was detected in this monitoring. In children under five years, rotaviruses were detected almost all round the year with a maximum prevalence in January in the dry season. *Shigella* species were highly detected in June to July in the rainy season.

## INTRODUCTION

Diarrheal disease is one of the commonest, but most important diseases that cause high mortality rate among children in Lao PDR. But only a few information has been available about microbial diarrheal agents in Lao PDR, except reports on enteropathogenic *E. coli* (Germani *et al.*, 1989) and pathogenic Enterobacteriaceae and Vibrios (Midorikawa *et al.*, 1996). However, there is no detailed report on seasonal patterns of diarrheal pathogens in Lao PDR in the last two decades. In this concern, a survey on identification of diarrheal agents was conducted in the first year of our project at Mahosot Hospital that was in cooperation with the National Institute of Hygiene and Epidemiology (NIHE) in Vientiane, Lao PDR.

In 1994 the monitoring was set up to study the etiological, clinical, and laboratory aspects on the diarrheal outpatients in the hospital. We were interested in identifying the relative importance of various enteropathogens, mainly on bacteria and rotavirus, and in observing the seasonal prevalence and severity of the disease associated with these agents.

## MATERIALS AND METHODS

### Monitoring methods

Diarrheal patients for monitoring was selected

at random based on the registration number of the outpatient. In the period from 5 January 1994 to 2 February 1995 inclusive, patients were selected immediately after registration, but in October 1994 the sample size was entirely decreased and the data were lacking. Official hospital staff members examined the monitored patients including emergency cases who were treated on a priority basis. All the patients were admitted to the diarrhea treatment unit of the hospital. After initial quick examination and care by a nurse or doctor, the patient monitor or an adult guardian was interviewed by a doctor who collected uniform information based on a formatted sheet on demographic background, recent medical history, presenting symptoms, previous treatment, and environmental conditions of his/her habitat. The environmental conditions were asked on water usage for drinking and washing, and on toilet type. Rectal swabs for bacteriological cultures were obtained from the patients, and stool samples for microscopic examination were taken.

### Laboratory methods

Rectal swabs or stool samples were plated directly on Salmonella Shigella (SS), MacConkey's, and TCBS agars. Specimens were also enriched in alkaline peptone water for vibrios and then plated on TCBS agar. The plates were examined for *Salmonellae*, *Shigellae* and Vibrios by standard methods. Non-lactose fermenting colonies from MacConkey's and SS agars were screened on Kligler's iron agar slants, Indole urea, SIM, Lysin, SC, and Voges-Proskauer media. Isolates of *Shigella* were

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biochemically identified and serologically grouped with the slide agglutination test using commercially available antisera (Denka Seiken, Japan). From January to February 1994, all specimens were cultured for *Campylobacter jejuni* using Campy-BAP charcoal agar (Oxoid, UK). A pool of 4 colonies of *Escherichia coli* from each patient was tested for O serogroup antigens by the slide agglutination test using commercially available antisera: Group 1; O1, O26, O86a, O111, O119, O127a, and O128, Group 2; O44, O55, O125, O126, O146, and O166, Group 3; O18, O114, O142, O151, O157, and O158, Group 4; O6, O27, O78, O148, O159, and O168, Group 5; O20, O25, O63, O153, and O167, Group 6; O8, O15, O115, and O169, Group 7; O28ac, O112ac, O124, O136, and O144, Group 8; O29, O143, O152, and O164 (Denka Seiken, Japan). Parasitic protozoas were identified by direct microscope observation of the smeared stools. Rotavirus antigen was identified by commercially available Reversed Passive Latex Agglutination test kit (Denka Seiken, Japan).

## RESULTS

In the period from 5 January 1994 to 2 February 1995, 588 diarrheal patients were treated at Mahosot Hospital, of whom 191 were monitored in this period (33.8% of the sample). Most patients treated were infants: 45.5% were under 1 year of age, 50.2% between 1 and 5 years, and 4.3% between 6 and 13. Although 60.2% of patients were male, there were no significant differences between the sexes in age distribution, duration of hospital access and severity of dehydration.

For the areal distribution, 167 sampled cases were from Vientiane municipality area and 24 from other provinces including Vientiane Province (Table 1).

Of 99 cases monitored, their living environmental conditions were shown in Tables 2 and 3. Concerning their drinking water resources, only 60% was dependent on tap water and the rest was mainly on well water. Their main type of toilet was latrine

(54%), and 10 cases were replayed defecation on outside ground.

Among 173 of the patients, 74 (42.8%) reported prior use of medicines, primarily bactrim (sulfamethoxazole-trimethoprim) and paracetamol (acetylsalicylic acid). Only 17 cases were confirmed to use oral rehydration fluid before coming to the hospital.

All patients (191) were cultured for *E. coli*, *Shigella*, and *Vibrio* and those seen during the period from January to February 1994, and 70 of them were also cultured for *C. jejuni*. From all patients *E. coli* was isolated and tested for the O serogroup for determining the enteropathogen, and all samples were tested for rotavirus.

### Agents associated with diarrhea

A recognized pathogenic organism was isolated and identified from 42.4% of patients screened for all pathogens, one-fourth of whom had a mixed infection with two or three agents. Table 4 shows that rotavirus (22%) was most frequently detected, fol-

Table 1  
Areal distribution of infant diarrheal cases during the period from January 1994 to February 1995.

District	Case
Chanthaboury	42
Sisattanak	38
Sikhottabong	26
Xaisetha	26
Hatxayfong	12
Naxaythong	8
Xaithani	14
Sangthong	0
Pakngum	1
<b>Subtotal</b>	167
Vientiane Province	10
Other Provinces	5
Unknown	9
<b>Subtotal</b>	24
<b>Totals</b>	191

Table 2  
Kinds of drinking water and washing water used among the cases.

Kind of water	Tap	Well	Public fountain	Pond	Tap, Pond	Well, Pond	Well, River	Pond, River	NA
Drinking water	57	30	1	3	3	2	1	1	1
Washing water	41	23	0	5	0	0	1	0	29

Table 3  
Types of toilet used among the cases.

Types of toilet					Total
Flush	Latrine	Outside ground	Flush, Latrine	NA	
34	53	10	1	1	99

Table 4  
Results of detected diarrhea pathogens identified.

Combination of pathogens	Age distribution			Total
	<1	1-5	5<	
EPEC	10	13	1	24
EPEC+Rotavirus	1	7	1	9
EPEC+S. flexneri		4	1	5
EPEC+C. jejuni	1			1
EPEC+S. flexneri+Rotavirus		1		1
EPEC+S. sonnei+Rotavirus	1			1
S. flexneri		3		3
S. sonnei	2	1		3
C. jejuni	1			1
Rotavirus	18	11		29
E. histolytica+Rotavirus		1		1
T. hominis+Rotavirus	1			1
G. intestinalis		1		1
T. hominis		1		1
Total	35	43	3	81

lowed by enterotoxigenic *E. coli* (21.5%), *Shigella flexneri* (4.7%), *C. jejuni* (2.9%) and *Shigella sonnei* (2.1%). No *Salmonella* species or *Vibrio* species were detected. *Trichomonas hominis* was detected in 1% of the patients examined. Trophozoites of *Entamoeba histolytica* and *Giardia intestinalis* were detected each in 0.5% of the patients.

Of all the enteropathogenic *E. coli*, 78% revealed the infected cases with single O sero-group varied from No.1 to No. 8., 14.6% with double O sero-groups, and 0.5% with triple O sero-groups (Table 5). Among them, identified O serotypes were O1, O6, O15, O44, O112ac, O126, O127, O148 and O157.

#### Age and sex distribution

Infants of 35 cases (<1 year) were infected most frequently with rotavirus (57.1%), followed by enterotoxigenic *E. coli* (37.1%), and *S. sonnei* (8.6%) and less with *C. jejuni* (5.7%), and *T. hominis*. (2.9%) (Table 4). Enteropathogenic *E. coli* (58.1%) and rotavirus (46.5%) remained the commonest pathogens in toddlers of 43 cases (1-4 years) followed by *S. flexneri* (18.6%), *S. sonnei* (2.3%), *E.*

*histolytica* (2.3%), *G. intestinalis* (2.3%), and *T. hominis* (2.3%). This group showed the highest detection rates of the pathogenic agents. Rotavirus and *S. flexneri* were rarely identified in children over 5 years. There was no significant sex difference for infections with any pathogenic agents.

#### Seasonality

The highest number of isolates detected was in January 1994. The number decreased sharply toward March. After brief high rates in April and in July, the numbers become reduced, reaching the lowest figures at the end of the rainy season (May to September) in September. The numbers remained low until the beginning of the dry season when they start to rise again (Fig 1).

This change of figures on detection of enteric pathogens was similar to that of whole diarrheal cases. The detection rates of rotavirus were markedly higher than those in bacteria, ranging from 37.5% to 29.2% in the period from January to February 1994, and 17.9% in January 1995. Rotaviruses were also detected during the rainy season (May to June, and August inclusive). While bacteria positives ranged

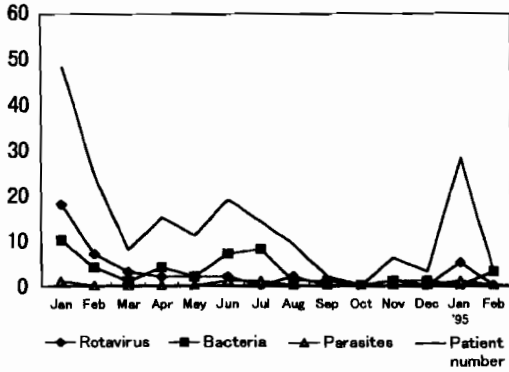


Fig 1-The seasonal changes of rotavirus, bacteria and parasites detected among diarrhea patients.

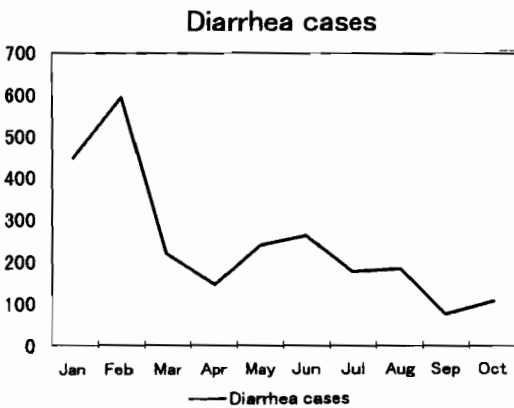


Fig 2-The seasonal occurrence of diarrhea cases under 5 years of age at Vientiane municipality, Jan-Oct, 1994.

from 57.1% to 36.8% from June to July in 1994, enteropathogenic *E. coli* was commonly detected during the period. *S. flexneri* and *S. sonnei* were common, and variable pathogens were detected in the rainy season (June to July inclusively).

**Clinical history**

Although full clinical information was not collected from all monitored patients (Tables 6 - 8). Most patients (68.6%) had an acute illness within six days, but 21% had been ill for more than a week before coming to the Mahosot Hospital. Watery diarrhea was the commonest chief complaint (33%) and was more frequent in patients with rotavirus (27.0%), enteropathogenic *E. coli* (22.2%) and *Shigella* (6.3%) than all other patients studied. Dysentery, defined as stool with blood, was the chief complaint of 15.7% of all patients but 16.7% of those were with *Shigella*. Frequency of pathogens on bacteria and rotavirus between fever and non-fever cases was not different in this monitoring. Death cases were not confirmed in this study.

Table 5  
O antigen groups of enteropathogenic *E. coli*.

O Ag group	Age distribution			Total
	<1	1-5	5<	
1	2	7	1	10
2	2	5		7
3	2	1	1	4
4		1		1
5	1	3		4
6		2		2
7	3		1	4
8		1		1
1, 7		1		1
2, 3	1			1
2, 4		1		1
2, 6	1			1
3, 7		1		1
3, 8	1			1
Unknown		2		2
Total	13	25	3	41

Table 6  
Duration of diarrhea (from onset to admission).

Age	<3days	4 - 6 days	>7days	Unknown
<5	64	63	38	18
5<	1	3	2	2

Table 7  
Chief complaint of diarrhea.

Age	Watery	Others	Unknown
<5	61	98	24
5<	2	6	0

Table 8  
Bloody stool and confirmed shigellosis.

Age	Bloody stool amongst cases			Confirmed shigellosis
	Yes	No	Unknown	
<5	27	144	12	5
5<	3	5	0	0

**DISCUSSION**

In the present study, the agents could be detected and identified only at 42% of diarrhea episodes. Positive detection was less than in Bangkok where pathogens were isolated from 60% of children with diarrhea (Echeverria *et al*, 1989), but similar to those in rural Thai communities (Echeverria *et*

al, 1994). During this monitoring, we confirmed that enteropathogenic *E. coli* was the commonest and most frequent bacteria pathogen among the cases. We were not confirmed enterotoxigenic *E. coli* (ETEC) using enterotoxin detection, even though these were prevalent at Vientiane area (Germani *et al*, 1989). Patients with *C. jejuni* had an illness that was determined for the first time and detected in Lao PDR. Diarrhea associated with *Shigella* infection was rare except the species of *S. sonnei* in infants (<1 year) and commoner in old children, suggesting short-lived transplacental immunity, protection due to breast-feeding or decreased exposure in infancy, which was mentioned in Bangladesh (Stoll *et al*, 1982). Breast-feeding rate amongst the patients was 89% (Suzuki *et al*, unpublished data).

Our figures of seasonal abundance on diarrhea revealed the same patterns of the results that were obtained during 1987-1991 at the Diarrhea Treatment Unit of the hospital (unpublished data). In addition, according to a report on diarrhea at Vientiane municipality from January to October in 1994 (unpublished data on a total of 2,419 under 5 years), the figures showed two distinct peaks in February (579) and in June (259), and revealed the similar tendency to our results (Fig 2). Rotavirus infection occurred during low temperature season (the dry season), especially in January, and the frequency was highest at age under 1 year, indicating the same tendency as in Thailand (Jayavasu *et al*, 1982).

Some bacterial agents may be missed because of the prior antibiotic use, failure to obtain an adequate rectal swab, or the examination of only one stool specimen. We could not detect *V. cholerae* and *Salmonella* spp. in this monitoring, however, this was not attributed to our technical failure, since there was a record of detecting these enteropathogens from adult cases in this laboratory (Midorikawa *et al*, 1996). We are considering that more agents will be identified from more patients with acute diarrhea in near future.

The health problems in Lao PDR are similar to those existing in other developing countries. Through this kind of survey, the laboratory functions were stimulated and improved, and obtained knowledge on the agents prevalent in a given population, their seasonality and the severity of the disease provided important information for considering treatment and prevention. Further more it was noticed that there is a need to develop more the laboratory diagnostic technology, even if there are

only limited resources. Despite this limitation, information useful for the control of diarrhea can be obtained from the continuous investigations of microbiologic monitoring.

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