20 YEARS OF PROGRESS IN TYPHOID RESEARCH

Narain H. Punjabi[1], Nancy D. Witham[1], Cyrus H. Simanjuntak[2], Murad Lesmana[1], Pratiwi Sudarmono[3], Atti R. Rivai[4], Swiandy Kumala[4], Soemarsono[5], and Donald H. Burr[1]

THE DISEASE

Awareness of the importance of *Salmonella typhi* as a disease producing agent in febrile patients in Indonesia has grown during the past twenty years. Numerous collaborative efforts from Ministry of Health, other health institutes, and NAMRU have played important roles in the recognition of this important cause of significant morbidity and mortality. Anderson *et al.* in 1976[1] studied the causes of occult febrile disease during a one year period in Jakarta. A surprising 43% of the 741 patients studied had either blood cultures positive for *Salmonella* spp., or positive *Salmonella* serology. This was convincing evidence to clinicians in Jakarta that typhoid fever was an important and treatable cause of fever of unknown origin (FUO).

THE EPIDEMIOLOGY

In 1981, the reported number of cases of typhoid fever in Indonesia was 19,596, increasing to 26,606 in 1986[2], most of the increase was due to improved detection and recognition of the pathogen. A more realistic estimate of incidence for this underreported and underdiagnosed disease would be from 540,000 to 1,210,000 cases per year. This was based on results of a household survey done by Budiarso R. *et al.* in 1980 and 1986[3], and on result of a community surveillance done by Ruwido[4] and Simanjuntak *et al.*[5,6] from 1983-1988. These studies were done in Plaju, South Sumatera, representing an urban area and Paseh, West Java, representing a semi-rural area. The results showed that the incidence rate of typhoid fever in the semi rural area was 358/100,000 population/year, and between 760-810/100,000 population/year in the urban area. These studies also indicated that the high risk group in the population was school age children between ages of 3-19 years. From other observations, it is also noted that the case fatality in urbanized and growing areas of Indonesia, may be higher than it is reported in other areas of the world[7,8].

LABORATORY DIAGNOSIS

Non-culture method

To overcome the delay of 3-7 days in laboratory diagnosis using conventional culture techniques, Sanborn and Lesmana, *et al.*[9-12],

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

[2] National Institute of Health Research and Development, Jakarta

[3] Department of Microbiology, Faculty of Medicine, University of Indonesia, Jakarta

[4] Infectious Disease Hospital, Jakarta

[5] Department of Internal Medicine, Faculty of Medicine, University of Indonesia, Jakarta

showed that salmonellosis could be detected by a more rapid coagulation method. Later, Rockhill *et al.* demonstrated that *Salmonella* C1, D and Vi antigens could be accurately detected in blood cultures after only 18 hours of incubation, by using *S. aureus* coagglutination methods which were rapid, cheap, and easy to perform[13]. Follow-up studies by Rockhill *et al.* using the coagglutination method on urine specimens, provided a presumptive diagnosis within 30 minutes after receiving the specimen, although false positives were a problem[14]. Studies with enrichment cultures from feces have been developed which detect the presence of typhoid antigens within four hours after inoculation[15].

The Widal test has been used extensively in Indonesia as an aid to diagnose typhoid fever instead of costlier and time-consuming *S. typhi* culture techniques. Two studies have been done to evaluate the Widal test for diagnosis of typhoid fever. In 1981 Rockhill *et al.* reported that in Indonesia a clinically significant Widal titer was not well defined, and that the test was not very sensitive, nor specific. It has certain restrictions and that diagnosis of typhoid fever still rested primarily on isolation of *S. typhi* from the patient[16].

Using serum from hospitalized febrile patients Hoffman *et al.* showed that the Widal slide agglutination test was highly specific with a high positive predictive value and low negative predictive value[17]. However, the sensitivity and specificity of this test for each particular laboratory and its patients population must be known in order to be able to calculate its predictive value. The results of these studies suggest the usefulness of the Widal test for diagnosis of typhoid fever in Indonesia is dependent on the procedure used in an individual hospital and its associated laboratory.

With the ever widening acceptance of genetic engineering technics, DNA probes offer a newer approach to the detection and identification of *S. typhi* in the blood of typhoid patients. Studies by Rubin *et al.* have shown that a Vi-antigen-specific probe can be used to detect *S. typhi* using about 2.5 ml of blood[18-20]. These results were the first demonstration of the use of a DNA probe to detect bacteria in blood.

Culture method

Our institutions have conducted various investigations of non-humoral body fluid and materials with the aim of improving isolation from typhoid patients. In 1984, Hoffman *et al.* determined the sensitivity of the duodenal string capsule culture for isolating *S. typhi* and *S. paratyphi* A from patients with enteric fever. They compared these results to those from bone marrow aspirate (BMA) culture, single blood culture, rectal swab culture, and various combinations of these[21]. Duodenal string culture was shown to have no advantage over the combination of rectal swab and blood culture, and was less sensitive than the BMA culture done alone. The addition of the duodenal string culture to blood and rectal swab cultures could improve the likelihood of isolation in cases when a BMA culture cannot be obtained.

The BMA culture was shown to be significantly more sensitive than an 8 ml blood culture (of a 1:10 ratio blood to broth), an 8 ml

streptokinase clot culture, a 3 ml blood culture (the routine culture for most Indonesian hospitals and clinics) and rectal swab culture[22]. When BMA culture cannot be performed Tjaniadi *et al.*[23] and Simanjuntak *et al.*[24] confirmed that standard whole blood cultures are of greater sensitivity than blood-clot cultures.

TREATMENT

In a landmark study, Hoffman and Punjabi in 1984[25,26] documented that the use of high dose dexamethasone in antibiotic-treated severely ill typhoid patients resulted in dramatic improvement in outcome. Although the drug is costly, its use for treatment of the severe typhoid cases has become standard in hospitals where admission for typhoid fever is common. Interestingly, this documentation stands alone and unchallenged in showing utility of steroid treatment in an acute infectious disease of bacterial origin.

PREVENTION

Typhoid vaccines have been used for a number of years in areas of Indonesia. However, the present vaccine is associated with unpleasant side effects and relatively short periods of protection. Recently, a large field trial was sponsored by the WHO to evaluate the efficacy of a different formulation of the Ty21a oral typhoid vaccine. The trial was performed as a collaboration between NIHRD, NAMRU and Pertamina, and involved over 20,000 participants.

Based on the high protection rates previously demonstrated in Egypt and Chile, the Indonesian trial was designed to confirm these results in a different population, and to evaluate the liquid vs. enteric coated capsule forms of the vaccine. In contrast to the high protection rates seen in the previous studies, the Indonesian trial demonstrated that at best, the vaccine (3 doses of the liquid formulation) provided a 53% protective efficacy lasting for a period of at least 30 months, somewhat more in adults over 19 years[6]. It must be noted that this relatively low protective efficacy was accomplished in a population having extremely high rates of disease transmission, compared with the studies done in Chile and Egypt which have one tenth and one quarter, respectively, the transmission rates of Indonesia.

In an ongoing collaboration with WHO the same group at NAMRU and NIHRD has been investigating the immunogenicity and side-effects of the newer injectable Vi-capsular polysaccharide vaccine. Side-effects have been minimal, and the immunogenicity conferred was greater than 95%[27] in children as young as 2 years of large.

REFERENCES

1. Anderson, K.E., Joseph, S.W., Nasution, R., Sunoto, Butler, T., Van Peenen, P.F.D., Irving, G.S., Sulianti Saroso, J. & Watten, R.H. (1976). Febrile illnesses resulting in hospital admission: A bacteriological and serological study in Jakarta, Indonesia. **Am. J. Trop. Med. Hyg., 25**: 116-121.

2. Simanjuntak, C.H. (1990). Perkembangan vaksin oral demam tifoid. **Medika, 3**(16): 213-218.

3. Budiarso, R., Putrali, J., & Muchtaruddin, R.L. (1986). Survei kesehatan rumah tangga 1980, Ed. Badan Penelitian dan Pengembangan Kesehatan Dep Kes R.I.

4. Darmowigoto, R., Hoffman, S.L., Suprawoto, **et al.** (1984). Typhoid anbd paratyphoid fever in Plaju, Sumatra, 1978-1983. Abstract and Poster of 11th. International Congress for Tropical Medicine and Malaria, September 16-22, 1984, Calgary, Canada.

5. Simanjuntak, C.H., Hoffman, S.L., Punjabi, N.H., Edman, D.C., Hasibuan, M.A., Sumarno, W., & Koiman, I. (1987). Epidemiologi demam tifoid di suatu daerah pedesaan di Paseh, Jawa Barat. **Cermin Dunia Kedokteran, 45**: 16-18.

6. Simanjuntak, C.H., Paleologo, F.P., Punjabi, N.H., Darmowigoto, R., Soeprawoto, Totosudirjo, H., Haryanto P., Suprijanto E., Witham, N.D., & Hoffman, S.L. (1989). The oral vaccine, Ty21a, protects against intense transmission of typhoid fever in Indonesia. Submitted for publication.

7. Punjabi, N.H. Typhoid fever in the developing world: is the clinical severity increasing? Presented at the International Workshop on Typhoid Fever, Washington, D.C., 29-30 November 1984.

8. Edelman, R. & Levine, M.M. (1986). Summary of International Workshop on Typhoid Fever. **Rev. Infect. Dis.**, 198(3): 329-336.

9. Sanborn, W.R., Lesmana, M., & Edwards, E.A. (1977). Coagglutination and culture methods for detecting salmonellae on agar plates. **Bact. Proc.**, C48.

10. Lesmana, M., Sanborn, W.R. & Edwards, E.A. (1977). Enrichment culture coagglutination test for rapid diagnosis of salmonellosis. **Bact. Proc.**, C49.

11. Lesmana, M., Rockhill, R.C. and Sanborn, W.R. (1980). A coagglutination method for presumptive identification of **Salmonella typhi**. **S. E. Asian J. Trop. Med. Pub. Hlth., 11**(2): 301-306.

12. Sanborn, W.R., Lesmana, M. & Edwards, E.A. (1980). Enrichment culture coagglutination test for rapid, low-cost diagnosis of Salmonellosis. **J. Clin. Microbiol., 12**(2): 151-155.

13. Rockhill, R.C., Lesmana, M., Moechtar, M.A. & Sutomo, A. (1980). Detection of **Salmonella** C1, D and Vi antigens, by coagglutination, in blood cultures from patients with **Salmonella** infections. **S. E. Asian J. Trop. Med. Pub. Hlth., 11**(4): 441-445.

14. Rockhill, R.C., Rumans, L.W., Lesmana, M. & Dennis, D.T. (1980). Detection of **Salmonella typhi** D, Vi and d antigens, by slide coagglutination, in urine from patients with typhoid fever. **J. Clin. Microbiol., 11**: 213-216.

15. Rockhill, R.C., Rumans, L.W. & Lesmana, M. (1981). Slide coagglutination for **Salmonella typhi** antigens in broths inoculated with feces from typhoid fever patients. **S. E. Asian J. Trop. Med. Pub. Hlth., 12**(4): 528-531.

16. Rockhill, R.C., Mochtar, A. & Soetomo, A. (1981). Comparison of the Widal test with **Salmonella typhi** isolation from typhoid fever patients in Jakarta, Indonesia. **Medika, 6**(7): 351-354.

17. Hoffman, S.L., Flanigan, R.P., Klaucke, D., Leksana, B., Rockhill, R.C., Punjabi, N.H., Pulungsih, S.P., Sutomo, A. & Moechtar, M.A. (1986). The widal slide agglutination test, a valuable rapid diagnostic test in typ' oid fever patients at the Infectious Diseases Hospital of Jakarta. **Am. J. Epidem., 123**(5): 869-875, 1986.

18. Rubin, F.A., Kopecko, D.J., Sack, R.B., Sudarmono, P., Marta, A.Y1.D., Meza, R., Moechtar, M.A., Edman, D.C. & Hoffman, S.L. (1988). Evaluation of a DNA Probe for identifying **Salmonella typhi** in Peruvian and Indonesian bacterial isolates. **J. Infect. Dis., 157**(5): 1051-1053.

19. Rubin, F.A., McWhirter, P.D., Punjabi, N.H., Lane, E., Sudarmono, P., Pulungsih, S.P., Lesmana, M., Kumala, S., K ecko, D.J. & Hoffman, S.L. (1989). Use of a DN probe to detect Salmonella **typhi** in the blood o. patients with typhoid fever. **J. Clin. Microbiol., 27**(5): 1112-1114.

20. Rubin, F.A., McWhirter, P.D., Burr, D., Punjabi, N.H., Lane, E., Kumala, S., Sudarmono, P., Pulungsih, S.P., Lesmana, M., Tjaniadi, P., Sukri, N. & Hoffman, S.L. (1990). Rapid diagnosis of typhoid fever through identification of **Salmonella typhi** within 18 hours of specimen acquisition by culture of the mononuclear cell-platelet fraction of blood. **J. Clin. Microbiol., 28**(4): 825-827.

21. Hoffman, S.L., Punjabi, N.H., Rockhill, R.C., Sutomo, A., Rifajati Rivai, A. & Sri Pandam Pulungsih. (1984). Duodenal string capsule culture compared with bone marrow, blood, and rectal swab cultures for diagnosing typhoid and paratyphoid fever. **J. Infect. Dis., 149**(2): 157-161.

22. Hoffman, S.L., Edman, D.C., Punjabi, N.H., Lesmana, M., Cholid, A., Sundah, S. & Harahap, J. (1986). Bone marrow aspirate culture superior to streptokinase clot culture and 8 ml 1:10 blood-to-broth ratio blood culture for diagnosis of typhoid fever. **Am. J. Trop. Med. Hyg., 35**(4): 836-839.

23. Tjaniadi, P., Lane, E.M., Lesmana, M., Edman, D.C. & Kostermans, D. (1988). Isolation of Salmonella typhi from standard whole-blood culture vs. blood-clot cultures. **S. E. Asian J. Trop. Med. Pub. Hlth., 19**(4): 623-627.

24. Simanjuntak, C.H., Hoffman, S.L., Darmowigoto. R., Lesmana, M., Soeprawoto & Edman, D.C. (1988). Streptokinase clot culture compared with whole blood culture for isolation of **Salmonella typhi** and **S. paratyphi** A from patients with enteric fever. **Trans. R. Soc. Trop. Med. Hyg., 82**: 340-341.

25. Hoffman, S.L., Punjabi, N.H., Kumala, S., Moechtar, A.M., Pulungsih, S.P., Rivai, A.R., Rockhill, R.C., Woodward, T.E. & Loedin, A.A. (1984). Reduction of mortality in chloramphenicol-treated severe typhoid fever by high-dose dexamethasone. **N. Engl. J. Med., 310**(2): 82-88.

26. Punjabi, N.H., Hoffman, S.L., Edman, D.C., Sukri, N., Laughlin, L.W., Sri Pandam, P., Rivai, A.R., Moechtar, A. & Woodward, T.E. (1988). Treatment of severe typhoid fever in children with high dose dexamethasone. **Pediat. Infect. Dis. J., 7**(8): 598-600.

27. Paleologo, F.P., Simanjuntak, C.H., Punjabi, N.H., Hardjining, S., Pudjarwoto, T. & Witham, N.D. (1989). Double blind controlled trial for side effects of parenteral Vi CPS typhoid vaccine in Indonesian children and adults. Presented at 38th Annual Meeting of the Amer. Soc. of Trop. Med. & Hyg., Honolulu, Hawaii, 10-14 December 1989.