

**Sexual Transmission of HCV Among Monogamous Heterosexual Couples: The HCV
Partners Study**

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J. Dodge: Provided statistical expertise and contributed to data interpretation, and writing of the manuscript.

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A. Reingold: Contributed to the study concept and design and contributed to manuscript revisions.

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Abbreviations:

HCV: hepatitis C virus

HIV: human immunodeficiency virus

IDU: injection drug use

PCR: polymerase chain reaction

RNA: ribonucleic acid

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ABSTRACT:

BACKGROUND: The efficiency of hepatitis C virus (HCV) transmission by sexual activity remains controversial. We conducted a cross-sectional study of HCV-positive persons and their partners to estimate the risk for HCV infection among monogamous heterosexual couples.

METHODS: 500 anti-HCV-positive, HIV-negative index persons and their long-term heterosexual partners were studied. Couples were interviewed separately for lifetime risk factors for HCV infection, within-couple sexual practices and sharing of personal grooming items. Blood samples were tested for anti-HCV, HCV RNA, and HCV genotype and serotype. Sequencing and phylogenetic analysis determined the relatedness of virus isolates among genotype-concordant couples.

RESULTS: HCV-positive index persons were mostly Non-Hispanic Whites, with median age 49 years (range 26-79) and median 15 years (range 2-52) of sexual activity with their partners. Overall, HCV prevalence among partners was 4% (n=20), and 9 couples had concordant genotype/serotype. Viral isolates in 3 couples (0.6%) were highly related, consistent with transmission of virus within the couple. Based upon 8377 person-years of follow-up, the maximum incidence rate of HCV transmission by sex was 0.07% per year (95% CI: 0.01, 0.13) or ~1 per 190,000 sexual contacts. No specific sexual practices were related to HCV-positivity among couples.

CONCLUSIONS: The results of this study provide quantifiable risk information for counseling long-term monogamous heterosexual couples in which one partner has chronic HCV infection. In addition to the extremely low estimated risk for HCV infection in sexual partners, the lack of association with specific sexual practices provides unambiguous and reassuring counseling messages.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection affects 3 to 4 million people in the United States, most of whom are sexually active adults (1). The primary means of transmission of HCV is by direct percutaneous exposures to infectious blood and there are clearly defined counseling messages for infected persons to prevent spread from such exposures (2). The accumulated epidemiological evidence indicates that HCV can be transmitted by sex with an infected partner, presumably by mucosal exposure to infectious blood or serum-derived fluids. However, sexual activity is much less efficient for transmitting HCV than for other blood borne, sexually-transmitted viruses, such as hepatitis B virus and human immunodeficiency virus (HIV) (3).

The association between sexual activity and HCV infection was first demonstrated by case-control studies of persons with acute hepatitis C (4). The few prospective cohort studies of monogamous heterosexual couples have reported incidence rates of HCV infection of 0 – 0.6% per year in seronegative partners of persons with chronic HCV infection (5-7). In cross-sectional studies, HCV prevalences among partners vary widely (0-27%) but are <5% in studies excluding partners with known percutaneous exposures (3). For HCV-infected persons in the United States, the risks quantified by previous incidence studies may not apply as they were performed in countries where the epidemiology of HCV infection differs from that in the United States due to potentially confounding by unmeasured non-sexual risk factors. Although several seroprevalence studies of monogamous heterosexual couples have been reported from the United States (8, 9), their sample sizes were insufficient to evaluate overall risk or that related to specific sexual practices, and detailed virologic analyses of antibody-concordant couples were lacking, leading to an overestimation of transmission risk.

While it is generally agreed that the risk for transmitting HCV to sex partners is very low, the lack of quantifiable data has been a limitation to clinicians counseling their patients. Thus, the major objectives of this study were to quantify the risk for sexual transmission of HCV infection from chronically infected persons to their long-term heterosexual partners and identify specific sexual practices associated with that risk.

STUDY METHODS

Study Population

The recruitment phase of the study was conducted in Northern California sites between January 2000 and May 2003. Recruitment began by first identifying a known HCV-positive person (referred to as the index person) from multiple sources, including liver clinics at the University of California at San Francisco, members of Kaiser Permanente Medical Care Plan in Northern California, California Pacific Medical Center and affiliated clinics, other community-based practices in the greater San Francisco Bay Area, and blood donors from Blood Centers of the Pacific/Blood Systems Research Institute. Researchers contacted index persons for study enrollment, and if eligible based on prescreening, contacted their sexual partner. Criteria for study participation by each couple included a heterosexual relationship for a minimum of 36 months, monogamy for the duration of the relationship reported by both partners, and a minimum of 3 sexual contacts by the couple in the preceding 6 months. Couples were excluded if either partner had known HIV or hepatitis B virus infection, prior organ transplantation, or was currently using antiviral or immunosuppressive therapy; or if both partners reported a history of injection drug use (IDU).

Partners of each couple were interviewed independently by phone (76%) or in-person (24%) by trained interviewers, with no difference in completing the questionnaire by interview type.

Detailed information was obtained on the sexual history with the study partner (Appendix 1),

non-sexual household exposures (sharing of personal items including nail-grooming tools, razors, and toothbrushes), and all other risk factors for HCV acquisition. The risk period for sexual transmission was defined using a uniform method to capture sexual activities over the entire duration of the couple's relationship. Sexual histories were collected in discrete time intervals defined by events in each participant sexual history and beginning from the time of first sexual contact with the current partner up to the time of interview. Each participant identified life events such as pregnancy, childbirth, medical illness, and absences that significantly changed sexual activities with their study partner and the corresponding year and age for each life event. Sexual practices, including type and frequency of sexual contact and use of protective barriers, were obtained during each of these defined intervals. When responses to questions about sexual or personal grooming practices were discordant between partners, responses were recoded for presence rather than absence of the practice.

This study was approved by the Institutional Review Boards for University of California at San Francisco, Blood Centers of the Pacific, California Pacific Medical Center, Kaiser Permanente Northern California, St. Louis University, and the Centers for Disease Control and Prevention.

Serologic Testing

Serum samples from index persons were tested for anti-HCV by enzyme immunoassay (EIA 2.0) (Abbott Laboratories, Abbott Park, IL) and for HCV RNA by qualitative PCR with detection limit ≤ 50 IU/mL (Roche Amplicor, Roche Molecular Diagnostics, Pleasanton, CA) (if not documented in medical records in prior 6 months). Serum samples from partners were tested for anti-HCV by EIA and positive results confirmed by recombinant immunoblot assay (RIBA 3.0, Chiron Corporation, Emeryville, CA). RIBA-positive samples were tested for HCV RNA by qualitative PCR. Serotyping of the antibody based on RIBA methodology was utilized in anti-HCV-positive concordant couples with HCV RNA-negative partners. Genotype was determined

in samples from anti-HCV-positive, HCV RNA-positive concordant couples using the InnoLipa assay (Innogenetics, Ghent, Belgium).

Sequencing

HCV RNA-positive specimens from genotype-concordant couples were amplified by reverse transcription nested PCR and the HCV consensus sequences were determined by directly sequencing uncloned PCR products from the 897 long nucleotide (nt) NS5B region for genotype 1a and 1b samples and from a 944 nt NS5B region for the 2b samples employing ABI dye-termination techniques (11). The 1a and 1b sequences correspond to H77 positions 7479 to 8375 (with genotype 1b sequences missing 3 nucleotides relative to the 1a sequences, resulting in a gap corresponding to 7566 to 7568 in the H77 sequence). These 1a/1b alignments cover the region of the ORF coding for the last 42 amino acids of NS5A and the first 258 amino acids of NS5B. The genotype 2b alignments correspond to the H77 sequence 8326-9269, encoding NS5B from amino acid 242 to 556. To evaluate the relatedness between isolates from genotype concordant partners, the consensus sequences from their isolates were compared to corresponding regions from reference sequences of the same subtype downloaded from the Broad Institute or from the NCBI; this included 99 genotype 1a and 97 genotype 1b sequences. The sequences were imported into the MEGA 4 sequence analysis package and the pair-wise distances and number of differences were calculated for each pair. These nucleotide sequences have been submitted to Genbank (Accession #'s HQ022864-HQ022879)

Phylogenetic Analyses and Estimation of Minimal Divergence Time

The pair-wise distance and number of nucleotide differences of sequences between partners in each couple were compared to the average pair-wise distance and average number of nucleotide differences between the subtype-specific reference sequences, using SPSS. The values for the reference sequences had normal distributions in all cases. The concordance

between isolates was measured by 2 different methods (genetic distance and number of differences) to ensure that there was no unexpected bias or skewing of the population average by one method. For the pair-wise distance calculation, all positions were used, the Maximum Composite Likelihood substitution model was used, and all sites were assumed to have the same rate of variation. The cut-offs used to establish statistical significance were 1.65 SD below the mean ($p = 0.05$) and 2.3 SD below the mean ($p = 0.01$) in a single-tailed analysis. A single tailed (left side) analysis was employed because we wished to determine whether the partner sequences were significantly more related to each other than to random HCV sequences.

We estimated the minimal divergence time needed to achieve the inter-pair nucleotide differences. These calculations are based on a nucleotide fixation rate for NS5 of 1.45×10^{-3} mutations per site per year (12, 13). When this rate was applied to both viruses from the partners, the divergence rate was 2.6 nucleotide positions per year for the target region analyzed. This rate provided minimal time estimates and was used only to establish a plausibility window for transmission.

Projected Sample Size and Other Statistical Analyses

Prior to study initiation, widths of confidence intervals around a prevalence estimate were calculated for a range of sample sizes. Given a 2-5% prevalence of HCV infection in sexual partners, the widths of confidence intervals would be between 1% and 2% for a projected sample size of 1000 couples and between 4% and 7% for a projected sample size of 300 couples.

Demographic characteristics and risk factors for HCV infection were summarized with frequency distributions (categorical variables) and medians and ranges (continuous variables) separately

for anti-HCV positive index persons and their partners. Data on sexual practices between partners are presented at the couple level. Duration of the sexual relationship was defined as the number of years between first sexual contact with the study partner and study enrollment, minus time intervals (in years) where sexual contact was absent within the couple. For example, a couple reporting a relationship start year of 1991, study enrollment in 2001 and no sexual activity between 1998 and 1999 would be assigned 9 years for the duration of the sexual relationship. To estimate the total number of sexual contacts for each relationship, the number of sexual contacts per month for each discrete time interval in their sexual history was multiplied by the duration of the time interval and summed over all intervals in the relationship. Changes in sexual activities over time were determined by comparing the types and frequencies of practices in the first year of the relationship relative to the year immediately prior to study enrollment (current year of sexual activity). Each partner independently reported time periods of sexual activity and the number of contacts per month during that time period. Since the time periods reported by each partner might not match perfectly, we calculated the number of contacts per time period per partner and summed the estimated number of contacts per each partner over the duration of the relationship. The average of the total number of contacts reported by partner 1 and partner 2 was used as the total number of contacts for the couple.

Prevalence of anti-HCV positivity and 95% confidence intervals were calculated for the partners of index persons. Incidence of sexually acquired HCV infection was estimated per number of sexual contacts (vaginal intercourse with and without menses and anal intercourse). Incidence density of HCV infection was calculated as the number of potential transmission events per total person-years of sexual relationship reported among partners. Duration of the sexual relationship was summed among the 500 partners to determine the total person-years of observation.

RESULTS

Eligible and Enrolled Couples

Of the 2077 couples screened for study inclusion, 672 (32%) were eligible. Reasons for study exclusion occurring in $\geq 5\%$ of the 1405 ineligible couples included lack of sexual activity (31%), prior organ transplant (12%), refused study participation (11%), doctor refused (8%), HIV or hepatitis B virus coinfection (8%), partnership less than 3 years or non-monogamous (6%), and history of IDU in both partners (6%). Of the 672 eligible couples, 500 (74%) enrolled and completed all the study requirements at which time study enrollment was halted. The primary reasons for failure to participate among the remaining 172 eligible couples were non-response (54%) or refusal (29%). Of the 500 enrolled couples, 43% were referred from tertiary referral practices, 34% from community sources, and 21% were blood donors.

Characteristics of Participating Couples

The 500 couples were predominantly non-Hispanic White race, educated, employed, and native-born (**Table 1**). The median duration of the couples' sexual relationships was 15 years (range 2 to 52). The most frequently reported risk factors for HCV infection among index persons were IDU (53.8%) and blood transfusion before 1992 (31.6%); these risks were infrequently reported by partners. Twenty or more lifetime sex partners prior to the current relationship were reported by 46.2% of index persons and 26.8% of partners.

The median number of sexual contacts per month was highest for vaginal intercourse during the first year of the relationship (12 contacts per month) (**Table 2**). The frequency of sexual contacts decreased over time for all types of sexual activity. Vaginal intercourse during menses and anal intercourse (≥ 1 occasion) were reported by 65.2% and 30.4% of couples, respectively. Condom use during vaginal intercourse was reported by 29.9% of couples and condom use decreased over time for vaginal and anal intercourse.

HCV Sequencing and Phylogenetic Analyses of HCV Strains

Among the 500 partners of anti-HCV positive index persons, 20 were confirmed anti-HCV positive and 13 of the 20 partners were HCV RNA positive. HCV genotyping/subtyping and HCV serotyping confirmed 9 couples to be concordant, 8 to be discordant and 3 couples to be of indeterminant status (**Table 3**).

Of the 9 genotype concordant couples, both partners of 6 couples were viremic, allowing phylogenetic analyses; 3 had strong evidence that the partners were infected with the same HCV isolate and 3 were consistent with infection by different HCV strains (**Table 4**). Couple 15 had HCV 1a strains that were more similar to each other than 99% of random pairings of HCV sequences of subtype 1a. Both partners of Couple 17 were infected with both HCV 1a and 1b strains, and their 1b strains were more similar to each other than 99% of random pairings of HCV 1b sequences, however, their 1a strains were no more closely related than to random HCV isolates in the population. Both partners of Couple 14 were infected with HCV strains 2b and 1a. The 2b strains were highly similar, with only 1.8% difference in basepairs over a 944 basepair region analyzed, whereas, their 1a strains were no more closely related than random pairs of 1a sequences in the population. The HCV isolates in couples 9, 11 and 13 were no more similar to each other than random HCV isolates of the same subtype in the population.

Among the partners with highly-related strains (Couples 14, 15 and 17), the estimated minimum divergence time was 6.5 years for couple 14, whose sexual relationship duration was 18 years; 14.6 years for couple 15, whose sexual relationship duration was 28 years; and 6.2 years for couple 17, whose sexual relationship duration was 10.0 years. The risk factor profiles of Couple 14 revealed that the female partner had a history of IDU and the male no identifiable risk factors for HCV infection other than contact with his female partner. In Couple 17, the female partner had a history of IDU and both partners reported more than 20 prior sexual partners, a history of

sexual transmitted diseases and snorting of drugs. In Couple 15, the male partner had a history of IDU, of being stuck by a sharp bloody object while working in a hospital, and more than 20 prior sexual partners; both partners reported snorting drugs and sharing snorting equipment with each other.

Prevalence and Incidence of HCV Infection in Partners

While the overall prevalence of HCV infection among the partners of anti-HCV-positive index persons was 20/500 (4%), the prevalence of HCV infection among partners potentially attributable to sexual contact was 3/500 (0.6%, 95% CI: 0.0%, 1.3%) assuming all HCV RNA negative partners were discordant (minimum estimate) and 6/500 (1.2%, 95% CI 0.2%, 2.2%) assuming all HCV RNA negative, antibody concordant couples were concordant (maximum estimate), respectively.

Based upon the frequencies of sexual contact and length of relationships reported, a cumulative 8,377 person-years of risk for acquiring HCV by sexual activity was calculated. With 3 viremic confirmed concordant couples and 3 possible concordant couples, the estimated incidence of HCV infection among partners ranged from 3.6 per 10,000 person-years (95% CI: 0.0, 7.7) (minimum estimate) to 7.2 per 10,000 person-years (95% CI: 1.3, 13.0) (maximum estimate). The estimated risk per sexual contact ranged from 1 per 380,000 (95% CI 1/600,000, 1/280,000) to 1 per 190,000 (95% CI 1/1.03 mil, 1/100,000).

Concordantly-infected couples were no more likely to share blood-contaminated objects, such as nail clippers, razors, and toothbrushes, than couples in which one partner remained uninfected (0.0% vs. 10.1%, $p=1.00$), but were more likely to have vaginal intercourse during menses (100.0% vs. 65.6%, $p= 0.55$) and anal intercourse (66.7% vs. 30.2%, $p=0.22$), and less

likely to use condoms (0.0% vs. 30.4%, $p=0.56$). These differences, however, were not statistically significant.

DISCUSSION:

Sexual transmission of HCV among monogamous heterosexual couples is an extremely infrequent event. The maximum prevalence of HCV infection among sexual partners of persons with chronic HCV infection was only 1.2%, and the maximum incidence of HCV transmission by sex was 0.07% per year or ~1 per 190,000 sexual contacts. Condom use was infrequent among the study participants and decreased over the duration of the sexual relationship, indicating that the very low rate of sexual transmission in our study population was not due to use of barrier methods during sexual activity.

This estimate includes couples who were antibody concordant by serotyping assays but without confirmation of HCV strain relatedness by phylogenetic analysis because at least one of the partners was HCV RNA negative. By including these couples, we minimized selection bias, but since couples with the same genotype/serotypes may not be infected with the same strain of HCV, we provided maximum (including aviremic serotype concordant couples) and minimum (based on viremic couples only) estimates of HCV prevalence and incidence. The minimum estimate of prevalence of HCV infection among viremic couples was 0.6% (95% CI: 0.0%, 1.3%) and the incidence was 0.04% per year.

Sexual transmission of HCV presumably occurs when infected serum-derived body fluids are exchanged across mucosal surfaces. Potential factors that may influence this exchange include the titer of virus, the integrity of the mucosal surfaces, and the presence of other genital infections (viral or bacterial). Studies to detect HCV RNA in semen (seminal fluid and cells), vaginal secretions, cervical smears, and saliva have yielded mixed results (14-20). Failure to

detect HCV RNA in body secretions from chronically infected persons may be due to technical factors including specimen collection and storage, and the inability to exclude cellular components and to overcome the presence of polymerase inhibitors in body fluids. Even in studies employing optimal methods to detect HCV RNA, the minority of samples were positive for HCV RNA and all positive samples were of low titer ($\leq 10^2$ IU/mL) (19-20). A low titer of virus in genital secretions may be one reason that HCV is transmitted less efficiently than hepatitis B virus or HIV (21-22). Additionally, transmission of infection by sex may require a specific genital tract environment such as disrupted mucosal integrity or the presence of viral or bacterial coinfections. These factors may explain the recent reports of HCV transmission by sex in HIV-infected men who have sex with men (23).

Epidemiologically, specific factors that facilitate sexual transmission of HCV have not been identified, although most studies were not large enough to do so. Our study is the largest conducted in the United States and the first to include a rigorous assessment of sexual practices, none of which were associated with concordant HCV-positivity in couples. Although a considerably larger sample size might yield different results, the very low estimated overall transmission risk indicates that any risk for infection from engaging in specific "high-risk" practices would be very low. Thus, this study supports the current recommendations that persons with HCV infection in long-term monogamous relationships need not change their sexual practices (2). Prospective studies from other countries of monogamous couples provide additional support for this recommendation (5, 6). An Italian study of 775 HCV-negative partners followed for 10 years average, identified new HCV infection in 3 partners but none of these partners had viral strains related to those in the HCV-infected partner, indicating an outside source of infection rather than possible sexual transmission (6). However, this study excluded 33 partners who were infected at baseline, introducing a potential bias into the study. It is possible that that risk period of HCV acquisition by sexual contact may be early in the

relationship and exclusion of infected partners in long-term relationships, excludes those partners at greatest risk. In contrast to the Italian study, we chose to include all anti-HCV positive partners and rely about the phylogenetic analysis and detailed risk histories to estimate likelihood of sexual transmission. The ideal prospective study to assess risk of HCV transmission among monogamous couples would target HCV-negative partners initiating a sexual relationship with an HCV-infected individual, but such a study would be extremely difficult to execute.

Interestingly, in two couples (Couples 14 and 17), each of the partners had evidence of HCV superinfection with only one of the strains phylogenetically similar in both partners. In Couple 14, it seems likely that the related strain was transmitted from the partner with a history of IDU to the partner who reported no risk factors for HCV infection other than contact with the infected partner. However, the origin of the unrelated HCV strain in the partner with no other HCV-related risk factors is unexplained. In Couple 17, the index person and partner both had different risk factors for HCV, which could explain why each member of the couple was infected with different HCV strains while they also shared a similar strain that was likely transmitted from one partner to the other. These cases highlight the complexity of using phylogenetic analysis to determine the direction or mode of transmission in individual situations when events occurred at unknown times in the past.

Among the 12 couples that had concordant (or indeterminate) HCV genotypes or serotypes, 50% were HCV RNA negative. This rate of spontaneous clearance is similar to that observed among persons infected at younger (<30 years) ages (by transfusion of whole blood, receipt of contaminated Rh immune globulin, IDU, or accidental needlestick injuries), and prospectively followed for 20 years (24-26). Although a younger age at infection might explain the high proportion of anti-HCV-positive, HCV RNA-negative partners in our study, one might speculate

that repeated exposures to small “doses” of HCV resulted in an immunization-like effect or facilitated viral clearance once infection occurred.

We acknowledge that we have not genetically “proven” transmission among the phylogenetically linked partners, but rather have presented strong evidence for such a transmission. The methods we used are much more effective for excluding possible transmission than it is for confirming it.

The consensus sequence of the virus is heavily dominated by a handful of dominant quasispecies, and it drifts relatively slowly. If the genetic distance is not significantly more similar between the pairs than to the rest of the population, then there is no realistic chance the dominant strains came from the same source. Proving (or providing strong evidence for)

infection with HCV from a common source is difficult for several reasons. 1. HCV passes a bottleneck upon infection (it has been estimated that only a dozen to <100 infectious particles initiate an infection, and these may not be randomly sampled from the donor quasispecies).

Therefore, it is possible even with deep sequencing that finding identical quasispecies variants shortly after infection may not be possible. 2. HCV rapidly adapts to a new host over the first 1-2 months of infection, leading to a burst of diversity and genetic drift. During the rapid expansion in a new host there is little constraining adaptive immunity, and consequently novel variants are not selected out as rapidly as in an established infection, and immune escape variants that were selected in the donor often revert to a more-fit sequence. 3. HCV's mutation rate is far higher than its fixation rate (i.e., the number apparent from population sequencing like we did). Therefore, at a quasispecies level, the viral sequence is essentially "shimmering" from the combined effects of random mutation and its opponent, negative selection. This mandates a rather careful genetic analysis to prove common-source infection, and this problem rapidly increases with time since infection. Finally, deep sequencing is quite error prone and consequently rather extensive statistical treatment of the data are required to be sure that rare variants actually exist in a sample. This means that even if identical reads are reported in 2

paired samples, one or both of them could easily be a sequencing error. The integration of these issues is that it would be perfectly possible by a careful quasispecies analysis or a deep sequencing analysis to prove an identical source for 2 infections shortly after transmission, but the ability to prove a common source decays relatively quickly with time and is difficult in situations where the transmission occurred many years in the past.

Limitations of this study include its cross-sectional nature. A prospective cohort would be the ideal study design to determine incident HCV infections among uninfected partners but the logistics and cost of undertaking such a longitudinal study are daunting, given the low incidence of infection. Unlike prior studies, we sought to overcome the limitations of the cross-sectional design by obtaining a detailed relationship history of sexual practices utilizing techniques similar to those used to obtain lifetime alcohol use histories. Since the partner's HCV status was unknown in the majority of cases prior to history-taking, there would be minimal effect of differential bias in recall of sexual or other shared practices. Regardless, some participants may have unacknowledged histories of IDU or other sensitive risk factors, a limitation we tried to minimize by screening each participant on multiple occasions. Recall bias is a potential limitation with any cross-sectional study, but we found no difference in completeness of the sexual histories among HCV-positive versus negative couples. Another potential limitation was the sample size and the small number of positive partners for stratified analysis. Finally, the study population may not be representative. While index persons were similar in age and gender distribution to HCV-positive adults identified in the general population (1), the study population was predominantly Non-Hispanic White and the majority had an education level beyond high school.

In summary, we conclude that HCV transmission by sex from chronically infected persons to their heterosexual partners in a long-term monogamous relationship likely occurs but is a rare

event. Our results provide a basis for specific counseling messages that clinicians can use with their patients. These messages should be qualified given the limitations of the sample size, but they support the current national recommendations that couples not change their sexual practices if they are in a monogamous heterosexual relationship.

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Table 1: Demographic Characteristics and Selected Risk Factors Reported by Anti-HCV-Positive Index Persons and Their Partners

	Anti-HCV-positive Index Persons		Sexual Partners of Index Persons	
	N=500	%	N=500	%
Duration of Sexual Relationship (median, range) yrs	15	2-52	N/A*	
Age (median, range) yrs	49	(26-79)	48	(21-80)
Male Sex	306	61.2%	194	38.8%
Race				
White	363	72.6%	381	76.2%
Black	26	5.2%	23	4.6%
Asian	12	2.4%	10	2.0%
Other	97	19.4%	84	16.8%
Ethnicity				
Hispanic	73	14.6%	65	13.0%
non-Hispanic	423	84.6%	434	86.8%
Education				
<12 years of education	34	6.8%	31	6.2%
High school graduate	99	19.8%	98	19.6%
College or technical training	367	73.4%	371	74.2%
Total Annual Household Income				
≤\$15,000	35	7.0%	N/A*	
\$15,001-\$30,000	60	12.0%		
\$30,001-\$50,000	109	21.8%		
\$50,001-\$100,000	178	35.6%		
> \$100,000	105	21.0%		
Country of Birth				
United States	458	91.6%	446	89.2%
Other	41	8.2%	53	10.6%
Lifetime Risk Factors				
Received blood transfusion before 1992	158	31.6%	55	11.0%
Ever stuck by sharp bloody object in	22	4.4%	10	2.0%

hospital job				
Ever injected illegal drugs	269	53.8%	12	2.4%
Number of lifetime opposite sex partners				
1	33	6.6%	48	9.6%
2-9	120	24.0%	185	37.0%
10-19	100	20.0%	118	23.6%
20-49	125	25.0%	89	17.8%
≥50	106	21.2%	45	9.0%

* N/A = not applicable, same as index person.

Table 2: Type and Frequency of Sexual Practices Among Couples According to Year of Relationship

Type and Frequency of Sexual Practice	Ever (%) N=500	First Year of Relationship	Current Year of Relationship
<i>Vaginal intercourse</i>	499 (99.8%)	499 (99.8%)	474 (94.8%)
Contacts per month (median, range)*		12 (0-300)	4 (0-60)
Ever condom use (n, %)		149 (29.9%)	109 (23.0%)
Frequent condom use (n, %)		84 (16.8%)	67 (13.4%)
<i>Vaginal intercourse during menses</i>	326 (65.2%)	297 (59.4%)	134 (26.8%)
Contacts per month (median, range)*		0 (0-10)	0 (0-4)
Frequent condom use (n, %)		45 (15.2%)	21 (15.7%)
<i>Anal intercourse</i>	152 (30.4%)	107 (21.4%)	68 (13.6%)
Contacts per month (median, range)*		0 (0-20)	0 (0-15)
Frequent condom use (n, %)		14 (13.1%)	5 (7.4%)
<i>Male receptive oral sex</i>	456 (91.2%)	428 (85.6)	370 (74.0%)
Contacts per month (median, range)*		3 (0-100)	<1 (0-100)
Frequent condom use (n, %)		6 (1.4%)	7 (1.9%)
<i>Female receptive oral sex</i>	462 (92.4%)	435 (87.0%)	374 (74.8)
Contacts per month (median, range)*		3 (0-100)	<1 (0-100)
Frequent barrier (dental dam) use (n, %)		2 (0.5%)	3 (0.8%)

* Summation of individual level data

Table 3: Serologic and Virologic Results in Confirmed Anti-HCV Positive Couples

** Couples determined to be highly related viral strains based on phylogenetic analyses are indicated in bold.*

Couple ID	Index Person Genotype	Index Gender	Partner Genotype	Partner Gender	Partner Serotype	Designation Based on Genotype* or Serotype
1	4a	F	Indeterminate	M	1	Discordant Couples
2	2a	F	1b	M		
3	1a	F	2b	M		
4	1b	F	1a	M		
5	1b	F	1a	M		
6	1a	F	1b	M		
7	3a	M	HCV RNA negative	F	1	
16	1a	F	1b	M		Concordant Couples
8	1a	F	HCV RNA negative	M	1	
9	1a	M	1a	F		
10	1a	M	HCV RNA negative	F	1	
11	1b	F	1b	M		
12	1a	M	HCV RNA negative	F	1	
13	1b	F	1b	M		
14	2b/1a	F	2b/1a	M		
15	1a	F	1a	M		
17	1a/1b	F	1a/1b	M		
18	Unavailable	M	HCV RNA negative	F	1	Unknown Status
19	1a	F	HCV RNA negative	M	Indeterminate	
20	1a	M	HCV RNA negative	F	1 or 3	

*Genotyping performed with InnoLipa with confirmation by sequencing for all genotype 1 samples.

Table 4: Phylogenetic Evaluation of Viremic, Genotype-Concordant Couples (N=6)

Couple ID (genotype)	Pairwise Distance between Sequences ¹	P Value	# Basepair Differences between Partners ²	P Value	Estimated Minimal Divergence Time ³
Couple 9 (1a)	0.059	p > 0.05	50	p > 0.05	19.2
Couple 11 (1b)	0.071	p > 0.05	59	p > 0.05	22.3
Couple 13 (1b)	0.069	p > 0.05	59	p > 0.05	22.3
Couple 14 (1a)	0.062	p > 0.05	53	p > 0.01	20.4
Couple 14* (2b)	0.018	p < 0.01*	17	p < 0.01*	6.5
Couple 15 (1a)	0.044	p < 0.01	38	p < 0.01	14.6
Couple 17 (1a)	0.076	p > 0.05	64	p > 0.05	24.6
Couple 17 (1b)	0.018	p < 0.01	16	p < 0.01	6.2

¹In the reference sets for subtypes 1a and 1b, the mean pairwise distance was 0.618 ± 0.0006 (SD) (<1.65 SD of mean = 0.052) for subtype 1a and 0.071 ± 0.007 (<1.54 SD of mean = 0.059) for subtype 1b.

²In the reference sets for subtypes 1a and 1b, the mean number of basepair differences was 52.33 ± 5.33 (<1.65 SD = 43.5) for 1a and was 59.53 ± 5.79 (<1.65 SD = 46.2) for 1b sequences.

³Minimal divergence time based on estimated divergence rate of 2.6 nucleotide positions per year for the target region analyzed.

*Significance was calculated relative to the subtype 1a distribution because an adequate background distribution could not be established with the publicly-available 2b sequences; these values are also significant when compared to the 1a distribution.

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