

20 YEARS OF PROGRESS IN DIARRHEAL DISEASE RESEARCH

Narain H. Punjabi¹, Nancy D. Witham¹, Donald H. Burr¹,
Cyrus H. Simanjuntak², Murad Lesmana¹, S. Suharyono³, Sunoto³,
Maramis A. Hisham⁴, Atti R. Rivai⁴, Swiandy Kumala⁴,
Y. Soenarto⁵, and S. Komalarini⁶

When NAMRU started its collaboration work with the National Institute of Health, Research and Development (NIHRD), it became apparent that diarrheal disease was one of the most important causes of morbidity and mortality in Indonesia, especially in children¹. Many of the most important etiologic agents of diarrhea were not known and the percentage of diarrheas with an identifiable etiologic agent was very low².

Since these early times NAMRU and NIHRD have worked together in all aspects of diarrheal disease research. Increased capabilities for the identification of bacteriologic, parasitic and viral enteropathogens, new vaccines, and better treatment via oral rehydration solutions are some of the results of this collaboration.

ETIOLOGY

The majority of the earlier work done by NAMRU and NIHRD was in the area of etiologies of diarrhea and gastrointestinal disease, focusing on cholera and other *Vibrio*

species, and intestinal parasitism. In 1971, Joseph and co-workers were the first to report in Indonesia the unsuspected association of *Vibrio parahemolyticus* with diarrheal disease³⁻⁵. The clinical picture of the patients with *V. parahemolyticus* infection was not significantly different from those with other commonly identified etiologies. Later in 1977, Komalarini *et al.* reported on a 21 month study of the etiology of diarrhea among infants and children admitted to Sumber Waras Hospital⁶. The major etiologic pathogen detected was *Vibrio cholerae* biotype El tor. Other important etiologies of diarrhea observed in this study were *Salmonella*, *Shigella*, *Vibrio cholerae* non-01, and *V. parahemolyticus*. A bimodal seasonal pattern of admitted cases occurred, with peaks during or immediately after the dry and rainy seasons. However, in later studies it was shown that these annual patterns of seasonality and morbidity were subject to significant long-term fluctuations^{6,10-11,13}.

The first isolation in Indonesia of *Campylobacter jejuni* from Indonesian patients

¹ U.S. Naval Medical Research Unit No. 2, Jakarta

² National Institute of Health Research and Development, Jakarta

³ Department of Child Health, Faculty of Medicine, University of Indonesia, Jakarta

⁴ Infectious Disease Hospital, Jakarta

⁵ Department of Child Health, Faculty of Medicine, Gadjah Mada University, Yogyakarta

⁶ Department of Child Health, Sumber Waras Hospital, Jakarta

with gastroenteritis⁷ was made by Ringertz *et al* in 1980. In this study, *Campylobacter* was isolated from feces of 15 of 144 diarrheic children less than 9 years of age, and from 4 of 251 adults with gastroenteritis. In contrast, *Campylobacter* spp. was isolated from only 1 child in a control group of 221 persons. Subsequent work, determined the susceptibility of this organism to a wide range of antimicrobial agents⁸.

Several other diarrhea studies were done in the following years with different objectives. A study done in Yogyakarta in 1975 aimed to determine the relation between bacteria isolated from stool culture and presence of leucocytes in feces⁹. Sunoto *et al.* in 1978 reported the effects of commonly utilized antibiotics on 2 groups of children infected with entero-pathogenic bacteria who were treated with oral rehydration solution (ORS). The result showed no significant difference between the group of children who received ORS plus antibiotic and the group who was given ORS only¹⁰. In 1980 Punjabi *et al.* compared the results of stool/rectal swab cultures from adult diarrheal patients to febrile patients hospitalized at Infectious Disease Hospital of Jakarta¹¹. Agents of diarrhea, including rotavirus and enterotoxigenic *E. coli* (ETEC), were detected in 84% of diarrhea patients. From the group of febrile patients positive isolation was observed in 44%, however, 39% were *Salmonella* spp., (27% *S. typhi*, 7% *S. paratyphi* and 5% *Salmonella* C1) which were leading cause febrile patients admission into this hospital. In 1982 Gracey *et al.* reported a 10% frequency of isolation for *Aeromonas* spp. among children who were hospitalized in Jakarta for diarrhea¹². *Aeromonas* spp., in much lower percentage, was

also reported by Rockhill *et al.* in 1982¹³ during a 16 month period of observation looking into enteropathogens from diarrhea patients. In 1986, Komalarini *et al.* reported the detection of rotavirus in stools from 31% of diarrhea children less than five years of age during a one year study. Other enteropathogens detected were: *Salmonella* (10%), *V. cholerae* (7%), *Shigella* spp. (0.8%) and *Campylobacter* spp. (0.3%)¹⁴.

Progress continued to be made toward better identification of pathogens responsible for diarrhea when Burr *et al.*, during 1988-1990 looked into the etiology of diarrhea among expatriates living in Jakarta. In this study, the list of identifiable pathogens was expanded to include new types of pathogenic *E. coli*: EHEC (entero hemorrhagic *E. coli*), EAEC (entero adherens *E. coli*), EIEC (entero invasive *E. coli*), *Yersinia enterocolica*, adenovirus and *Cryptosporidium* spp.¹⁵.

In addition to the identification of enteropathogen in human materials, NAMRU and collaborators also investigate environmental and food contamination by diarrheal pathogens. Results from testing water samples collected during 1973-74 from the Ciliwung River, Jakarta's main river, Tjaniadi and Hadiputranto *et al.* in 1974 showed the presence of large numbers of coliform bacteria, including *Salmonella* spp. and *S. typhi*¹⁶. Similar levels of contamination reported by Gracey *et al.* in 1976, indicated fecal pollution of Jakarta's rivers was a significant public health problem¹⁷. While investigating food and ice sold in Jakarta's streets, Hadiputranto and Rockhill in 1982¹⁸ reported that 38% of samples had total and fecal

coliform contamination, while others had lesser but significant contamination with *Ps. aeruginosa*, *S. epidermis*, and *B. cereus*. However, there were no isolates of common bacterial enteropathogens such as *Salmonella* spp., *Shigella* spp., *V. cholerae*, *Aeromonas* or *C. botulinum*¹⁸.

RAPID DIAGNOSIS

The development of rapid methods for the detection of enteropathogens has concentrated primarily on the rapid diagnosis of *V. cholerae*. Lesmana and Rockhill *et al* in 1982 reported on a coagglutination test for the rapid identification of *V. cholerae* using heat treated, stabilized protein A containing staphylococci to which anti-cholera antiserum is attached²⁰. This highly sensitive and specific method was able to detect *V. cholerae* antigen in enrichment cultures from feces after only 8 hours of incubation. Lesmana *et al.* also showed that the coagglutination test could be used as a preliminary screen to detect *V. cholerae* on primary isolation plates, even when overgrown with other bacteria²¹⁻²².

TREATMENT

A collaborative effort between NAMRU, NIHRD and Infectious Diseases Hospital of Jakarta to study treatment of infectious diarrhea has focused principally on continued evaluation of the WHO program for oral rehydration solutions. In a randomized, double-blind controlled trial of almost 200 secretory diarrhea cases including cholera patients, it was shown

that a citrate-based ORS was superior compared to bicarbonate based ORS in reducing all the parameters clinicians consider important in assessing improvement from an episode of severe diarrhea. Use of the citrate-based ORS was associated with a reduced incidence of vomiting, decreased stool output, and faster clinical normalization²³. This result and other results from similar studies performed in different centers, led to the world-wide recommendation by WHO for use of the citrated ORS preparation instead of bicarbonate base.

A glycine supplemented ORS formula was evaluated in Jakarta during 1987 and was shown to be no more effective than the standard citrated ORS²⁴. More recently, a similar trial compared the standard citrated with glucose based ORS to a powdered, pre-cooked, rice based ORS. In addition to improved palatability, the rice ORS was associated with greater reductions in stool output²⁵.

PREVENTION

Until recently, NIHRD and NAMRU had not been actively involved in research for the prevention of diarrhea. In 1989, the Center for Vaccine Development in Baltimore, Maryland, USA solicited our involvement in a safety and immunogenicity trial of a recombinant DNA oral cholera vaccine in Indonesia. Preliminary results have shown a well-tolerated vaccine that with lower dose has a poorer immunogenicity in Indonesian compared to North American volunteers. Utilizing higher dose, the immunogenicity was much improved without significant increase in side effect.

REFERENCES

1. Brotoawasisto. Epidemiology of diarrheal disease. Diare, masalah dan penanggulangannya. DEPKES RI 20: 75.
2. Bachtin, M., Nelwan, Suprpto, Sebodo, T., & Ismangun. 1979. Tropical Pediatrics and Environmental Child Health, 101-103.
3. Joseph, S.W., Goke, D.L., Nadrifil, S., Van Peenen, P.F.D. & Widyaharsana, J. (1971). **Vibrio parahemolyticus** related gastroenteritis in Djakarta, Indonesia. Proc., 6th Singapore-Malaysia Congress of Medicine, pp. 44-47.
4. Joseph, S.W. (1973). Observations on **vibrio parahaemolyticus** in Indonesia. Proc., First International Symposium on **Vibrio parahaemolyticus**, Tokyo, pp. 35-40.
5. Komalarini, S. & Joseph, S.W. (1975). Gastroenteritis due to **Vibrio parahaemolyticus**. **Mod. Med. Asia**, 11: 9-11.
6. Komalarini, S., Adisuwirjo, K. & Sanborn, W.R. (1977). Diarrhoeal disease of bacterial origin in Jakarta. **Asian J. Trop. Med. Hyg.**, 8: 4.
7. Ringertz, S., Rockhill, R.C., Ringertz, O. & Sutomo, A. (1980). **Campylobacter fetus** subsp. **jejuni** as a cause of gastroenteritis in Jakarta, Indonesia. **J. Clin. Microbiol.**, 12(4): 538-540.
8. Ringertz, Rockhill, R.C., Ringertz, O. & Sutomo, A. (1981). Susceptibility of **Campylobacter fetus** subsp. **jejuni**, isolated from patients in Jakarta, Indonesia to ten antimicrobial agents. **J. Antimicrob. Chemother.**, 8: 333-336.
9. Sebodo, T., Soetarjo, Sadjimin, T., Soenarto, Y., & Sanborn, W.R. (1978). Study on the etiology of diarrhea. **J. Trop. Pediatr.**, 24: 107-109.
10. Sunoto, Pusponegoro, T.S., Suridwan & Sanborn, W.R. (1978). A question on the use of antibiotics in the treatment of acute diarrhoeal disease. **Paediat. Indon.**, 18: 191-198.
11. Punjabi, N.H., Hoffman, S.L., Krassner, R.C.J., Rockhill, R.C., Moechtar, A., Sutono, A., Kuntjoro & Echeverria, P. (1984). Infeksi berganda pada penderita-penderita diare dewasa yang dirawat di R.S. Khusus Penyakit Menular di Jakarta: Adakah synergisme dari pathogen. Presented at VI National Congress of Internal Medicine (KOPAPDI VI), July 24-28, 1984. Jakarta.
12. Gracey, M., Burke, V., Rockhill, R.C., Suharyono & Sunoto. (1982). **Aeromonas** sp. as enteric pathogens. **Lancet**, 23rd January.
13. Rockhill, R.C., Hadiputranto, H., Moechtar, A. & Soetomo, A. (1982). Prevalence of enteric bacteria pathogens among gastroenteritis and febrile illness patients in Jakarta, Indonesia: A 16 month study. **Medika**, 4(8): 287-290.
14. Komalarini, S., Setiawan, J., Lane, E.M., Lesmana, M., Bartz, C.R. & Sie, A. (1987). Pola kuman patogen penyebab diare di bagian anak R.S. Sumber Waras. 20th Bagian Ilmu Kesehatan Anak Rumah Sakit Sumber Waras. pp. 153-160.
15. Burr, D.H., Punjabi, N.H., Burr, P.Q., Subekti, D., Tjaniadi, P., Witham, N.D., Echeveria, P., Bayuk, T., Arthur, B., & Lesmana, M. (1990). Etiology of diarrhea in expatriates living in Jakarta. Presented at the 39th Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 4-8, 1990, New Orleans, LA.
16. Tjaniadi, P., Hadiputranto, H., Rockhill, R.C. & Sanborn, W.R. (1980). The presence of total coliform, **Salmonella typhi**, and other **Salmonella** in Jakarta river water. Presented at the Seminar Ilmiah/Kongress ke III Ikatan Ahli Kesehatan Masyarakat Indonesia (I.A.K.M.I.), November 27-29, 1980, Semarang, Central Java.
17. Gracey, M., Stone, D.E., Sutoto & Sutejo. (1976). Environmental pollution and diarrhoeal disease in Jakarta, **Ind. Environ. Child Hlth.**, 18-23.
18. Hadiputranto, H. & Rockhill, R.C. (1982). Bacterial content in food, drinks and ice collected from street food handlers in Jakarta, Indonesia. **Medika**, 8(3): 213-216.
19. Lesmana, M., Rockhill, R.C., Sutanti, D. & Sutomo, A. (1982). A coagglutination test to detect **Vibrio cholerae** in feces alkaline peptone water cultures. **S. E. Asian J. Trop. Med. Publ. Hlth.**, 13(3): 377-379.
20. Rockhill, R.C., Lesmana, M. & Moechtar, A. (1983). Improved method, using staphylococcal beta-hemolysin, for detection hemolysin(s) produced by **Vibrio cholerae** biotype El Tor. **Asian J. Trop. Med. Publ. Hlth.**, 14(2): 181-185.
21. Lesmana, M. & Rockhill, R.C. (1983). A rapid method to identify **vibrio cholerae** in primary culture by coagglutination. **Medika**, 9(11): 933-935.

22. Lesmana, M., Rockhill, R.C., Sutanti, D. & Sutomo, A. (1985). An evaluation of alkaline peptone water for enrichment of *Vibrio cholerae* in feces. *S. E. Asian J. Trop. Med. Pub. Hlth.*, 16(2): 265-267.
23. Hoffman, S.L., Asril Moechtar, M., Simanjuntak, C.H., Punjabi, N.H., Kumala, S., Sutoto, Silalahi, P., Sutopo, B., Kuncoro, Y.S., Soriano, M., Plowe, C., Paleologo, F.P., Edman, D.C. & Laughlin, L.W. (1985). Rehydration and maintenance therapy of cholera patients in Jakarta: Citrate-Based versus bicarbonate-based oral rehydration salt solution. *J. Infect. Dis.*, 152(6): 1159-1165.
24. Rasidi, C., Punjabi, N.H., Moechtar, M.A., Paleologo, F.P., Pulungsih, S.P., Kumala, S., Sukri, N., Edman, D.C., & Lesmana, M. (1989). Perbandingan penggunaan larutan garam oralit sitrat dengan dan tanpa glycine pada penderita kholera. Presented at Kongres Nasional BKGAI, Jakarta, June 1989.
25. Punjabi, N.H., Rasidi, C., Sundah, S., Pulungsih, S.P., Moechtar, M.A., Sukri, N., Burr, D.H., Witham, N.D., & Paleologo, F.P. (1989). Precooked rice powder ORS compared to standard citrate-glucose ORS in maintenance therapy of rehydrated hospitalized cholera patients in Jakarta. Presented at the 38th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Honolulu, HI, December 10-14, 1989.

