

SEATO MEDICAL RESEARCH STUDY ON DIARRHEAL DISEASES

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Period of Report: 1. April 1965-31 March 1966

Study Reports

- Title: Bacteriologic Survey of Stools from Patients with Acute Diarrhea
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Objective - The objectives of this study were to determine the types, frequency of occurrence and distribution of salmonellae and shigellae in stools of patients with acute or chronic diarrhea and to determine the incidence of other Enterobacteriaceae and their relationships to this disease.

Description - Specimens included in this study were from inpatients and outpatients of both sexes from hospitals throughout Thailand. Most were hospitalized for acute diarrhea, and most specimens were taken during the acute stage of the disease. For the first 9 months of this reporting period the laboratory procedure was as follows: In the Bangkok area 2 rectal swabs were taken from each patient. One was placed in a screw-capped tube of selenite-F enrichment broth and the second into a tube of alkaline peptone broth. Specimens from outside of Bangkok were submitted in a holding medium designed for transport of enteric bacteria. Upon arrival at the laboratory tubes of selenite-F and alkaline peptone broth were inoculated from the holding medium. Each selenite-F broth was subcultured on Desoxycholate-Citrate (DC) and MacConkey (MC) agar plates. After overnight incubation, the selenite-F broth was again subcultured, this time to salmonella-shigella (SS) and eosin-methylene blue (EMB) or MC agar plates. The alkaline peptone broth was subcultured on alkaline lauryl sulfate tellurite agar plates. All incubations were at 37 C. During the last 3 months the procedure was modified. Three rectal swabs moistened with alkaline peptone broth were obtained from each patient. Two swabs were placed in enrichment broth and the third streaked directly on SS and MC agar plates. The alkaline peptone broth was subcultured to alkaline lauryl sulfate tellurite agar. After overnight incubation at 37C the selenite-F broth was subcultured on SS and DC plates. Specimens received in holding media were streaked directly on SS and MC and tubes of selenite-F and alkaline peptone broth were inoculated from the holding medium. These enrichment broths were subcultured exactly as described immediately above.

With both procedures, all plates were examined after 24 and 48 hours incubation. Lactose-negative colonies were transferred to Kligler's iron agar slants and subsequently into a variety of media to determine patterns of biochemical activity. Colonies typical of vibrios on alkaline lauryl sulfate tellurite agar were processed in the same way. Those isolates showing biochemical patterns typical of salmonellae, shigellae or vibrios were definitively identified serologically in accordance with methods described by Edwards and Ewing.

The survey method used for parasite detection consisted of mixing approximately 0.5 gm of feces with freshly prepared MIF solution and subsequent examination of the material in Bangkok. Although the advantages of MIF fixed material for survey work are obvious and well known there are some limitations. Foremost of these is that light infections are not readily detected and to gain a true picture of the extent of intestinal parasitism a concentration technique, such as the zinc sulfate method, should also be employed. The diagnosis of Entamoeba histolytica cysts is not always easy and any future survey should employ PVA-Hematoxylin stained material as well as concentration techniques. Despite these limitations, a preliminary picture of intestinal parasitism can be gained from these surveys.

Progress-Recognized diarrheal agents were isolated from approximately 18 percent of all specimens (Table 1). The incidence of diarrhea associated with salmonellae was 14.3 percent, a percentage that has been reasonably constant for the last 4 years. Only 6 of 65 specimens from Americans were positive for salmonellae. The results in table 2 show that at least 16 species were represented among the 482 positive specimens. S. paratyphi B and S. derby were isolated every month from specimens submitted from Children's Hospital, Bangkok, Thailand and isolates of the latter increased to 85 of 186 specimens submitted in March 1966. Each of the 6 isolates of S. typhosa occurred in different months. The number of isolates of S. montevideo decreased substantially during the past year but 41 of the 54 were isolated during the first 3 months of 1966.

Among Americans, there were 8 shigellae isolates representing four serotypes isolated from 65 specimens. Overall, a total of 115 shigellae isolates representing 11 species were made during this period. (Table 3) More than half of these were isolated during the first three months of 1966 when the revised isolation procedures were in use and might indicate better laboratory techniques rather than increase of shigellosis. There were outbreaks of shigellosis in Thailand which will be discussed later.

Agglutinating and non-agglutinating vibrios were rarely isolated in the Bangkok area during this period. An outbreak of cholera in central Thailand is discussed in the Study Report on Cholera.

While their role in the etiology of diarrheal diseases is unproven, Paracolobactrum sp. were listed in Table 1 for the sake of completeness. About 39 percent of the specimens were positive for one or more species of this genus. There was no pattern of distribution of these organisms that suggested any role as an enteric pathogen except that no recognized pathogen was isolated from most of these patients.

Studies on the kinds and distribution of enteropathogenic Escherichia coli were limited to patients under 6 years of age. A total of 137 strains were identified from 2574 isolates typed. The finding that 11 of 12 enteropathogenic serotypes searched for were found in infants and small children suffering from acute diarrhea emphasizes the potential danger of outbreaks and continued sporadic cases of enteritis caused by these organisms.

By use of the tube dilution method, 102 isolates of salmonellae representing 15 species, and 41 isolates of shigellae representing 10 species and serotypes were tested for sensitivities to 5 antibiotics. Overall the most effective antibiotic for the salmonellae isolates was colistin followed in order of decreasing effectiveness by chloramphenicol, the tetracyclines, kanamycin sulfate and neomycin sulfate. Almost all strains of the 3 salmonella species isolated most frequently, i.e. S. paratyphi B, S. derby, and S. montevideo were resistant to all antibiotics tested except colistin. All shigellae strains were sensitive to colistin followed in order of decreasing effectiveness by neomycin sulfate, kanamycin sulfate, chloramphenicol and tetracycline.

No outbreaks of acute diarrhea were noted among U.S. personnel during this reporting period. However a series of outbreaks in Thai nationals occurred in several northeastern provinces. Observations

made in Nakorn Phanom province by a team from SMRL found that some patients had bloody mucus stools and others had watery stools. In the more severely ill patients, there was only a small amount of mucus with much straining at the stools and their stools contained many leukocytes and erythrocytes. Patients had a high fever which persisted in spite of treatment with tetracycline for 48 hours (1 gm/day). At the time of the fever the patients were extremely weak and without appetite. Malnutrition was severe in most of the children and many had bronchopneumonia.

Cultures were taken from villagers and all hospitalized patients with a history of diarrhea. It was evident from the swabs that most of the patients did not have diarrhea when cultured. The pathogen found most frequently was Shigella dysenteriae 1 which was resistant to chloramphenicol, tetracycline, sulfathiazole and sulfadiazine but sensitive to colistin and kanamycin. Other pathogens isolated were 2 serotypes of pathogenic Escherichia coli, some of which were non-motile and did not ferment lactose until the 12th day of incubation at 37°C.

At least 8 provinces reported similar outbreaks of acute diarrhea and isolates of Sh. dysenteriae 1 with the antibiotic sensitivity pattern described above were identified in those provinces where cultures were taken. As a result it was decided to conduct surveys to determine types of dysentery bacteria and intestinal parasites which represent potential hazards to military troops operating in those areas. Surveys were carried out in Korat, Udorn, Ubol and will be carried out in Nakorn Phanom.

Bacteriological findings from the 3 provinces already surveyed indicate that carrier rates among Americans and Thais were very low. (Table 5) Among the pathogens isolated were 10 serotypes of shigellae, 7 species of salmonellae and 8 serotypes of enteropathogenic E. coli. Parasitological studies of stool specimens obtained in Ubol have not yet been analyzed. In both Udorn and Korat the most common parasite was found to be hookworm. An unusual finding was the relatively low infection rates of both Ascaris and Trichuris. In many parts of the tropical East these are the predominant parasites. Furthermore although a few heavy infections were noted, as evidenced by the standard smear egg-count, the majority of infections appeared to be light. It would be of interest to examine an adjacent rural community to determine whether ascariasis would be more prevalent than in city-dwellers although the rate of hookworm in the latter indicates that they too have ample opportunity to acquire soil transmitted helminthiases. Because of the possible importance of amoebiasis as a cause of disease in Thailand it is suggested that more detailed studies be carried out in the future. It would also be of interest to determine the role of Strongyloides and Giardia as a cause of diarrhoea in this country.

Summary - A new procedure for processing stool specimens was evaluated and adopted when results indicated that the new procedure resulted in recovery of more serotypes of enteric bacterial pathogens. Approximately 18 percent of stool specimens from diarrhea patients were positive for known bacterial pathogens. In the Bangkok area the organisms most frequently isolated were S. paratyphi B and S. derby. In the northeastern part of Thailand there was a series of outbreaks of acute diarrhea. The enteric pathogens most frequently isolated were entero-pathogenic E. coli and Sh. dysenteriae 1, the latter being resistant to chloramphenicol tetracycline, sulfathiazole and sulfadiazine but sensitive to colistin and kanamycin. In general, most enteric bacterial pathogens were resistant to the tetracyclines but sensitive to colistin. A survey is in progress to determine types of dysentery bacteria and intestinal parasites which might represent potential hazards to military personnel operating in those areas. Incomplete results indicate very low rates of parasitological infestation and few carriers of bacterial pathogens. This study will be completed during the next quarter.

Publication, Noyes, H.E., Benjadol, P., Thareesawasdi, M. and Senadisai, P.: Antibiotic Sensitivities and Cross-Resistance Patterns of El Tor Vibrios and Other Enteric Pathogens Indigenous to Thailand. In Antimicrobial Agents and Chemotherapy. 1964 p. 415-422.

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