The seroprevalence of hepatitis C virus (HCV) among 559,890 first-time volunteer blood donors in China reflects regional heterogeneity in HCV prevalence and changes in blood donor recruitment models

Yongshui Fu, Wenjie Xia, Yizhong Wang, Linwei Tian, Oliver G. Pybus, Ling Lu, and Kenrad Nelson

BACKGROUND: A decrease in the prevalence of hepatitis C virus antibody (anti-HCV) has been reported among voluntary blood donors in some regions of China. However, the prevalence of HCV among volunteer blood donors in other regions of China has not been reported. The aim of this study was to investigate the seroprevalence of HCV among 559,890 first-time volunteer blood donors recruited during 2004 through 2007 at the Guangzhou Blood Center, China.

STUDY DESIGN AND METHODS: Anti-HCV was detected using two different third-generation enzyme immunoassay kits. HCV RNA was detected using reverse transcription–polymerase chain reaction (RT-PCR) targeting the 5′-untranslated region of HCV.

RESULTS: Among 559,890 donors, 1877 (0.335%) were positive for anti-HCV. The anti-HCV+ rate was significantly higher in males than females (0.37% vs. 0.28%; p < 0.001) and significantly lower among donors living in Guangdong Province than donors who had migrated from other locations (0.30% vs. 0.40%; p < 0.001). Among the 1877 anti-HCV+ donors, 450 were randomly selected for HCV nucleic acid amplification by RT-PCR. Of these, 270 (60%) were HCV RNA+ and 180 (40%) were HCV RNA−.

CONCLUSIONS: Many donors from outside Guangdong Province were migrant laborers from other areas in China, suggesting that there is regional heterogeneity in HCV prevalence within China. The overall anti-HCV+ rate reported here is among the lowest reported among blood donors in China reflecting the effect of the current recruitment of exclusively volunteer donors.

Chronic infection with hepatitis C virus (HCV) is a major and growing public health problem. Globally, approximately 170 million people are infected with HCV; however, the prevalence varies greatly among countries, from 0.2% to 26%.1–6 The rapid global spread of HCV is believed to have occurred primarily because of efficient transmission through blood transfusion and parenteral exposures with contaminated equipment.7 In countries where HCV antibody screening and nucleic acid amplification testing (NAT) are mandatory for all blood donors, new HCV-infected donors often have a history of injecting illicit drugs, contaminated medical procedures, or other parenteral exposures. Risk

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factors also include tattooing, ear and body piercing, household or sexual contact, and unknown sources of infection. One of the most consistent characteristics of HCV is its ability to cause chronic infection in a high percentage of individuals. Chronic HCV infection often leads to liver cirrhosis and hepatocellular carcinoma.

As blood-borne pathogens, both HCV and the human immunodeficiency virus (HIV) were frequently detected among paid blood donors in China during the early 1990s. To improve the safety of blood supply and reduce the risk of transfusion-transmitted diseases, the Chinese government has, since 1998, outlawed the use of paid blood donors. As a result, Chinese blood banks now rely on various other methods to recruit blood donors; these include employer-organized donor recruitment and the use of student donors, replacement donors, and in the recent past, only volunteer donors. This transition in the blood donor recruitment methods in China has been associated with a gradual decrease in the seroprevalence of anti-HCV among donors. The HCV seroprevalence among the general population of China has been estimated to be about 3.2%, while among paid blood donors the prevalence has been 5.7% or higher. However, among employer-organized donors and volunteer donors, the prevalence has ranged between 1.1, 2.3, and 0.46%, respectively. Although a progressive decrease in the overall prevalence of anti-HCV has been observed among donors in some regions, the HCV prevalence among first-time volunteer blood donors in China has not been reported. From January 2004 to December 2007, a total of 559,890 first-time volunteer blood donors were evaluated at the Guangzhou Blood Center, in Guangdong Province. In this study, we report the prevalence of anti-HCV in this cohort of donor population.

MATERIALS AND METHODS

Subjects and samples
From January 2004 to December 2007, a total of 559,890 first-time volunteer blood donors were recruited at the Guangzhou Blood Center. Before donation each volunteer was asked to answer a standardized risk factor questionnaire created by the Chinese Ministry of Public Health. The questionnaire identifies donors for exclusion who had a history of blood product transfusion, injection drug use, receipt of a tattoo, ear or body piercing, surgery, or other invasive medical procedures. Before blood collection, all donors underwent rapid testing for hepatitis B surface antigen (HBsAg; colloidal gold strip method). After a negative result was obtained, donors free of reported risk on the questionnaire were accepted to donate blood. Donated blood was stored at 4°C for less than 24 hours before serum was tested using routine screening assays for alanine aminotransferase (ALT; ≥40 IU/L by the speed rate method or ≥25 IU/L by the Reitman method), anti-HIV-1, anti-HIV-2, HBsAg, and anti-HCV and a serologic test for syphilis. These assays were performed in two rounds with different reagents from two different manufacturers. All reagents used in the screening assays are licensed by the Chinese National Food and Drug Administration and have passed the lot release process of the Chinese National Institute for the Control of Pharmaceutical and Biological Products with the Chinese National Reference Panels. If a donor gave blood more than once, only the first-time donation was included in this analysis.

Anti-HCV assays
Anti-HCV was assayed using third-generation enzyme immunoassay (EIA) reagents from two different manufacturers, following the manufacturers’ protocols. The first assay was the enzyme-linked immunosorbent assay diagnostic kit for anti-HCV, from Kehua Biotech Co. Ltd (Shanghai, China), and the second was the Ortho HCV 3.0 enzyme-linked immunosorbent assay kit from Ortho-Clinical Diagnostic, Inc. (Raritan, NJ). Samples reactive in either or both tests were considered as anti-HCV+ and were not used for transfusion.

Reverse transcription–polymerase chain reaction to detect HCV RNA
From each 140 μL of serum, total RNA was extracted using a viral RNA mini kit following the manufacturer’s protocol (QIAamp, Qiagen, Chatsworth, CA). After extraction, RNA was converted into cDNA in 20 μL of volume containing random hexamers (Promega, Madison, WI) and 15 units of AMV reverse transcriptase (Promega) and the reaction was incubated at 37°C for 60 minutes. With the converted cDNA as a template, the 5’-untranslated region of the HCV genome was amplified. This was done using the Primer STAR HS (Premix) kit (Takara, Dalian, China) and the primers previously described. Each polymerase chain reaction (PCR) volume was 25 μL. The first-round PCR contained 2 μL of cDNA and 10 pmol of each outer primer; the second-round PCR contained 1 μL of the first-round PCR product and 10 pmol of each inner primer. The amplification was performed for one cycle at 94°C for 2 minutes, followed by 40 cycles, each consisting of 94°C for 40 seconds, 50°C for 40 seconds, and 72°C for 40 seconds, with the last cycle of extension at 72°C for 10 minutes. After amplification, PCR products were resolved in 2% agarose gel stained with ethidium bromide.

Statistical analysis
Participants in this study were classified into groups before being subjected to statistical analyses: 1877 of 559,890 volunteer blood donors were identified as being anti-HCV+. Of the 1877 anti-HCV+ donors, 450 were
randomly selected for reverse transcription (RT)-PCR assays, which subsequently classified the 450 donors into those that were HCV RNA+ and those that were HCV RNA−. Donors were further classified by sex (male/female) and location of origin (Guangdong/outside Guangdong).

Chi-square tests and analysis of variance tests were performed, as appropriate, to test the associations between HCV prevalence and donor age, sex, and location of origin. In the comparison of HCV prevalence rates of donors originating from Guangdong and migrants from outside Guangdong, the rates were standardized according to the overall sex and age distribution of all the 559,890 donors. Statistical analyses were performed using computer software (SPSS, Version 14.0 for Windows, SPSS, Inc., Chicago, IL). Comparisons at the p < 0.05 level were judged to be significant.

RESULTS

Characteristics of the donor population and anti-HCV seroprevalence

Of the 559,890 first-time volunteer blood donors, 357,673 (63.88%) were male and 202,217 (36.12%) were female; 345,385 (61.69%) were from Guangdong Province, and 214,505 (38.31%) were from areas other than Guangdong. The latter included donors from all provinces, municipalities, and special administrative regions of China, most of them representing off-season migrant laborers traveling from rural areas to work in factories around the Pearl River Delta (see Table 1). The 559,890 donors were between 18 and 55 years of age and had a mean age of 29.7 years (standard deviation [SD], 7.79 years). Overall, 1877 of the 559,890 donors were positive for anti-HCV: of these, 1308 (0.298%) were male donors, 574 males and 275 females were from Guangdong Province and 144,893 (67.5%) male and 69,612 (32.5%) female donors were from other areas. The sex ratios and mean ages were both significantly different (p < 0.05) between these two geographic groups. When the 1877 anti-HCV+ donors were similarly stratified, 734 males and 294 females were from Guangdong Province, and 574 males and 275 females were from elsewhere; the sex ratios and mean ages were not significantly different between the two geographic groups. The anti-HCV+ prevalence among donors of Guangdong origin was significantly higher (p < 0.001) in males (0.345%) than females (0.222%). However, the anti-HCV+ prevalence was similar (p > 0.05) in males (0.396%) and females (0.395%) from areas outside Guangdong Province (Table 1).

According to the age distribution of all donors, the age-standardized anti-HCV+ rates were calculated for different groups. Although a birth cohort effect was observed, the age-standardized anti-HCV+ rates did not significantly deviate from the crude rates (Table 2 and Fig. 1).

Of the 1877 anti-HCV+ donors, 450 were selected for NAT assay. Among the 450 donors, 307 (68.2%) were male and 143 (31.9%) were female; 265 (58.9%) were from Guangdong Province and 185 (41.1%) were from elsewhere. The ages of these 450 donors ranged between 21 and 54 years, with a mean age of 33.3 years (SD, 6.93 years). Comparing with the 1877 anti-HCV+ donors, the 450 selected donors showed no significant differences in sex, age, and location of origin. Using an in-house RT-PCR amplification assay targeting the 5′-untranslated region of the HCV genome, HCV RNA was detected among 270

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<th>Table 1. HCV seroprevalence among 559,890 first-time voluntary donors at the Guangzhou Blood Center by age, sex, and location of origin, 2004 through 2007</th>
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<td>Donor group</td>
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* p < 0.001.
† Comparing with the total number of donors from Guangdong Province.
‡ p < 0.05.
§ Comparing with the anti-HCV+ rate among donors from Guangdong Province.
(60%) of the 450 donors and was undetectable in 180 (40%). There was no difference in the age and location of origin of these two groups (Table 3). However, females were significantly more common ($p < 0.001$) in the HCV RNA− group than in the HCV RNA+ group.

Commonly, an elevated ALT level is considered to be a biochemical marker for active hepatitis. To differentiate biochemical hepatitis from past HCV infection, we examined the ALT levels of the 180 anti-HCV+ but HCV RNA− donors: 13 had elevated ALT levels (10 male, three female; 11 from Guangdong, two from elsewhere).

**DISCUSSION**

Blood transfusion was a major risk factor for HCV infection before the virus was identified in 1989. This transmission route remained common in China during 1992 through 1994, when more than 500,000 donors were found to be infected with HCV due to contaminated blood collection, mostly plasma collection in for-profit facilities. To eliminate this risk, the Chinese government has outlawed the use of paid blood donors since 1998. Blood donor recruitment models in China have therefore changed from the use of paid donors to selecting donors from employer-organized recruitment drives, donors among students, and volunteer donors among low-risk populations. In recent years, the proportion of volunteer donors have rapidly increased, from 5% in 1998 to 71.5% at the end of 2004. They now constitute the main source of the blood supply in all major blood banks in China. Although data on anti-HCV prevalence have not been reported among donors recruited by different methods from a single geographic region, temporal trends of decreasing HCV seroprevalence have been observed as the methods of donor recruitment changed. In 1993 through 1994, the HCV prevalence among blood donors was 6.5%. From 1997 to 2000, it decreased to 4.6%. The prevalence was 2.3% in 2000 through 2001 and it decreased further to 0.41% to 0.46% in 2003 and 0.335% in the current study during 2004 through 2007. These trends indicate that the changes in blood donor populations resulting from different methods of recruitment have resulted in a decreasing seroprevalence of HCV among donors. These trends also likely reflect the reduced proportion of donors who are in the seronegative window period or who have a false-negative serologic test for other reasons. The residual risk of transmission of HCV from a seronegative donor was recently estimated to be approximately 1 in 40,000 to 60,000 donations in China. A decrease in anti-HCV+ donations has been reported among donors in the United States (from 0.63% to 0.40%...
A nationwide anti-HCV rate was estimated from a study of 66,975 individuals screened in 1992 who were a representative sample of the general population of China. These subjects were aged 1 to 59 years and originated from all provinces of China. The overall anti-HCV rate was estimated to be 3.2% and a strong birth cohort effect was observed. The anti-HCV prevalence has been replaced with younger individuals with lower anti-HCV frequencies. Persons over the age of 55 are not accepted for blood donation in China. In addition, the majority of the donors in the current study were from the south of China and all were between 18 and 55 years of age when they volunteered to donate blood. Taken together, these temporal and spatial variations could have contributed significantly to the lower anti-HCV seroprevalence found in this study. In the United States and Canada, the anti-HCV prevalence among first-time volunteer blood donors has been estimated to be approximately one-third to one-fifth of the HCV infection among the general population. If this ratio of HCV prevalence among blood donors to that in the general population also exists in China, approximately 1.0% to 1.68% of the Chinese population might be currently infected with HCV, representing approximately 13 million to 22 million people. These estimates are lower than those previously reported in 1992.

Several other factors could affect the anti-HCV prevalence among this cohort of donors. A proportion of the volunteer blood donors were among migrants from rural areas, who were temporarily employed in Guangzhou city. During the “farming-busy” seasons and holidays, these individuals typically travel back and forth from the city to remote rural areas. Nationally, the total number of such internal migrants is estimated to be approximately 100 million; they have among the highest rates of HIV and HCV transmission in the country, among the general population. These individuals can be identified when they donate blood because their permanent rural addresses are shown on their identification documents that must be presented at the time they register for donation. More than 30 million migrants are employed around the Pearl River Delta; they may be motivated to donate blood because, if they have a record of volunteer blood donation in Guangzhou, they or their family members will receive free blood transfusions anywhere in the country in the future, if this is needed. Migrant laborers may be at higher risk for HCV infection than permanent city residents because of their lower socioeconomic status.
and more frequent parenteral exposures in higher-risk locations.

Third-generation EIAs to detect anti-HCV are now routinely used for screening potential donors in nearly all blood banks in China. These screening assays are quite sensitive to detect infections with HCV after the early infection-seronegative window period has passed. A positive screening HCV EIA test is used as a criteria for exclusion of a blood donor in China. Nevertheless, not all donors who have reactive EIA tests have active HCV infections. To increase the sensitivity of the HCV EIA screening assays and to improve the positive predictive value of the testing results, the Guangzhou blood bank routinely screens prospective donors using EIA assays from two different manufacturers. Donors who are reactive with either screening assay are excluded and their blood is not transfused. However, some donors who are reactive on only one assay may more commonly have false-positive tests. Other persons with a reactive EIA may have cleared a previous HCV infection spontaneously and be noninfectious. The overall frequency of spontaneous clearance of an HCV infection has been reported to be approximately 20% to 30% in areas where Genotype 1 infection predominates. The proportion is higher after infection with HCV Genotypes 2 and 3. The HCV genotype distribution in China and other areas in Asia differs from that in the United States and Western Europe. Genotypes 3 and 6 are more common in Asia, contributing approximately 25% to 30% or more to all HCV infections, whereas 65% to 80% of infected persons in Western countries are infected with Genotype 1. Variations in the natural history of infection by different HCV genotypes in our population could account for the somewhat greater proportion of RNA-negative, EIA repeatedly reactive donors (40%) than has been reported among blood donors in the United States and Europe.

Females also have higher rates of spontaneous clearance of HCV infections than males. Spontaneous virus clearance of an HCV infection could account for a large proportion of the anti-HCV+, RNA− donors found in this study. This is supported by the finding that HCV RNA−, EIA+ donors were statistically more common among women in our study.

Although confirmatory testing with recombinant immunoblot assay or a NAT assay is recommended and routinely used by blood banks in the United States and Europe to confirm a repeat-reactive EIA HCV screening result, the availability of these tests is very limited in China due to the high cost and the requirement for qualified personnel and specialized equipment. Therefore, blood banks in China report HCV infection rates among donors based only on an anti-HCV+ screening assay. The substantial reduction in the prevalence of HCV infection among donors at the Guangzhou blood collection center in the years after the regulation requiring the collection of blood only from voluntary donors, suggests that an important reduction in the risk of the transmission of HCV by blood transfusion has occurred in China in the past decade.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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