DIRECTION OF HEPATITIS SUPPORTED RESEARCH AT NAMRU-2

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ABSTRAK

ARAH PENELITIAN HEPATITIS DI NAMRU-2

Titik berat penelitian hepatitis di NAMRU-2 pada saat ini adalah: 1) penelitian binatang untuk mengetahui populasi reservoar HEV; 2) penggunaan model hewan untuk lebih diketahuinya transmisi HEV pada populasi reservoar yang dicurigai; 3) diketahuinya akurasi alat diagnostik yang digunakan (untuk semua marker); 4) diketahuinya lama pengeluaran virus hepatitis E pada kotoran manusia yang menderita hepatitis akut maupun dari model hewan; 5) diketahuinya insidens hepatitis E akut melalui pencarian kasus dengan menggunakan metode penelitian lapangan di masyarakat; dan 6) serokonversi infeksi hepatitis E pada anak-anak.

Hubungan erat dengan berbagai universitas dan instansi pemerintah yang terkait dengan penanganan kasus-kasus hepatitis, telah dan masih menjadi bagian penting bagi suksesnya penelitian hepatitis di NAMRU-2. Hasil yang dicapai berdasarkan program penelitian di atas antara lain: 1) seminar hepatitis E di Kalimantan Barat; 2) tersedianya kemampuan diagnostik pada laboratorium setempat; 3) pembinaan peneliti di Indonesia dan Asia Tenggara dalam hal epidemiologi; serta 4) alih teknologi dalam pelaksanaan penelitian yang dapat melibatkan minat para peneliti pada penyakit hepatitis di Indonesia.

HEPATITIS

Viral hepatitis is one of the principal causes of acute morbidity and mortality worldwide. The important role of viral hepatitis in producing chronic and progressive liver diseases has been well documented. The risks factors associated with the spread of hepatitis are generally virus specific. Water-borne and person-to-person contact, and needle sharing and exchange of bodily fluids via sexual exposure, are related to the transmission of hepatitis A (HAV) and hepatitis B viruses (HBV) respectively.

Previously, serological capability was limited to HAV and HBV detection. However, recent advances in laboratory diagnostic technologies have provided for the differentiation non-A, non-B hepatitis into separate viruses: C, D, E, and F/G. The U.S. Naval Medical Research Unit No.2 (NAMRU-2), in

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partnership with NIHRD, and Prof. H.A. Sulaiman (Medical Faculty, University of Indonesia, Cipto Mangunkusumo Hospital), has conducted a hepatitis research program to better outline all epidemiological profile of the newly recognized hepatitis viruses.

METHODS

Methods in studies supported by NAMRU-2 involve both community and hospital based design concepts. Community-based investigations include cross-sectional surveys, case follow-up (after initial outbreak) surveys, disease outbreak investigations, and longitudinal studies of both active and passive case detection. Hospital-based studies are uniform in targeting: 1) high risk groups, i.e., recipients of multiple blood transfusions, and 2) patients with acute hepatitis disease. Hepatitis cases in hospital based studies are generally age/sex matched for comparative purposes.

HEPATITIS C VIRUS (HCV) has been documented as an important cause of both acute hepatitis and hepatic cirrhosis[8]. A significant risk has also been associated between HCV infection and hepatocellular carcinoma[9]. Blood and blood product mediated transmission has been implicated in the spread of HCV[1]. In Egypt, HCV infection was detected among 55% of pediatric cases with histories of multiple blood transfusions, compared with less than 1% in healthy, non-transfused controls [11]

Overall prevalence of HCV among 7572 healthy Indonesian blood donors from (21 out of 27 Indonesian provinces) was 2.1% [17]. Figure 1 shows the geographical mapping of prevalence of antibody against HCV (anti-HCV) from throughout the archipelago. Java and Bali had the highest proportion of anti-HCV positives with 2.5%, followed by Sulawesi, Sumatra, Kalimantan, and Eastern Indonesia: 1.8%, 1.7%, 1.5%, and 1.0%, respectively. There were no significant differences in anti-HCV prevalence between male and female populations. The proportion of anti-HCV positive reactives increased significantly ($p<0.001$) with age, ranging from 1.0 to 10.3 percent. Also, the mean age of anti-HCV positives (42.0) was significantly higher ($p<0.001$) than for negatives (32.7), but this may simply be the result of increasing risk of surgery and transfusion that occurs as individuals grow older. Risk factors found in association with the presence of anti-HCV were history of surgery, acupuncture, intravenous medication ($p<0.001$), and blood transfusion(s) ($p<0.0001$).

A study of Indonesian children with hematological conditions from the Pediatrics Departments, Dr. Cipto Mangunkusumo, University of Indonesia Hospital, was carried out to determine if transmission of HCV was related to receipt of blood and blood products and the contribution it makes to the spread of HCV in Indonesia. Seventy-six blood product recipients and 74 age/sex matched controls (85 males and 65 females) with no history of clinical jaundice or transfusions were tested for anti-HCV. The mean age of cases and controls was 8.1 years, ranging from less than 1 to 13 years. Mothers (75) of hematological case subjects were also evaluated for anti-HCV. Overall, anti-HCV among all 225 study participants was 15.6% using anti-HCV ELISA testing. The percent of anti-HCV positives among cases (39%) was significantly higher ($p<0.0001$) than for controls (2.7%) or case-mothers (4%). Anti-HCV prevalence was significantly lower ($p=0.05$) among males (36%) than for females (44%). The mean age of anti-HCV positive blood product recipients (9.3 years) was significantly higher ($p<0.05$)

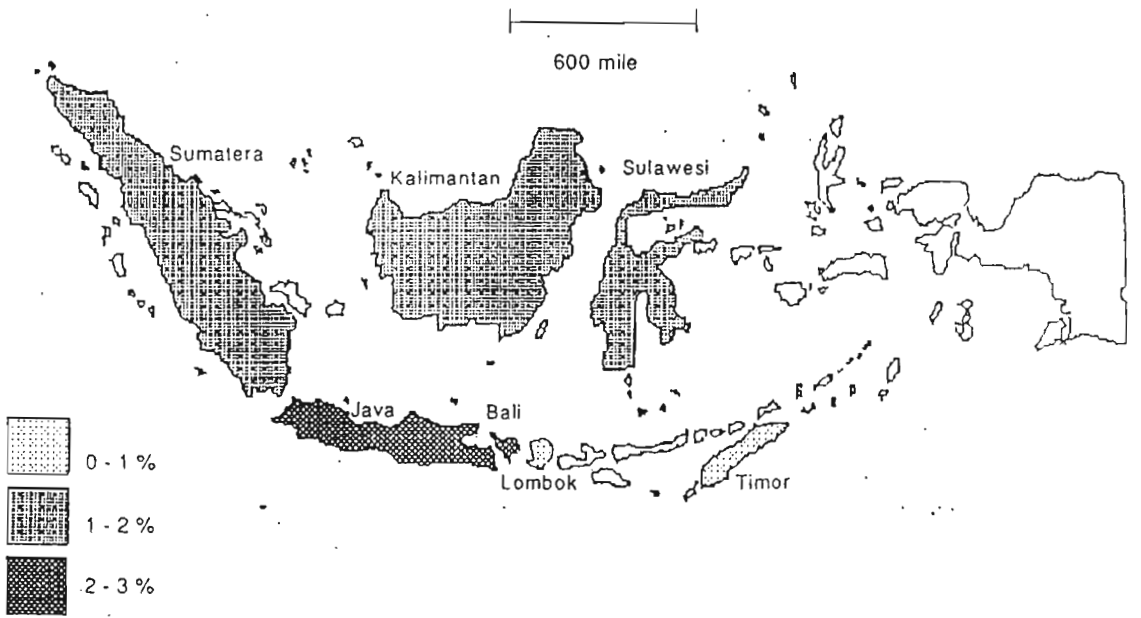

Figure 1. The Prevalence of HCV among blood donors, by location, November 1992 - February 1993.

than those of anti-HCV negatives blood product recipients (7.7 years). There was no increase in case risk with the number of blood only and blood product transfusions ($p>0.05$). However, the mean number of blood only transfusions among anti-HCV positive Blood Protect Recipient (10.8) was significantly higher ($p<0.05$) than for anti-HCV negative Blood Protect Recipient (8.8). Circumcision was shown to be strongly associated with anti-HCV case reactivity for both males ($p=0.05$) and females ($p<0.05$).

An investigation as to the risk of HCV among renal-dialysis patients was conducted in collaboration with Gatot Subroto Army Hospital. Renal-dialysis cases (150) were age/sex matched with hospital controls (150). Sixty-nine percent of dialysis patients were anti-HCV positive. Anti-HCV reactivity was negligible (<2%) among controls.

The costs of testing blood and related products for anti-HCV must be weighed against those of acute and chronic disease in formulating screening policies. However, high anti-HCV prevalence among healthy blood donors, particularly in the population aged >40 years (5.6%), warrants concern. Additionally, blood-related exposure via multiple transfusions and dialysis clearly constitutes significant risk relative to HCV infection. Also, the large percentage of anti-HCV positive non-hematological-related pediatric controls (2.7%) suggests that over 100,000 children (≤ 12 years) from the Jakarta area may have been infected with hepatitis C virus (extrapolated from cencus data). Efforts to insure safe (avoidance of multiple needle sharing) along with effective immunization should be emphasized.

HEPATITIS E VIRUS (HEV) infection has been well documented throughout Asia. First identified during the 1956 outbreak in India involving at least 29,000 cases, enterically transmitted HEV has since been detected in epidemic form from the neighboring countries of Pakistan, Nepal and Myanmar[3,13]. Most cases of enterically transmitted non-A, non-B hepatitis (ET-NANBH), acquired in both epidemic and endemic settings, can be attributed to HEV infections[5]. Like hepatitis A virus (HAV), water-borne spread of HEV is associated with fecal contamination of water supplies. However, in contrast with HAV, epidemic HEV is generally characterized by: 1) a longer incubation period; 2) higher case-fatality rates, particularly among pregnant women (10-24%); and 3) poor protective value of gamma globulin[4,15].

In Indonesia, HEV was the causative agent in an outbreak reported from West Kalimantan during October/November 1987[4,16]. The HEV virus was implicated as responsible in approximately 2500 acute hepatitis cases on the basis of evaluation of 28 (out of 34) acutely jaundiced patients who tested negative for both IgM anti-HAV and IgG anti-HBc, but positive for anti-HEV in acute sera[14]. A second outbreak (following that reported in 1987) of HEV transmission was recognized from West Kalimantan in September, 1991, among a different, though contiguous, group of isolated communities further downstream along a 20 kilometer stretch of the same river system. An attack rate of 9% was estimated among a population of approximately 20,000. Overall, the case-fatality rate was 9.5/1000 persons with higher rate among pregnant women, 14% (3 out of 22) (BERITA EPIDEMIOLOGI, 1991). Laboratory testing was based on the serological presence of IgG anti-HEV in the absence of IgM anti-HAV and IgM anti-HBc[12].

A two-year follow-up investigation of the 1991 hepatitis E virus (HEV outbreak in West

Kalimantan, Indonesia, was carried out to describe the epidemiology of HEV transmission and documented persistence of IgG antibody response[6]. This study was performed in collaboration with Dinas Kesehatan Kabupaten Sintang, West Kalimantan; Kantor Wilayah Departemen Kesehatan, Propinsi Kalimantan Barat, Pontianak, West Kalimantan; and NIHRD, Jakarta. Cases (60) identified as IgG anti-HEV positives during the actual outbreak in 1991 were matched with controls (67) and surveyed along with their respectively family members (318). Overall, the prevalence of IgG anti-HEV among the 445 study subjects representing 127 study households was 59%. There was no significant differences in anti-HEV prevalence between cases (72%) and controls (61%). Loss of detectable IgG anti-HEV after 2 years was demonstrated in 28% of case subjects who were originally screened as reactive for anti-HEV during the 1991 outbreak. Cross-sectional prevalence was found to increase with age ($p=0.01$). When communities were grouped into areas of low (<40%), medium (40-59%) and high (≥ 60%) anti-HEV prevalence, use of river water for 1) drinking and cooking; 2) bathing; and 3) human waste disposal were associated with high prevalence communities ($p<0.001$, for all comparisons). Conversely, boiling of water for drinking purposes was negatively associated ($p<0.05$) with increased prevalence. There is evidence to suggest that lower dilution of the virus with river water further upstream probably led into a more concentrated exposure, and consequently, high attack rates (infection accompanied by disease); attack rates decreased in communities downstream, although community prevalence 2 years later did not. Finally, subnormal rainfall during the month August leading up to the 1991 outbreak (19 centimeters compared to 209 centimeters for monthly mean (August) for the years 1985-1993) may have contributed to favorable epidemic conditions.

A cross-sectional survey of communities affected during the 1987 outbreak and a control area further downstream was conducted in 1994, again, in cooperation with provincial and local health authorities from Pontianak and Sintang, West Kalimantan. Sera collected from 885 persons were assayed (EIA) for IgG anti-HEV and IgG anti-HAV. Overall IgG anti-HEV prevalence was 40% (both in outbreak affected and control areas), whereas 90% of prior study subjects had IgM anti-HAV markers. Prevalence of IgG anti-HEV in the study areas (50%) was significantly higher than in the control areas (23%, $p<0.0001$); community prevalence declined from a high of 55% upriver to a low of 15% downriver (control area). Prevalence in the affected area increased by age ($p<0.01$); no significant rise in age specific prevalence was noted in the control area (Figure 2). Hepatitis E virus prevalence in the population aged ≥7 years (53%) from the outbreak area (alive during the actual outbreak) was significantly higher ($p<0.01$) than among children aged <7 years (born after the outbreak) (15%). In contrast, there was no significant difference in IgG anti-HEV prevalence between the ≥7 (23%) and <7 (20%) age groups from the control area. This study indicates continuing (sporadic) infections given IgG anti-HEV prevalence in children <7 years from the outbreak area (15%).

A summation of data from Indonesia, and more recently from NAMRU-2 supported hepatitis investigations in Vietnam (where the first outbreak HEV in Indo-China was documented), indicates that universal usage of non-treated river water for drinking purposes (in the absence of boiling) in a rural environment, favors epidemic HEV

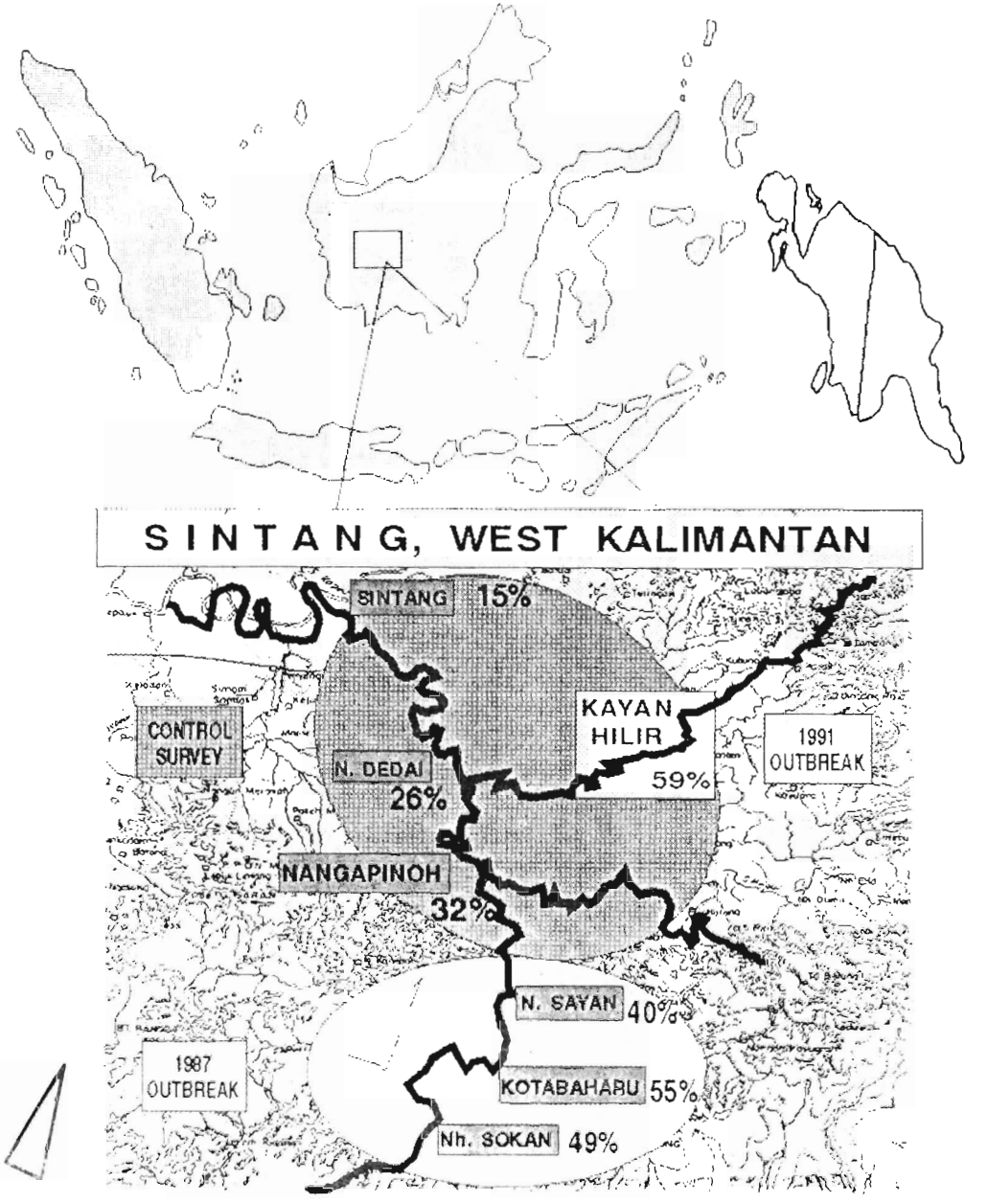

Figure 2. Anti-HEV Prevalence, Sintang, West Kalimantan, 1993-1994.

transmission[7]. Moreover, HEV should be weighed against HAV as the major cause of water-borne, enteric outbreaks, particularly in Southeast Asia, were over 95% of most rural populations are exposed to HAV as young children[15].

HEPATITIS G VIRUS (HGV) has recently been identified in hepatitis patients from the Americas' and Africa[2]. NAMRU-2, working closely with diagnostic manufacturers, is planning to determine the presence of HGV in different populations. Preliminary (yet to be confirmed) data suggest the first detection of this newly recognized virus in Indonesia (*CORWIN et al., 1995, unpublished data*).

CONCLUSION

Emphasis of hepatitis research at NAMRU-2 now includes : 1) animal surveys to identify HEV reservoir populations; 2) animal modeling to better understand HEV transmission in suspected reservoir populations; 3) reliability assessment of diagnostic tests (all markers); 4) persistence of viral (HEV) shedding from human patients with acute hepatitis and animal models; 5) incidence of acute HEV cases through community-based case detection; and 6) seroconversions of HEV infection in children. Strong relationships have been and remain essential to NAMRU-2's successful hepatitis research. This program has supported hepatitis seminars in West Kalimantan and provided diagnostic laboratory and epidemiological training to collaborating investigators throughout the archipelago, as well as Southeast Asia. The transfer of research capabilities allows NAMRU-2 to expand its support of independent projects involving shared hepatitis research interests with would-be Indonesian investigators.

REFERENCES

1. Aach RD, Stevens CE, Hollinger FB, Mosley JW, Peterson DA, Taylor PE, Johnson RG, Barbosa LH, Nemo GJ (1991). Hepatitis C virus infection in post-transfusion hepatitis: an analysis with first and second-generation assays. New England Journal of Medicine, 325, 1325-1329.

2. ABBOTT LABORATORIES (1995). ABBOTT scientist identify new hepatitis viruses. Diagnostics Division, Public Affairs, April 11.

3. Arankalle VA, Tichehurst J, Sreenivasan MA, Kapikian AZ, Popper H, Pavri KM (1988). Etiologic association of virus-like particle with enterically-transmitted non-A, non-B hepatitis. LANCET, 1, 550-554.

4. Balayan MS (1991). HEV infection: historical perspectives, global epidemiology, and clinical features in Viral Hepatitis and Liver Disease. Proceedings of the 1990 International Symposium on Viral Hepatitis and Liver Disease by Hollinger FB, Lemon SM, Margolis H, 498-501 (Williams and Wilkins, Baltimore).

5. Bradley, DW (1992). Hepatitis E: epidemiology, etiology and molecular biology. Reviews Medical Virology, 2, 19-28.

6. Corwin AL, Jarot K, Lubis I, Nasution K, Suparmawo S, Sumardiati A, Widodo S, Nazir S, Orndorff G, Choi Y, Tan R, Sie A, Wignall S. Graham R, Hyams K (1995). Two year follow-up investigation of epidemic hepatitis E virus transmission in West Kalimantan (Borneo). Transactions of the Royal Society of Tropical Medicine and Hygiene, IN PRESS.

7 Corwin AL, Khiem HB, Clayson ET, Sac PK, Nhung VTT, Yen VT, Cuc CTT, Vaughn DL Merven J, Richie TL, Putri MP, He J, Graham R, Wignall FS, Hyams KC (1995) A water-borne outbreak of Hepatitis E virus transmission in the South-West of Vietnam Journal of Infectious Disease, IN PRESS.

8. Chen DS, Kuo GC, Sung JL, Lai MY, Sheu JC, Chen PJ, Yang PM, Hsu HM, Chang MH, Chen CJ, Hahn LC, Choo QL, Wang TH, Houghton M (1990). Hepatitis C virus infection in an area hyperendemic for hepatitis B and chronic liver disease: the Taiwan experience Journal of Infectious Diseases, 162, 817-822.

9. Dazza MC, Meneses LV, Girard PM, Astagneau P, Villaroel C, Delaporte E, Larouze B (1993). Absence of a relationship between antibodies to hepatitis C virus and hepatocellular carcinoma in Mozambiqui. American Journal of Tropical Medicine & Hygiene, 48, 237-242.

10. Hasil Penyelidikan KLB hepatitis di Kec. Kayanhilir Kab. Sintang, Kalimantan Barat. Berita Epidemiologi - Epidemiology Bulletin Kwartal III (1991). 21-30.

11. Hibbs RG, Corwin AL, Hassan NF, Kamel M, Darwish M, Edelman R, Constantine NT, Oao MR, Khalifa AS, Mokhtar S, Fam NS, Ekladious EM, Bassily SB (1993). The epidemiology of antibody to hepatitis C in Egypt. Journal of Infectious Disease, 168, 789-790.

12. Jennings GB, Lubis I, Listiyaningsih E, Buran JP, Hyams K (1994). Hepatitis E virus in Indonesia. Transactions of the Royal Society of Tropical Medicine and Hygiene, 88, 57.

13. Kane MA, Breadley DW, Shrestha SM, Maynard JE, Cook EH, Mishra RP, Joshi DD (1984). Epidemic non-A, non-B hepatitis in Nepal: Recovery of a possible etiologic agent and transmission studies of marmosets. Journal of the American Medical Association, 252, 3140-3145.

14. Lubis I.(1989). Epidemik virus hepatitis non-A, non-B di Sintang, Kalbar. Berita Epidemiologi - Epidemiological Bulletin, August, 3-12.

15. Mast E, Alter MJ (1993). Epidemiology of viral hepatitis: an overview. Seminars in Virology, 4, 273-283.

16. Ticehurst J (1991). Identification and characterization of hepatitis E virus in viral Hepatitis and Liver Disease, Proceedings of the 1990 International Symposium on Viral Hepatitis and Liver Disease by Hollinger FB, Lemon SM, Margolis H, 501-513 (Williams and Wilkins, Baltimore).

17 Sulaiman HA, Julitasari, Annie Sie, Rustam M, Corwin AL, Jennings GB (1995). Prevalence of hepatitis B and C viruses in healthy Indonesian blood donors. Transactions of the Royal Society of Tropical Medicine and Hygiene, 89, 167-170.