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Markers of Hepatitis C and B Virus Infections among Blood Donors in Ho Chi Minh City and Hanoi, Vietnam

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Blood donors in two cities in Vietnam were tested for markers of hepatitis C virus (HCV) and hepatitis B virus infections. Antibody to HCV was detected by passive hemagglutination with antigens of the second generation in 101 (20.6%) of 491 donors in Ho Chi Minh City; it was detected less frequently ($P < 0.001$) in donors in Hanoi (4 [0.8%] of 499). HCV RNA was tested for in donors with antibody by PCR with nested primers from the 5′-noncoding region and detected in 79 donors in Ho Chi Minh City and 4 donors in Hanoi. HCV RNA was genotyped by PCR with type-specific primers from the core gene. Of 83 HCV carriers from Vietnam, 24 (29%) were infected with HCV of genotype I/1a, 19 (23%) were infected with II/1b, 4 (5%) were infected with III/2a, and 2 (2%) were infected with mixed genotypes (I/1a and II/1b); HCV genotypes in the remaining 34 (41%) donors, including all 4 donors in Hanoi, were not classifiable into I/1a, II/1b, III/2a, IV/2b, or V/3a. Of the 10 isolates with unclassifiable genotypes, 2 showed substantial sequence divergence within the 5′-noncoding region from reported isolates with known genotypes (I/1a to 6a). An analysis of part of the core gene sequence indicated that six of the remaining isolates most likely represented new HCV genotypes. Hepatitis B surface antigen and the corresponding antibody, respectively, were detected in 15 (3.1%) and 234 (47.7%) donors in Ho Chi Minh City as well as in 15 (3.0%) and 248 (49.7%) donors in Hanoi. These results indicate an extensive spread of HCV among Ho Chi Minh City donors and HCV of novel genotypes in Vietnam.

The discovery of hepatitis C virus (HCV) by Choo et al. (11) led to the development of enzyme immunoassays with recombinant HCV proteins for the detection of antibody to HCV (anti-HCV) (19, 22). PCRs have been developed for the detection of HCV RNA in sera; they use primers deduced from structural and nonstructural regions (39), as well as the well-conserved 5′-noncoding region of the HCV genome (5, 9, 29), to establish an ongoing HCV infection. These virus-specific tests have shown that HCV is the major etiologic agent of blood-borne non-A, non-B hepatitis worldwide (2, 3, 19) and are useful in preventing posttransfusion hepatitis C (1, 18, 38).

Reflecting the rapid evolution of RNA viruses (15), HCV variants which show significant divergence in both nucleotide and deduced amino acid sequences of the entire genome have been reported (12, 26–28, 37). On the basis of sequence similarity over the entire genome and within parts of it, HCV isolates have been classified into genotypes I, II, III, IV, V, and VI (24, 27, 31, 32). Another classification which groups HCV into three major types, 1, 2, and 3, and divides each type into subtypes a, b, c, etc., has been proposed (10, 34). Genotype I corresponds to 1a, II corresponds to 1b, III corresponds to 2a, IV corresponds to 2b, V corresponds to 3a, and VI corresponds to 3b. In a third classification, devised by Houghton et al. (16) and Cha et al. (8), group I corresponds to genotype I/1a, II corresponds to II/1b, III includes III/2a and IV/2b, IV encompasses V/3a, and V includes HCV variants clustered exclusively in South Africa. Until an official nomenclature for HCV grouping is worked out, a genotyping scheme reflecting these provisional classifications would be appropriate.

HCV genotypes have distinct epidemiological distributions (6–8, 10, 23, 24, 31, 32, 35), like subtypes of hepatitis B surface antigen (HBsAg) (13), and they are correlated with severity of liver disease and response to interferon therapy (14, 17, 23, 33, 36, 40). In the present study, markers of HCV and hepatitis B virus (HBV) infections, including HCV genotypes and HBsAg subtypes, were surveyed among blood donors in Ho Chi Minh City and Hanoi, Vietnam. The results obtained indicated different patterns of HCV transmission in these two major cities and HCV variants not classifiable into known genotypes prevailing in Vietnam.

MATERIALS AND METHODS

Serum samples. A total of 491 consecutive commercial blood donors (male, 142; female, 349) who visited the blood bank of Cho Ray Hospital in Ho Chi Minh City during 5 days in September and October 1992 and 499 donors (male, 202; female, 297) visiting the blood bank of Viet Duc Hospital in Hanoi during 8 days in August 1992 were studied. Ho Chi Minh City donors were older than Hanoi donors (mean ages, 52.9 ± 13.0 and 38.7 ± 9.7 years, respectively), and a higher proportion of them were ≥50 years old (67.8 and 18.2%, respectively). Tests for HBsAg, malaria, and syphilis are mandatory in blood banks in Vietnam. Donors who had given blood units previously were registered, and those with these markers were rejected from further donations. Markers for HCV infection are not screened for at present. There were 57

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TABLE 1. Markers of HCV, HBV, HIV, and HTLV-I infections in blood donors in Ho Chi Minh City and Hanoi, Vietnam

Donors (*n*)	No. (%) with the following marker:					
	Anti-HCV	HCV RNA[a]	Anti-HBs	HBsAg	Anti-HIV	Anti-HTLV-I
Ho Chi Minh City						
Total (491)	101 (20.6)	79	234 (47.7)	15 (3.1)	1 (0.2)	3 (0.6)
Male (142)	61 (43.0)	46	63 (44.4)	4 (2.8)	1 (0.7)	2 (1.4)
Female (349)	40 (11.5)	33	171 (49.0)	11 (3.2)	0	1 (0.3)
Hanoi						
Total (499)	4 (0.8)	4	248 (49.7)	15 (3.0)	0	0
Male (202)	4 (2.0)	4	96 (47.5)	6 (3.0)	0	0
Female (297)	0	0	152 (51.2)	9 (3.0)	0	0

[a] HCV RNA was determined only in sera testing positive for anti-HCV.

(11.6%) first-time donors in Ho Chi Minh City and 18 (3.6%) in Hanoi. Sera were transported on dry ice to Japan and tested for viral markers.

Markers of HCV infection. Anti-HCV was determined by passive hemagglutination (PHA) with recombinant HCV antigens of the second generation (c100-3, pHCV-31, and pHCV-34) (38) by use of a commercial kit (Abbott HCV PHA 2nd Generation; Dainabot, Tokyo, Japan). The test was performed with serial twofold dilutions of serum, and hemagglutination titers of $\geq 2^5$ were considered positive. Only sera with anti-HCV were tested for HCV RNA. Nucleic acids were extracted from 100 μl of serum, reverse transcribed to cDNA, and amplified by a two-stage PCR with nested primers deduced from the 5′-noncoding region of the HCV genome (30). An antisense primer (primer 36) used in the original method (29) for cDNA synthesis and the first stage of PCR was replaced by primer 299, which has the sequence 5′-ACCCAACACTACT CGGCTAG-3′ representing nucleotides 250 to 269 (25); nucleotides were numbered from the putative 5′ end of the HCV genome. Primer 299 matches isolates of all known genotypes, including V/3a, while primer 36 does not match V/3a in nucleotides 247 to 249. The modified PCR amplified a product of 225 bp with a sensitivity comparable to that of the original method (29).

Genotypes of HCV. HCV genotypes were determined by a modification of a method described previously (31). Part of the HCV core gene (272 bp) was amplified on HCV cDNA with universal primers. A portion of the product was then amplified by PCR with universal sense primers and a mixture of five antisense primers deduced from HCV core gene sequences specific for genotypes I/1a, II/1b, III/2a, IV/2b, and V/3a (31, 32). PCR products were electrophoresed and stained with ethidium bromide. The five genotypes were distinguished from one another by the sizes of the PCR products: 49 bp for genotype I/1a, 144 bp for II/1b, 174 bp for III/2a, 123 bp for IV/2b, and 88 bp for V/3a.

To avoid contamination during PCR procedures, the guidelines of Kwok and Higuchi (20) were strictly observed. RNA extraction and PCR were performed with two negative controls and one positive control with a known genotype for every 17 test samples. Results were recorded only when false-positive results were not obtained for the negative controls and HCV RNA of the proper genotype was detected in the positive control.

Markers of HBV and other virus infections. HBsAg and antibody to HBsAg (anti-HBs) were tested by PHA with a commercial kit (MyCell; Institute of Immunology Co., Ltd., Tokyo, Japan). HBsAg samples in sera with hemagglutination titers of $\geq 2^5$ were classified into the four major subtypes (4, 21), adw, adr, ayw, and ayr, by an enzyme immunoassay with a commercial kit (Immunis HBsAg/Subtype EIA; Institute of Immunology). Antibody (anti-HIV) to human immunodeficiency virus type 1 (HIV) and antibody (anti-HTLV-I) to human T-cell leukemia virus type I (HTLV-I) were determined by agglutination of gelatin microparticles coated with the respective viral antigens by use of commercial kits (SERODIA · HIV and SERODIA · HTLV-I; Fuji Rebio, Tokyo, Japan). The results for anti-HTLV-I were confirmed by staining for the viral antigen by the immunofluorescent-antibody technique.

Sequencing of parts of the 5′-noncoding region and the C gene. Parts of the 5′-noncoding region and the C gene of the 4 nontypeable HCV isolates from Hanoi donors and 6 of the 30 nontypeable HCV isolates from Ho Chi Minh City donors were sequenced. Products of the first-stage PCR with primers 32 and 299 (225 bp spanning nucleotides 45 to 269) and those of the PCR used for genotyping (272 bp spanning nucleotides 480 to 751) were obtained by methods reported previously (27, 28, 31, 32) and sequenced on both plus and minus strands.

Statistical analysis. Frequencies were compared by use of the χ^2 test and Fisher's exact probability test.

RESULTS

Markers of HCV infection in blood donors in Ho Chi Minh City and Hanoi, Vietnam. There were striking differences in the prevalence of anti-HCV in the two major cities of Vietnam (Table 1). Anti-HCV was detected in 101 (20.6%) of 491 donors in Ho Chi Minh City but in only 4 (0.8%) of 499 donors in Hanoi ($P < 0.001$). HCV RNA was tested for in the donors positive for anti-HCV and was detected in 79 donors in Ho Chi Minh City and 4 donors in Hanoi. In Ho Chi Minh City, anti-HCV was more prevalent in male donors than in female donors (61 of 142 [43.0%] and 40 of 349 [11.5%], respectively [$P < 0.001$]). Donors with anti-HCV in Ho Chi Minh City were

TABLE 2. Genotypes of HCV RNA in blood donors in Vietnam

Donors (*n*)	No. (%) with the following genotype[a]:						
	I/1a	II/1b	III/2a	IV/2b	V/3a	I/1a + II/1b	Nonclassifiable
Total (83)	24 (29)	19 (23)	4 (5)	0	0	2 (2)	34 (41)[b]
Male (50)	15 (30)	11 (22)	3 (6)	0	0	1 (2)	20 (40)
Female (33)	9 (27)	8 (24)	1 (3)	0	0	1 (3)	14 (42)

[a] Genotypes were determined by PCR with type-specific primers deduced from the HCV core gene (31, 32).

[b] Including all four HCV RNA-positive samples from Hanoi donors.

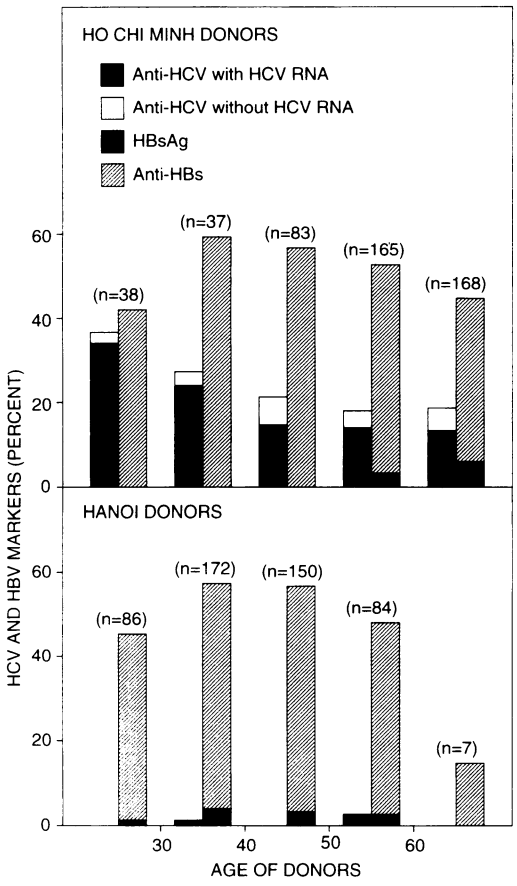

FIG. 1. Markers of HCV and HBV infections in blood donors in Ho Chi Minh City and Hanoi grouped by age. HCV RNA was tested for only in donors with anti-HCV.

younger than those without (49.7 ± 13.8 and 53.7 ± 12.7 years old, respectively) and had donated more blood units before (8.5 ± 12.8 and 7.8 ± 8.3 units, respectively). There were 57 first-time donors in Ho Chi Minh City; 9 (16%) were positive for anti-HCV. In contrast, none of the four donors who had anti-HCV in Hanoi were first-time donors.

HCV RNA samples from 83 donors, including 79 in Ho Chi Minh City and 4 in Hanoi, were genotyped (Table 2). There were no appreciable differences in the distribution of HCV genotypes in male and female donors. Genotypes I/1a (29%) and II/1b (23%) accounted for the majority; III/2a accounted for a minority (5%). Two donors were infected with HCV of different genotypes (I/1a and II/1b). Genotypes in the remaining 34 (41%) samples, including all 4 from Hanoi, were not classifiable into I/1a, II/1b, III/2a, IV/2b, or V/3a.

Markers of HBV, HIV, and HTLV-I infections. The prevalences of anti-HBs, reflecting a past HBV infection, and of HBsAg, reflecting an ongoing infection, were comparable between donors in Ho Chi Minh City and Hanoi, at 47.7 and 49.7%, respectively, for anti-HBs and at 3.1 and 3.0%, respectively, for HBsAg (Table 1). There were no sex-related differences for these HBV markers in both cities. Subtypes of HBsAg were determined for 10 samples from Ho Chi Minh City and 8 samples from Hanoi; there were no apparent differences in the distribution of HBsAg subtypes in samples from the two cities. Subtypes adr, adw, and ayw were detected in 4 (22%), 1 (6%), and 13 (72%) samples, respectively.

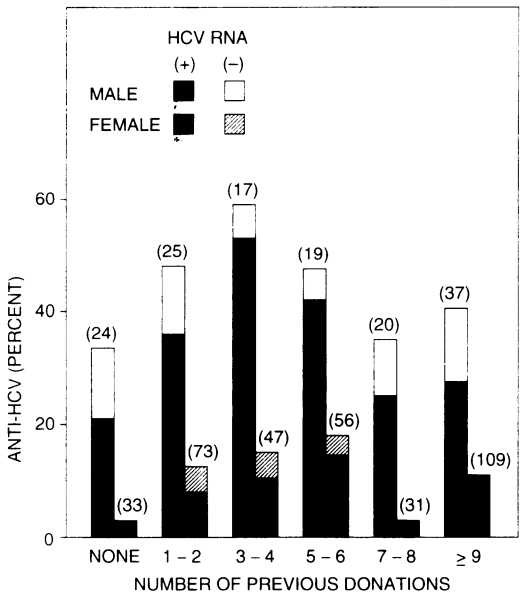

FIG. 2. Frequency of anti-HCV in male and female donors in Ho Chi Minh City in relation to the number of previous donations. HCV RNA was tested for only in donors with anti-HCV. Numbers in parentheses indicate the numbers of male and female donors.

Anti-HIV (0.2%) and anti-HTLV-I (0.6%) were very rare in Ho Chi Minh City donors and were not detectable in Hanoi donors.

Age-specific prevalence of markers of HCV and HBV infections. The prevalences of anti-HCV with HCV RNA, reflecting an ongoing HCV infection, anti-HCV without HCV RNA, reflecting a past HCV infection, HBsAg, reflecting an ongoing HBV infection, and anti-HBs, reflecting a past HBV infection, were determined for donors in Ho Chi Minh City and Hanoi divided into five age groups (Fig. 1). The prevalences of anti-HCV and HCV RNA in anti-HCV-positive donors in Ho Chi Minh City decreased in parallel with age and were much higher than those in Hanoi donors for all age groups. There was no apparent relationship between HBsAg and age in donors in the two cities. The prevalence of anti-HBs was the lowest in donors younger than 30 years in both cities. It was the highest for donors in their thirties in both Ho Chi Minh City and Hanoi and then gradually decreased with added age in decades.

Figure 2 illustrates frequency of markers of HCV infection in Ho Chi Minh City donors stratified by number of previous donations. The frequencies of anti-HCV and HCV RNA in anti-HCV-positive donors increased as previous donations increased up to four units in male donors and up to six units in female donors; HCV markers were less prevalent in donors with more than those numbers of previous donations.

Nucleotide sequences of HCV isolates from Vietnam not classifiable into known genotypes. Figure 3 compares the sequences of part of the 5′-noncoding region (185 bp spanning nucleotides 65 to 249 [primer sequences excluded]) of 10 HCV isolates from Vietnam not classifiable into known genotypes. Two Vietnamese isolates, VN506 and VN538, had sequences closely resembling that of a Hong Kong isolate (HK2) of genotype 6a (6, 7). Two other isolates, VN004 and VN405, had sequences different from the others or from each other because of a combination of unique mutations. The remaining six isolates, VN085, VN235, VN507, VN530, VN531, and VN540,

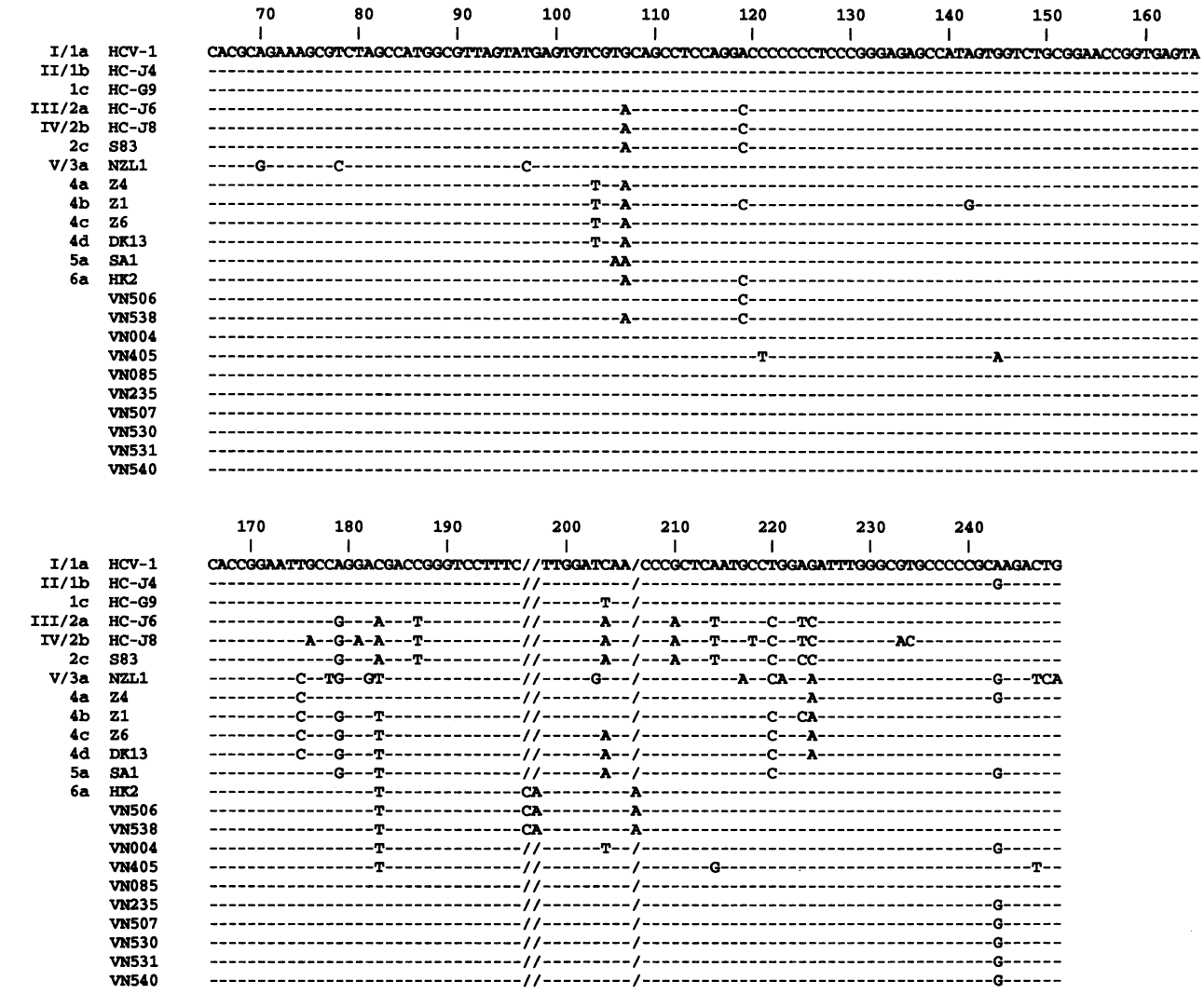

FIG. 3. Nucleotide sequences of part of the 5′-noncoding region of HCV isolates of known genotypes and those from Vietnamese donors. Ten Vietnamese isolates were compared with HCV-1 (12), HC-J4 (30), HC-G9 (26), HC-J6 (28), HC-J8 (27), S83 (6, 7), NZL1 (32), and Z4, Z1, Z6, DK13, SA1, and HK2 (6, 7). Nucleotides were numbered from the putative 5′ end of the HCV genome (27).

resembled HCV-1 of genotype I/1a and HC-J4 of genotype II/1b within the 5′-noncoding region compared. However, they were substantially different from the genotype I/1a and II/1b isolates within part of the C gene (232 bp spanning nucleotides 500 to 731 [primer sequences excluded]), sharing only 81.0 to 81.9% nucleotides with HCV-1 and 78.0 to 80.2% nucleotides with HC-J4.

DISCUSSION

Anti-HCV was detected in 101 (20.6%) of 491 commercial blood donors in Ho Chi Minh City but in only 4 (0.8%) of 499 donors in Hanoi ($P < 0.001$). The prevalence of anti-HCV in Ho Chi Minh City, detected by PHA with recombinant antigens of the second generation (38), by far exceeded that in voluntary blood donors in the United States (0.2 to 0.5% [2, 19]) and in Japan (1.5% [38]), to whom donors in Hanoi were comparable. Tests for anti-HCV are not mandatory in Vietnam. Because of the high prevalence of HCV infection among Vietnamese blood donors, screening of donors for anti-HCV to exclude contaminated blood units is urgently required. It is not clear why the prevalence of anti-HCV was much higher in blood donors in Ho Chi Minh City than in those in Hanoi. Ho Chi Minh City donors were older than Hanoi donors; the frequency of anti-HCV was high in Ho Chi Minh City irrespective of age, however.

Hanoi is located in northern Vietnam, and ethics and disciplines were maintained fairly well during and after the Vietnam War. Ho Chi Minh City is located in the south, which includes Saigon, and the influence of the Vietnam War was much greater there, with many sailors and soldiers visiting. Intravenous drug abuse has been common since the Vietnam War. Ho Chi Minh City donors with HCV markers were younger than those without them and more likely to be males than females, a distribution suggesting transmission by intravenous drug abuse. Since donors had not been screened for anti-HCV, it was detected frequently in both first-time and repeat donors. The prevalence of HCV infection in Ho Chi Minh City donors increased with the number of previous

donations, a fact which might reflect HCV transmission during donation.

Genotypes in 79 HCV RNA-positive samples from Ho Chi Minh City were mostly I/1a and II/1b, which accounted for the majority (52%); III/2a accounted for a minority (5%). Genotypes in the remaining 34 (41%) samples were not classifiable into I/1a, II/1b, III/2a, IV/2b, or V/3a, like the four HCV RNA-positive samples from Hanoi donors. Possible limitations in the applied typing system notwithstanding, some nontypeable HCV RNA-positive samples may belong to novel genotypes. The sequences of the 5′-noncoding region of 2 of the 10 nontypeable HCV isolates from Vietnam tested were substantially different from those recently reported for that region of isolates of genotypes 2c, 4a to 4d, 5a, and 6a (6, 7), indicating that these two isolates probably belong to novel genotypes. Although six other Vietnamese isolates were similar within part of the 5′-noncoding region to isolates of genotypes I/1a and II/1b, they were substantially different within part of the C gene. The remaining two isolates were similar to a Hong Kong isolate of genotype 6a (6, 7). Thus, 8 of the 10 Vietnamese isolates sequenced partially probably belong to novel genotypes not described previously. Determining novel HCV genotypes not only cross-sectionally, as in the present study, but also retrospectively and prospectively will disclose a more precise epidemiology of HCV in Vietnam.

HCV RNAs of possibly novel genotypes are of virological, clinical, and epidemiological interest. It is likely that HCV variants of particular genotypes evolved in restricted areas of the world, because HCV isolates with sequences not seen in other parts of the world have been reported frequently (6–8, 10, 24, 26, 34–36). HCV genotypes appear to influence virulence, because HCV of genotype II/1b induces more severe liver disease than HCV of genotype III/2a and patients with chronic hepatitis C and carrying HCV of genotype II/1b are less responsive to interferon than those carrying HCV of genotype III/2a (14, 17, 33, 36). Whether HCV genotypes intrinsic to Vietnam have any clinical relevance needs to be investigated.

Anti-HBs was detected in approximately half of commercial donors from both Ho Chi Minh City and Hanoi. The high frequency of anti-HBs contrasted with the rather low frequency of HBsAg, 3%, found for donors in both cities. The rejection of donors once found to be HBsAg positive would be responsible for this discrepancy.

Anti-HIV was detected very rarely in Ho Chi Minh City donors (0.2%) and not at all in Hanoi donors. This fact is very fortunate, considering the spread of HCV infection in a mode similar to that of HIV infection. Anti-HTLV-I is at present also very rare in blood donors in both Ho Chi Minh City and Hanoi. A low prevalence of present HIV infection notwithstanding, anti-HIV needs to be screened for in blood donors in Vietnam to protect recipients and control the possible future spread of HIV among blood donors.

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