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THE IMPORTANCE OF LEPTOSPIROSIS IN SOUTHEAST ASIA

KANTI LARAS, CAO BAO VAN, KHANTHONG BOUNLU, NGUYEN THI KIM TIEN, JAMES G. OLSON, SISOUK THONGCHANH, TRAN NGUYEN VAN ANH, HOANG KIM LOAN, NARAIN PUNJABI, HA BA KHIEM, UNG SAM AN, SITHAT INSISIENGMAY, DOUGLAS M. WATTS, H. JAMES BEECHAM, AND ANDREW L. CORWIN

U.S. Naval Medical Research Unit No. 2 (US NAMRU-2), Jakarta, Indonesia; Pasteur Institute, Ho Chi Minh City, Socialist Republic of Vietnam; Center of National Laboratory and Epidemiology, Vientiane, Lao PDR; U.S. NAMRU-2/NIPH, Phnom Penh, Cambodia; National Institute of Public Health, Phnom Penh, Cambodia; U.S. Naval Medical Research Center, Silver Spring, Maryland.

Abstract. The importance of leptospirosis in Southeast Asia was assessed in conjunction with other studies supported by the U.S. Naval Medical Research Unit No. 2 (US NAMRU-2), Jakarta, Republic of Indonesia. These included studies of hospital-based, acute clinical jaundice in Indonesia, Lao PDR, and Socialist Republic of Vietnam; nonmalarial fever in Indonesia; and hemorrhagic fever in Cambodia. Background prevalence estimates of leptospiral infection were obtained by a cross-sectional, community-based study in Lao PDR. Laboratory testing methods involved serology, microscopic agglutination test, and reverse-transcriptase polymerase chain reaction. Suggestive evidence of recent leptospiral infections was detected in 17%, 13%, and 3% of patients selected on the basis of non–hepatitis A through E jaundice, nonmalarial fever, and hemorrhagic fever (in the absence of acute, dengue viral infections). Leptospiral IgG antibody, reflective of prior infections, was detected in 37% of human sera, collected in Lao PDR. The predominant leptospiral serogroups identified from cases with clinical jaundice were Hurstbridge, Bataviae, and Icterohaemorrhagiae *tonkini* LT 96 69. Among the nonmalarial febrile cases, Bataviae was the most frequently recognized serogroup. Pyrogenes and Hurstbridge were the principal serogroups among the hemorrhagic fever case subjects. These findings further attest to the relative importance of clinical leptospirosis in Southeast Asia. The wide spectrum of clinical signs and symptoms associated with probable, acute, leptospiral infections contributes to the potential of significant underreporting.

INTRODUCTION

Leptospirosis is caused by bacteria from the genus *Leptospira,* which comprises 12 species (*L. alexanderi, L. biflexa, L. borgpetersenii, L. fainei, L. inadai, L. interrogans, L. kirschneri, L. noguchii, L. santarosai, L. weilii, L. meyeri,* and *L. wolbachii*). To date, only *L. interrogans* and *L. fainei* have been reported as pathogenic in humans.[1,2] Common symptoms of leptospirosis in humans are sudden onset of fever, headache, chills, severe myalgia, and conjunctival suffusion.[3]

Southeast Asia is an endemic area for leptospirosis, and infection in humans has been reported throughout the region.[4–10] Of the serovars included in the major pathogenic species, 70% have been isolated in Asia[4]. The nonspecificity of signs and symptoms and the limited availability of laboratory confirmation in endemic areas probably have contributed to significant underreporting, however, most notably in association with jaundiced disease.[7] High regional endemicity of other diseases, such as malaria and dengue hemorrhagic fever, also leads clinicians to assign presumptive diagnostic status in the absence of supportive laboratory testing and alternative diagnostic considerations (e.g., leptospirosis).

The purpose of this study was 4-fold: (1) to measures the relative importance of leptospiral infection in association with various clinical presentations; (2) to measure the relative importance of leptospiral infections in conjunction with jaundice case criteria from diverse localities in Southeast Asia; (3) to recognize possible clustering of leptospiral serogroups, as a function of specific clinical presentations (signs and symptoms); and (4) to determine background endemicity of leptospirosis (in Lao PDR).

METHODS

Investigation of leptospirosis was sponsored by the U.S. Naval Medical Research Unit No. 2 (US NAMRU-2) Jakarta (Indonesia), in collaboration with partner institutions throughout Southeast Asia. This investigation was carried out in conjunction with the following related studies: (1) a study of causes of acute, hospital-recognized jaundice disease (conducted in Lao PDR, Socialist Republic of Vietnam, and Indonesia); (2) a study of causes of nonmalarial febrile disease (conducted in Irian Jaya, Indonesia); (3) a study of causes of hemorrhagic fever disease (conducted in Cambodia); and (4) a cross-sectional, community-based study of background *Leptospira* infection (conducted in Lao PDR) (Figure 1, Table 1). All studies, hospital-based (clinical) and community-based (nonclinical), were carried out in the period 1993–2001.

Sera collected during acute jaundice disease studies were tested for hepatitis A, B, C, and E virus markers by enzyme-linked immunosorbent assay (ELISA) (Abbott Laboratories, Abbott Park, IL) at U.S. NAMRU 2, Jakarta. Sera negative for hepatitis A–E viruses were tested at the Pasteur Institute in Ho Chi Minh (HCM) City for evidence of recent leptospiral infections by microscopic agglutination test (MAT)[11] and polymerase chain reaction (PCR).[12,13]

Sera collected from clinical patients with nonmalarial fever and nondengue hemorrhagic febrile manifestations first were screened by PanBio Leptospira IgM ELISA kit (Panbio Ltd, Brisbane, Australia) at the National Institutes of Public Health (NIPH)/U.S. NAMRU-2 laboratory, Phnom Penh, Cambodia. Positive ELISA specimens were tested by MAT and PCR at Pasteur Institute, HCM City. Before testing for recent evidence of leptospiral infections, all sera collected during the nonmalarial febrile study were examined for malaria parasitemia by microscopy at U.S. NAMRU-2, and sera from the hemorrhagic fever disease study were tested for dengue antibody using IgM capture ELISA reagents supplied by the U.S. Armed Forces Research Institute of Medical Sciences (U.S. AFRIMS), Bangkok, Thailand.

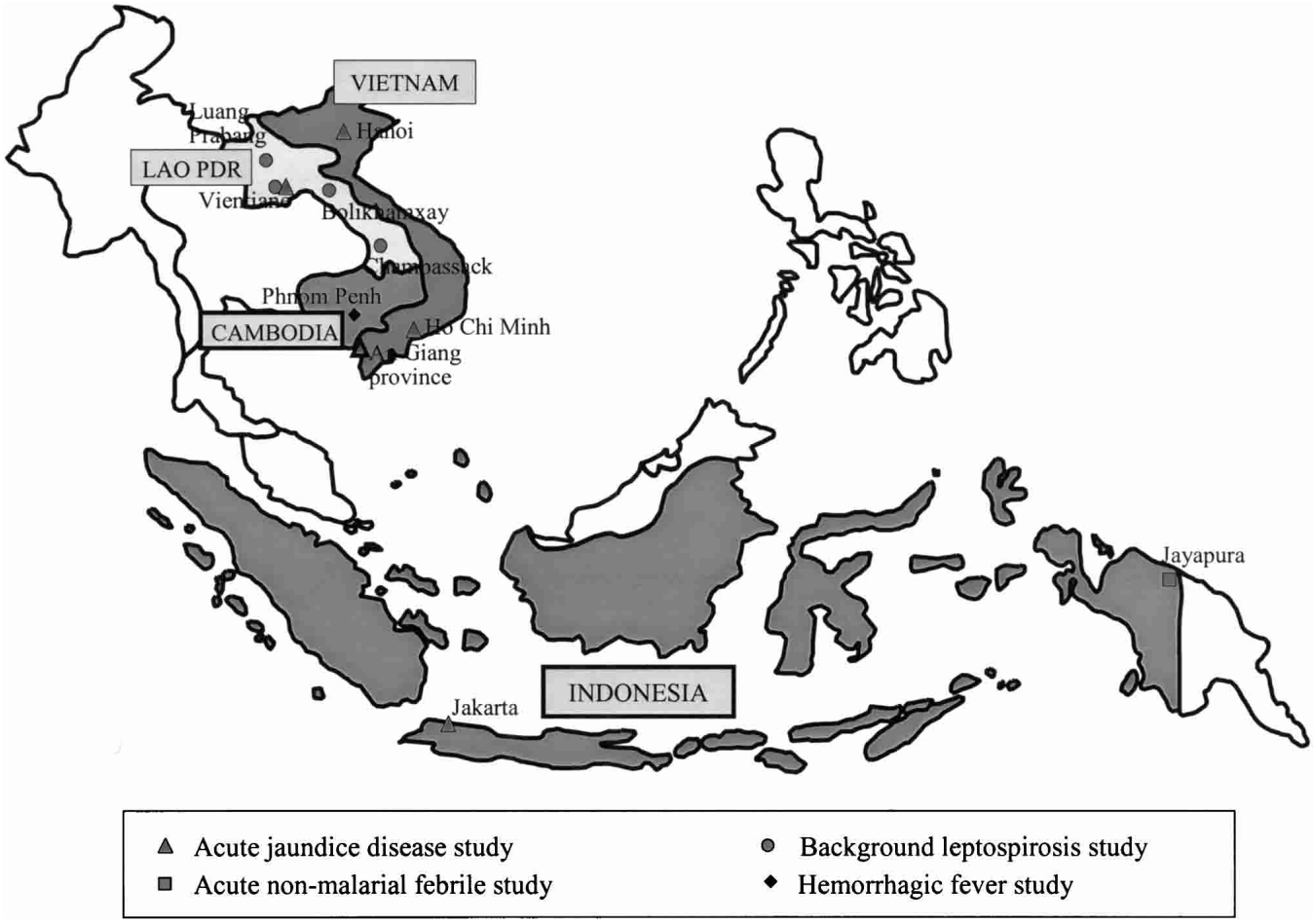

FIGURE 1. Geographic profile of leptospirosis study sites contributing to regional findings.

The MAT was performed by standard method[11] using a battery of 24 reference and field live antigen strains (provided by the WHO Collaborating Center for Leptospirosis, Pasteur Institute, Paris, France), representing 16 major serogroups of *Leptospira* (Table 2). The strains used represented the serogroups most frequently found in Southeast Asia.

The PCR was performed by purifying DNA from 100 μl of serum sample, using silica particles and guanidinium thiocyanate lysis buffer.[12] Amplification of a 331 base pair-fragment was performed using a pair of primers (A, 5′-GGC GGCGCGTCTTAAACATG-3′; B, 3′-TTCCCCCCATT GAGCAAGATT-3′) designed from the 16S rRNA gene of *Leptospira* species, and the PCR products were analyzed by dot-blot hybridization as previously described.[13]

Leptospiral infection was defined in all study instances using case selection criteria based on (nonhepatitis) jaundice disease, (nondengue) hemorrhagic disease, and (nonmalaria) febrile disease and by positive PCR or antibody titer ≥1:200 by MAT or both.[1,14,15] The criteria for concluding evidence of recent leptospiral infection, using MAT and PCR results, are described in Table 3.[16,17]

In describing the distribution and potential clustering of leptospiral serogroups, by clinical selection criteria and location, all serogroups with MAT titer values ≥1:200, regardless of PCR (positive or negative) findings, were included for study purposes. Additionally, serogroups with expressed MAT titers <1:200, but positive by PCR, were added to distribution analysis.

Sera obtained during the cross-sectional, community-based study, to determine the prevalence of *Leptospira* infection in Lao PDR, were tested with a modified version of the Panbio Leptospira IgM ELISA kit, adopted specifically for IgG detection, at the Center of National Laboratory and Epidemiology (CNLE), Vientiane, Lao PDR. The IgM-to-IgG transformation was accomplished by changing the horseradish peroxidase (HRP) conjugated sheep antihuman IgM to HRP conjugated goat IgG fraction to human Ig (molecule) (Organon Teknika, Durham, NC). Preliminary testing in predetermining optimal conjugate dilutions was done for every batch of reagent used at U.S. NAMRU-2/NIPH (Phnom Penh) and CNLE (Vientiane). All other procedures were carried out in accordance with test kit insert instructions, as applied to IgM detection.

All research protocols were reviewed and approved by the NAMRU-2's Committee for the Protection of Human Subjects and by the respective human use committees at each of the cooperating institutions (Table 1). Informed voluntary consent was obtained from all study subjects before enrollment. In each study, leptospirosis was identified for inclusion in the diagnostic algorithm, by name or inference (e.g., associated with jaundice disease), in addition to acute hepatitis A through E markers.

TABLE 1
Study-specific information, from which leptospirosis findings are based, pertaining to location, institutional affiliation, testing methodology, and year of collection

Study	Location	Institution	Test	Year of collection
Acute jaundice disease	Jakarta, Indonesia	Persahabatan Hospital	MAT PCR	1993–1995
	Vientiane, Lao PDR	Mahosoth Hospital, Settathirath Hospital, Friendship Hospital, Center of National Laboratory and Epidemiology, Vientiane	MAT PCR	1995–1996
	Hanoi, Vietnam	Institute for Clinical Research in Tropical Diseases/Bach Mai Hospital, Institute for Protections of Children's Health	MAT PCR	1993–1995
	Ho Chi Minh city, Vietnam	Pasteur Institute, Cho Quan Hospital, Ho Chi Minh City	MAT PCR	1995–1997
	An Giang province, Vietnam	Pasteur Institute, An Giang Province Health Authority	MAT PCR	1997
Acute nonmalarial febrile disease	Jayapura, Irian Jaya, Indonesia	Jayapura Provincial Hospital	IgM ELISA MAT* PCR*	1997–2000
Hemorrhagic fever disease	Phnom Penh, Cambodia	National Pediatric Hospital, Phnom Penh	IgM ELISA MAT* PCR*	1999–2001
Background leptospirosis infection	Luang Prabang, Bolikhamxay, Vientiane, Champassack (Lao PDR)	Center of National Laboratory and Epidemiology, Vientiane	IgG ELISA	2000–2001

* Only for IgM *Leptospira* ELISA-positive sera.

RESULTS

Laboratory findings. *Acute disease episodes.* Table 4 shows the proportional representation of examined specimen with leptospiral infections (acute and nonacute), by study type and location. Overall, 17% (95 of 577) of case sera (in the absence of acute hepatitis A through E markers) from jaundiced clinical study subjects exhibited serologic evidence of recent leptospiral infections. The highest proportions of MAT or PCR positive results were registered from collections made in Jakarta: 28% (28 of 100) case sera. The relative importance of leptospirosis in jaundiced disease by location (highest to lowest) was determined on the basis of total, area-specific collections, without regard to absence or presence of acute hepatitis markers: 14% (33 of 232) of cases from Vientiane, Lao PDR; 11% (28 of 253) of cases from Jakarta; Indonesia; 8% (23 of 288) of cases from Hanoi, Socialist Republic of Vietnam; 2% (7 of 464) of cases from An Giang Province, Socialist Republic of Vietnam; and 2% (4 of 266) of cases from HCM City, Socialist Republic of Vietnam.

The study of acute, nonmalarial febrile disease, conducted in Irian Jaya (Indonesia), indicated evidence of recent leptospiral infections in 13% (30 of 232) of the case sera tested by MAT or PCR or both (Table 4). There was no denominator value from which to base a conclusion from among all febrile case admissions: (Leptospirosis) study inclusion criteria were predicated on a (repeated) negative smear for parasitemia.

Examination of sera obtained from hemorrhagic fever disease case episodes (Cambodia) showed that 3% (5 of 194) were positive by MAT or PCR test results, in the absence of an acute dengue viral infection. Overall (regardless of dengue

TABLE 2
Antigen set used in MAT*

No.	Serogroup	Serovar	Strain
1	Australis	*australis*	Ballico
2	Autumnalis	*autumnalis*	Akiyama A
3	Bataviae	*bataviae*	Van Tienen
4	Canicola	*canicola*	Hond Utrecht IV
5	Ballum	*castellonis*	Castellon 3
6	Icterohaemorrhagiae	*copenhageni*	Wijnberg
7	Pyrogenes	*pyrogenes*	Salinem
8	Icterohaemorrhagiae	*tonkini*	LT 96 68
9	Icterohaemorrhagiae	*icterohaemorrhagiae*	Verdun
10	Cynopteri	*cynopteri*	3522 c
11	Grippotyphosa	*grippotyphosa*	Moskva V
12	Sejroe	*hardjo*	Hardjo Bovis
13	Hebdomadis	*hebdomadis*	Hebdomadis
14	Javanica	*javanica*	Veldrat Bataviae 46
15	Panama	*panama*	CZ 214K
16	Semaranga	*patoc*	Patoc I
17	Pomona	*pomona*	Pomona
18	Tarassovi	*tarassovi*	Mitis Johnson
19	Tarassovi	*vughia*	LT 09 68
20	Sejroe	*hardjo*	Hardjoprajitno
21	Sejroe	*saxkoebing*	Mus 24
22	Canicola	*canicola*	Chiffon
23	Louisiana	*louisiana*	LSU 1945
24	Hurstbridge		

* MAT carried out at Pasteur Institute, HCM City, Vietnam.

TABLE 3
Study criteria, by methods, in assigning positive or negative acute (leptospiral) infection status

MAT	PCR	Conclusion
<1/200	Negative	Negative
<1/200	Positive	Positive
>1/200	Negative	Positive
>1/200	Positive	Positive
Negative	Positive	Positive

TABLE 4
Relative importance of leptospiral infections, by clinical presentation or study design

Study	Location	Test	Percent positive in the absence of markers	Overall percent positive, regardless of markers
Acute jaundice disease	Jakarta, Indonesia	MAT	28/100 (28%)†	28/253 (11%)†
		PCR		
	Vientiane, Lao PDR	MAT	33/151 (22%)†	33/232 (14%)†
		PCR		
	Hanoi, Vietnam	MAT	23/113 (20%)†	23/288 (8%)†
		PCR		
	Ho Chi Minh City, Vietnam	MAT	4/112 (4%)†	4/266 (2%)†
		PCR		
	An Giang province, Vietnam	MAT	7/101 (7%)†	7/464 (2%)†
		PCR		
Nonmalarial febrile disease	Jayapura, Irian Jaya, Indonesia	IgM ELISA	37/232 (16%)‡	NA‖
		MAT*	30/232 (13%)‡	NA‖
		PCR*		
Hemorrhagic fever disease	Phnom Penh, Cambodia	IgM ELISA	16/194 (8%)§	24/202 (12%)§
		MAT*	5/194 (3%)§	6/202 (3%)§
		PCR*		
Background leptospirosis infection	Luang Prabang, Bolikhamxay, Vientiane, Champassack (Lao PDR)	IgG ELISA	763/2.083 (37%)	NA

* Only for IgM *Leptospira* ELISA-positive sera.
† Acute hepatitis A–E markers.
‡ Malaria parasitemia.
§ Acute dengue marker.
‖ No denominator for febrile patients before exclusion on the basis of malaria smear for parasitemia.
NA = not applicable.

status), 6 of 202 (3%) instances of acute hemorrhagic fever from the case population were likely attributed to leptospiral infections; 1 case serum yielded IgM dengue antibody and *Leptospira* DNA.

Background prevalence. Overall, the prevalence of leptospiral IgG antibody was 37% (763 of 2,083) in Lao PDR. Findings reflected significant geographic variability in antibody prevalence between targeted provinces: 45% in the northern highland area of Luang Prabang, 49% in Vientiane, 37% in Bolikhamxay, and 19% in the southernmost region of Champassack, bordering with neighboring Cambodia (Figure 2).

Demographic profile. *Acute disease episodes.* The age distributions of patients with evidence of recent leptospiral infections are shown in Table 5. The mean age of patients identified with leptospirosis, against background selection criteria of acute jaundice disease, by location, was 25.7 ± 14.3 years for Jakarta, 29.5 ± 16.7 years for Vientiane, 27 ± 9.8 years for Hanoi, 35 ± 16.6 years for HCM City, and 26.6 ± 13.3 years for An Giang province ($P = 0.696$, analysis of variance [ANOVA], no significant difference). There was no significant difference ($P = 0.919$, ANOVA) in mean ages when comparing jaundiced with nonmalaria febrile study populations: 27.8 ± 14 and 28.1 ± 11.8 years. The mean age of hemorrhagic fever case subjects with evidence of recent leptospiral infections was 10.5 ± 3.7 years—that a pediatric population was targeted for hemorrhagic fever study purposes precluded any comparative (age) analysis.

The overall male-to-female patient ratio for clinically expressed, acute disease, with demonstrative leptospiral infections (1:0.66), differed significantly ($P < 0.05$). There was, however, no significant difference ($P < 0.05$) in sex ratio of jaundiced, leptospiral positive cases (1:0.75); the male-to-female ratio of patients identified with leptospirosis, against a background selection criteria of acute jaundice disease, by location, was 1:0.8 ($P > 0.05$) for Jakarta, 1:1.3 ($P > 0.05$) for Vientiane, 1:0.44 ($P < 0.05$) for Hanoi, 1:0.33 ($P > 0.05$) for HCM City, and 1:0.4 ($P > 0.05$) for An Giang province. Among nonmalarial febrile patients with recognized leptospi-

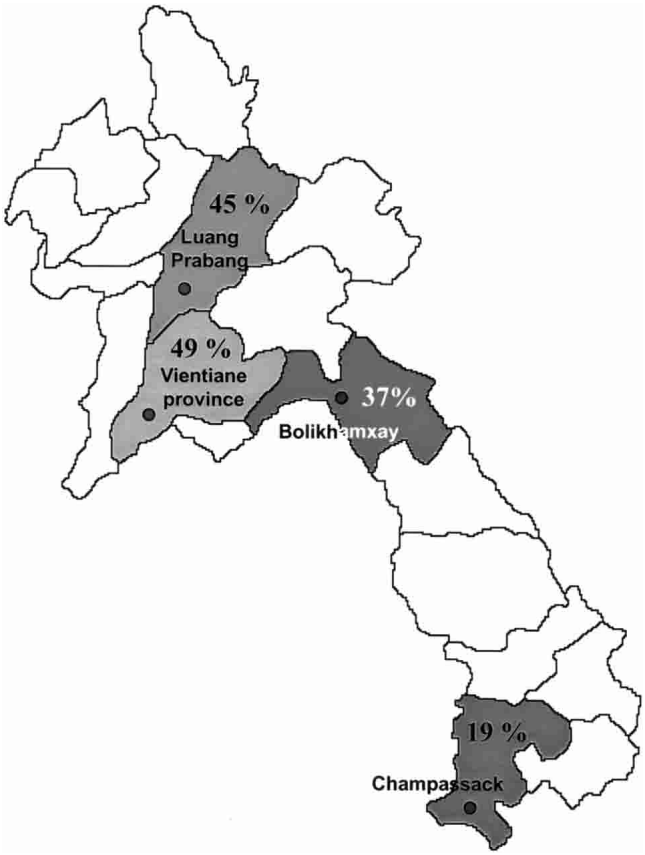

FIGURE 2. Geographic distribution of *Leptospira* in Lao PDR.

TABLE 5
Proportional distribution of leptospirosis, by age and location

	Percent of leptospirosis infection by age and location							
	Acute jaundice disease (without hepatitis A–E markers)					Acute non-malarial febrile	Febrile Hemorrhagic disease	Background leptospirosis infection
Age group (y)	Jakarta* (n = 100)	Vientiane (n = 144)†	Hanoi* (n = 113)	HCM city* (n = 106)†	An Giang* (n = 101)	Irian Jaya* (n = 228)†	Cambodia* (n = 202)	Lao PDR‡ (n = 1,082)
0–9	0/7 (0%)	4/20 (20%)	0/10 (0%)	0/56 (0%)	1/15 (7%)	0/6 (0%)	3/25 (2%)	22/290 (8%)
10–19	6/25 (24%)	5/19 (26%)	5/19 (26%)	1/23 (4%)	0/27 (0%)	8/58 (14%)	4/67 (6%)	22/290 (17%)
20–29	8/38 (21%)	4/36 (11%)	9/48 (9%)	0/9 (0%)	3/31 (10%)	11/86 (13%)	NA	66/164 (40%)
30–39	6/15 (40%)	8/40 (23%)	7/25 (28%)	1/7 (14%)	2/18 (11%)	6/37 (16%)	NA	63/157 (40%)
≥40	5/12 (42%)	33% (n = 30)	2/11 (18%)	2/11 (18%)	1/10 (10%)	5/41 (12%)	NA	124/240 (52%)
Mean age	25.7 ± 14.3	29.5 ± 16.6	27 ± 9.8	35 ± 16.6	26.6 ± 13.3	28.1 ± 11.8	10.5 ± 3.7	34.1 ± 18.9

* Based on MAT and PCR result.
† Number of specimens with age data.
‡ Based on *Leptospira* IgG ELISA result.
NA = not applicable.

ral infections, the sex ratio (1:0.42) differed significantly ($P < 0.05$), whereas no such difference was noted among hemorrhagic fever patients (1:1).

Background prevalence. The overall mean age of surveyed participants expressing IgG leptospiral antibody was 34.1 ± 18.9 years. Mean ages varied significantly ($P = 0.00058$, ANOVA) by location: 39.63 ± 1.97 years for Champassack, 36.7 ± 19.04 years for Bolikhamxay; 32.3 ± 19 years for Vientiane, and 31.9 ± 17.5 years for Luang Prabang. The percent of IgG positives significantly ($P = 0.0224$, chi square) increased with age: 10%, 30%, 45%, 51%, 56%, and 64%, in the 0–9, 10–19, 20–29, 30–39,40–49, and >49 age groups.

The overall percentage of IgG antibody *Leptospira*-positive males was 43% (out of 941) compared with 32% (out of 1141) of females ($P < 0.05$). This demographic feature was found in each of the provinces surveyed: 35% of males versus 14% of females in Champassack, 42% of males versus 30% of females in Bolikhamxay, 49% of males versus 41% of females in Luang Prabang, and 56% of males versus 43% of females in Vientiane.

Molecular epidemiology. Table 6 presents the distribution of *Leptospira* serogroups and associated titers from acute jaundice disease study sites. Overall, Hurstbridge (16%) was the predominant serogroup identified from the 5 locations, followed by Bataviae (15%) and Icterohaemorrhagiae *tonkini* LT 96 69 (13%). Site-specific findings show area differences as to serogroup (proportional) representation. The predominate serogroup in Jakarta, HCM City, An Giang, Hanoi, and Vientiane was Hurstbridge (23%), Bataviae (50%), Bataviae (60%), Icterohaemorrhagiae *copenhageni* Wijnberg (36%), and Icterohaemorrhagiae *tonkini* LT 96 69 (18%).

Predominant *Leptospira* serogroups and titers, expressive of clinical case selection criteria involving nonmalarial febrile and hemorrhagic fever disease, are shown in Table 7. Bataviae (27%) was the most common serogroup found in the population of acute nonmalarial febrile patients. The principal serogroups represented from among hemorrhagic fever cases were Pyrogenes (50%) and Hurstbridge (50%).

Testing uniformity. Agreement measures between MAT and PCR test results are presented in Table 8. Overall, only 12% (16 of 131) of *Leptospira*-positive sera were positive by MAT and PCR. Of case specimens, 63% (83 of 131) were concluded to be positive by PCR only (with negative MAT criteria: with an absence of titer, or <1:200). Conversely, 25% (33 of 131) of sera were positive only by MAT, with positive titer criteria of ≥1:200.

Acute jaundice study. Of *Leptospira*-positive sera, 11% (10 of 95) were positive by MAT and PCR. Of sera, 66% (63 of 95) were positive by PCR, but negative by MAT; 23% (22 of 95) were positive by MAT only. Sera from acute jaundice studies were not screened before MAT and PCR testing by ELISA for *Leptospira* IgM antibody.

Nonmalarial febrile study. Of sera, 16% (37 of 232) screened positive, with detectable *Leptospira* IgM antibody: (1) 41% (15 of 37) had MAT titers ≥1:200, with negative PCR results; (2) 41% (15 of 37) were positive by PCR, with negative MAT results; and (3) 18% (7 of 37) were positive by ELISA only. None of the 37 ELISA-positive sera were positive by both MAT and PCR.

Hemorrhagic fever study. Of sera, 12% (24 of 202) proved positive by ELISA. Only 1 serum (4%) was positive by MAT only, and 5 (21%) sera were positive by PCR only. Eighteen sera (75%) were positive only by ELISA. None of the sera were positive by both MAT and PCR, using study criteria.

DISCUSSION

The impressive representation of causative leptospirosis in association with acute, non–hepatitis A through E jaundiced disease (17% of all cases) and nonmalarial febrile (13% of all cases) disease is notable. That only a small proportion of nondengue hemorrhagic fever cases were likely attributed to leptospiral infections (3%) nevertheless attests to the potential of this cause in conjunction with hemorrhagic disease manifestations, particularly in the absence of an acute dengue immunologic response. The relationship between leptospiral infection and (pulmonary) hemorrhagic disease was documented in Nicaragua, during epidemic occurrence.[18] Suggestive underreporting of leptospirosis from the region has been interpreted from other studies.

Leptospirosis was identified as the contributing cause for a jaundice outbreak investigated in South Sumatra, Indonesia.[19] In a study of fever (of unknown origin) in patients at Bangkok's Children's Hospital, 36% (26 of 73) of pediatric disease episodes resulted from leptospiral infections.[6] In Jakarta, leptospirosis was shown as a contributing cause among 6% of adult febrile hospitalized patients.[10]

Community-based, background prevalence findings from the 4 surveyed provinces in Lao PDR offer a (geographically)

TABLE 6
Serogroup and titer of leptospirosis in acute jaundice disease cases, by location

	Jakarta titer				Vientiane titer					Hanoi titer				HCM City titer		An Giang Province titer		
Serogroup	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:800	1:50	1:100	1:200	1:800	1:50	1:400	1:50	1:100	1:400
Australis			1		2	1	1	2		1								
Autumnalis																		
Bataviae			2	1	3								1	1	1		1	2
Canicola																		
Ballum									1									
Icterohaemorrhagiae *copenhageni* Wijnberg					3					2								
Pyrogenes	2				1	1				1				1				
Icterohaemorrhagiae *tonkini* LT 96 69		2			1	4	1	1		1								
Icterohaemorrhagiae *icterohaemorrhagiae* Verdun	2				1	1	1											
Cynopteri	1																	
Grippotyphosa	1				1													
Sejroe *hardjo* Hardjo Bovis					1									1				
Hebdomadis			2		3								1					
Javanica					1													
Panama	1				3							1						
Semaranga*	7				3		1	1		4	1	2						
Tarassovi *tarassovi* Mitis Johnson	1																	
Tarassovi *vughia* LT 09 68																		
Sejroe *hardjo* Hardjoprajitno							1											
Sejroe *saxkoebing* Mus 24																		
Louisiana *louisiana* LSU 1945	1																	
Hurstbridge	3	2			4				1	1						2		

* Not included in the analysis of serogroup distribution.

TABLE 7
Serogroup and titer of leptospirosis in acute nonmalarial febrile cases and hemmorhagic fever disease cases

Serogroup	Acute nonmalarial febrile titer							Hemorrhagic fever disease titer
	1:50	1:100	1:200	1:400	1:800	1:1,600	1:3,200	1:50
Australis	3			1				
Autumnalis	1							
Bataviae	1		4		3	1	1	
Canicola								
Ballum								
Icterohaemorrhagiae *copenhageni* Wijnberg								
Pyrogenes	1							1
Icterohaemorrhagiae *tonkini* LT 96 69	1	1			1			
Icterohaemorrhagiae *icterohaemorrhagiae* Verdun	1							
Cynopteri								
Grippotyphosa								
Sejroe *hardjo* Hardjo Bovis		1	1	1				
Hebdomadis				1				
Javanica				1				
Panama								
Semaranga*								
Tarassovi *tarassovi* Mitis Johnson		1	2					
Tarassovi *vughia* LT 09 68				1	1			
Sejroe *hardjo* Hardjoprajitno			1		1			
Sejroe *saxkoebing* Mus 24			1		1			
Louisiana *louisiana* LSU 1945								
Hurstbridge			2	1				1

* Not included in the analysis of serogroup distribution.

TABLE 8
Agreement between MAT and PCR result in 131 *Leptospira*-positive sera

MAT	PCR	Conclusion	Acute jaundice* (n = 95)	Nonmalarial febrile† (n = 30)	Hemorrhagic fever† (n = 6)	Total (n = 131)
<1:200	Positive	Positive	38 (40%)	4 (13%)	1 (17%)	43 (33%)
>1:200	Negative	Positive	22 (23%)	10 (33%)	1 (17%)	33 (25%)
>1:200	Positive	Positive	10 (11%)	5 (17%)	—	16 (12%)
Negative	Positive	Positive	25 (26%)	11 (37%)	4 (66%)	40 (30%)

* Without hepatitis A–E markers.
† Only for IgM *Leptospira* ELISA-positive sera.

diverse area representation, highlighting the endemic status of leptospirosis: north, south, east, and west. Similar prevalence estimates were made from a cross-sectional study in neighboring Vietnam, in the Mekong Delta region.[4] In the Philippines, endemicity in rice-farming villages was 44%.[20]

The notable increase in age-specific prevalence has been recognized from other studies.[21] This demographic finding may reflect an occupational risk associated with infection. Alternatively, background prevalence is likely to be higher among the older population, in that this demographic group has had a longer temporal window of exposure opportunity.

That leptospirosis prevalence was significantly more apparent in males versus females suggests a possible occupational component associated with exposure opportunities (e.g., working in the rice fields). Other prevalence type studies also documented this sex-biased phenomenon, with males, particularly in the 20–40-year age range, affected more so than females: Higher male-to-female ratios usually correlated with sex-specific occupational and behavioral factors.[2,20] In contrast to such reported sex bias shown in the described investigations, no differences were observed in male and female prevalence findings described from the Mekong Delta region of Vietnam[4]; exposure opportunities probably were related to frequent flooding conditions, in which both sexes were subjected to the same water-associated risks. In clinical case studies described in this article, information pertaining to patient occupations was not available for analytical purposes in risk assignment.

There was an apparent association as to predominance of certain serogroups, with specific, clinical case (syndromic) presentations: Hurstbridge with acute jaundice, Bataviae with nonmalarial fever, and Pyrogenes and Hurstbridge with hemorrhagic fever. A possible explanation for these differences in serogroup findings, especially between jaundiced and febrile patients, may be that specific serogroups elicit a unique clinical response as to signs and symptoms. There were negligible differences in predominate serogroup representation by geographic location, as evident from the various acute jaundice studies. The predominance of Hurstbridge serogroup, against a background of jaundiced disease, varies with previously documented findings from Indonesia. In an outbreak of jaundiced disease in South Sumatra (Indonesia), Australis, Grippotyphosa and Icterohaemorrhagiae were the most frequently recognized serogroups.[19] Lastly, Bataviae was predominant among (febrile, nonmalarial) leptospirosis cases identified in Irian Jaya, Indonesia (as described in this article), whereas cases exhibiting febrile symptoms in Jakarta (1970–1971) were principally associated with the Pyrogenes serogroup.[10]

The detection of serogroup Hurstbridge among jaundiced patients with evidence of recent leptospiral infections, regardless of location, is notable. That *L. fainei* serovar Hurstbridge serogroup has not been linked previously with leptospirosis in association with jaundice disease case presentation may reflect on the newness of this serogroup, first discovered and isolated from animal sources in 1994[22] and more recently documented from clinical human sources from patients with suspected leptospirosis (from the Seychelles Island grouping[2] and Australia[22]). In the context of these findings, Hurstbridge serogroup has been implicated in severe forms and possibly in fatal forms of the disease.[2]

In analyzing serogroup distribution, subjects who exhibited agglutinins against serogroup Semaranga nevertheless were excluded: Significant cross-reactivity linked with Semaranga precludes serogroup identification in association with MAT-recognized infections. Problematically, cross-agglutinating antibodies may suggest the circulation of other pathogenic serogroups, some of which may not have been included in the MAT antigens set used for this study.[11] Overall, findings from acute jaundice studies showed that 13 of 94 (14%) leptospirosis sera, positive by MAT or PCR or both, exhibited agglutinins against the saprophytic serogroup Semaranga.

There is no *established* fixed cutoff value for the assessment of recent leptospiral infection by MAT, owing to different serogroup titer responses associated with MAT reactions. In adding to testing sensitivity, a combination of ≥2 detection techniques is suggested: MAT and PCR evaluation for the purpose of this study.[23] Because of the long conservation of serum samples, the current Centers for Disease Control and Prevention laboratory supportive case definition of (titer) ≥1:200 was used as the MAT cutoff value in reflecting clinical leptospirosis.[24,25] In the absence of a MAT titer of ≥1:200, PCR provided acceptable criteria in determining probable (recent) leptospiral infection.

Most notably, there was significant discordance associated with test results. Different test methods provide measures of different immunologic responses, accounting for testing variability.

The lack of conformity between MAT and PCR results demands critical scrutiny in interpreting clinical and study findings. Only 12% of 131 sera that satisfied positive testing criteria (suggestive of a recent leptospiral infection) were reactive by MAT and PCR. Most of the *Leptospira*-positive sera (63%) in these study findings were determined reactive on the sole basis of PCR results: negative by MAT. This finding shows the advantages of using this assay (DNA replication) to differentiate clinical leptospirosis in endemic areas compared with the MAT, as substantiated from other studies.[16] A negative PCR result in conjunction with a positive MAT result could be attributed to the following: (1) a low or a short leptospiremia during the acute phase of the disease, (2) a blood sample taken late in the clinical course of the

disease, (3) the self-administration of antibiotics before hospital admission, or (4) poor specimen conservation.[2,13,16]

CONCLUSION

In much of the developing world, where laboratory capabilities for confirmation of acute leptospiral infection are lacking, leptospirosis as an acute disease is rarely considered in clinical differential diagnoses. In many such locations, there is a tendency to identify (and often overrepresent) only what historically has been proved through serologic testing (e.g., hepatitis and dengue). Although our study data reflect probable cause, signs and symptoms, compatible with leptospirosis, add to the validity of supportive laboratory findings. That leptospirosis significantly contributes to acute disease in Southeast Asia, as reflected in many clinical presentations (e.g., jaundice, hemorrhagic, and febrile illness), has been highlighted from this investigation. Finally, leptospirosis can present with or without jaundice so that presumptive antibiotic therapy (doxycycline) for leptospirosis may be warranted in treatment algorithms when signs and symptoms suggest this disease cause, particularly when no supportive laboratory evidence is available.

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Authors' addresses: Kanti Laras, Narain Punjabi, James Beecham, and Andrew L. Corwin, U.S. Naval Medical Research Unit No. 2 (US NAMRU-2), P.O. Box 3, Unit 8132, Jakarta, Indonesia, FPO AP 96520-8132. Cao Bao Van, Nguyen Thi Kim Tien, Tran Nguyen Van Anh, Hoang Kim Loan, and Ha Ba Khiem, Pasteur Insitute, 167, Pasteur Street, District 3, Ho Chi Minh City, Vietnam. Ung Sam An, National Institute of Public Health (NIPH), P.O. Box 1300, Phnom Penh, Cambodia. James G. Olson (current), Centers for Disease Control, Atlanta, GA. Khanthong Bounlu, Sisouk Thongchanh, and Sithat Insisiengmay, Center of National Laboratory and Epidemiology (CNLE), Km3, Thadeua Road, Vientiane, Lao PDR. Douglas M. Watts, U.S. Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500.

Reprint requests: Andrew L. Corwin, PhD, U.S. Naval Medical Research Unit No. 2 (U.S. NAMRU-2 Jl. Percetakan Negara No. 29, Kompleks P2M-PLP/LITBANGKES, Jakarta Pusat, Indonesia; or via U.S. NAMRU-2, American Embassy Jakarta, Unit 8132 NAMRU TWO, FPO AP 96520-8132 USA, E-mail: corwinal@namru2.med.navy.mil

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