ORIGINAL ARTICLE

TJG
The Turkish Journal of Gastroenterology

Risk factors for higher anti-HCV positivity in a border city in southern Turkey with unique population characteristics

Birol ÖZER[1], Gülşah SEYDAOĞLU[2], Akatlı Kürşat ÖZŞAHİN[3], Hakan DEMİRHİNDİ[4]

Departments of [1]Gastroenterology and [3]Family Medicine, Başkent University, Adana
Departments of [2]Biostatistics and [4]Public Health, Çukurova University School of Medicine, Adana

Background/aims: *This study was conducted as a seroprevalence study on hepatitis C virus infection in a small city located in southern Anatolia, to estimate the prevalence of hepatitis C virus and to explore the potential risk factors for hepatitis C virus infection at this population level.* ***Material and Methods:*** *A total of 1427 (685 male (48%), 742 female) subjects agreed to participate in the study. Risk factors were examined using a questionnaire. All blood samples were tested using third-generation anti-hepatitis C virus enzyme-linked immunosorbent assays.* ***Results:*** *The overall anti-hepatitis C virus prevalence was 3.1% (44/1427). There was a steady rise in the prevalence of anti-hepatitis C virus positivity with age; the anti-hepatitis C virus prevalence was slightly higher in men (3.6%) than women (2.6%). The prevalence of anti-hepatitis C virus positivity was significantly higher in primary school graduates (3.4%) (odds ratio [OR]: 4.1, 95% confidence interval [CI]: 1.5-11.6, p=0.0001) and in illiterate subjects (5.0%) (OR: 2.7, 95% CI: 1.1-7.4, p=0.021) compared to secondary-plus graduates. Anti-hepatitis C virus positivity was higher (3.7%) in married subjects (OR: 8.7, 95% CI: 1.2-63.7, p=0.003) compared to single subjects (0.0%). Having dental procedure, delivery at home, provocative abortion, working abroad, hypertension, and diabetes mellitus were factors found to increase the anti-hepatitis C virus positivity significantly.* ***Conclusions:*** *In the region of the current study, the anti-hepatitis C virus seroprevalence was higher compared to the whole country. Illiteracy, previous dental procedures, and working abroad in neighboring countries seem to be factors that relate to this high ratio.*

Key words: Seroprevalence, hepatitis C virus, risk factors

Antakya yöresinde yüksek anti-HCV seroprevalansı ve risk faktörleri

Amaç: *Bu çalışma güney Anadolu'nun bir sınır şehrinde anti-hepatit C virus seroprevalansını saptamak, ikincil olarak da bu popülasyondaki potansiyel risk faktörlerini belirlemek amacıyla yapılmıştır.* ***Gereç ve Yöntem:*** *Çalışmaya 685 erkek, 742 kadın toplam 1427 birey alındı. Risk faktörleri bir anket formu ile sorgulandı. Tüm kan örnekleri anti-hepatit C virus için 3. jenerasyon enzyme-linked immunosorbent assays yöntemi ile analiz edildi.* ***Bulgular:*** *Toplam anti-hepatit C virus prevalansı 3.1% (44/1427) saptandı. Erkeklerde anti-hepatit C virus pozitifliği (3.6%) kadınlara göre daha fazla (2.6%) idi ve prevalans yaşla birlikte artmaktaydı. Anti-hepatit C virus prevalansı ilkokul mezunları (3.4%) (OR: 4.1, 95% CI: 1.5-11.6, p= 0.0001) ve eğitimsizlerde (5.0%) (OR:2.7, 95% CI: 1.1-7.4, p=0.021) ortaokul ve üstü eğitimlilere göre anlamlı ölçüde daha yüksekti. Anti-hepatit C virus pozitifliği evlilerde (3.7%) bekarlara (0.0%) göre daha fazla idi (OR:8.7, 95% CI: 1.2-63.7, p=0.003). Diş müdahalesi, evde doğum, provakatif düşük, yurt dışında çalışma, hipertansiyon, diabetes mellitus öyküsüne sahip olmak artmış anti-hepatit C virus pozitifliği için anlamlı bulundu.* ***Sonuç:*** *Çalışmanın yapıldığı bölgede anti-hepatit C virus seroprevalansı ülke geneli ile karşılaştırıldığında daha yüksekti. Eğitimsiz, öncesinde diş müdahalesi ve komşu ülkelerde çalışmış olmak bu yüksek oranda etkili faktörler olarak gözükmektedir.*

Anahtar kelimeler: Seroprevalans, hepatit C virus, risk faktörleri

INTRODUCTION

Hepatitis C virus (HCV) is a positive strand RNA virus, which is related to the flavivirus family. Choo and his colleagues (1) first characterized HCV in 1989. HCV was the most common cause of

Address for correspondence: Birol ÖZER
Başkent University, School of Medicine, Department of Gastroenterology,
Adana, Turkey
E-mail: birolozer@yahoo.com

Manuscript received: 20.03.2012 **Accepted:** 17.06.2012

Turk J Gastroenterol 2012; 23 (5): 574-579
doi: 10.4318/tjg.2012.0554

post-transfusion hepatitis before the screening of all blood donors for HCV (2). According to the World Health Organization (WHO) estimates, approximately 3% of the world population, or about 170 million people, may be infected with HCV (3,4). In the United States (US), antibodies to HCV are encountered in 0.1%-1.8% of the general population. However, in different regions of the world, the incidence of HCV infection both in the general population and blood donors may be much higher (5). In the general population, the prevalence of HCV in Turkey varies between 0% and 3.9% (6). HCV infections lead to acute hepatitis in 20% of cases and chronic hepatitis in 50% of cases, 20% of whom develop cirrhosis (7). There is also a strong relationship between HCV and hepatocellular carcinoma (8). The parenteral route is the most efficient route of HCV transmission and has been well studied and documented; however, many individuals with HCV infection report no blood or blood product contact, needle sharing, dental or surgery procedure, or other known risk factors (9). Therefore, a number of other routes of transmission, such as sexual or household exposure to an infected contact, are postulated but not widely accepted, since conflicting data have emerged regarding the presence of HCV in body fluids other than blood. However, some authors have found total absence of the virus in sperm, saliva, vaginal secretions, and other body fluids (10,11).

We conducted a seroepidemiological study on HCV infection in a small city located in southern Anatolia, firstly, to estimate the prevalence of HCV, and secondly, to explore the potential risk factors for HCV infection at this population level.

MATERIALS AND METHODS

Antakya is a small border city in southern Anatolia. This city is quite unique in the country in that many people living in the region have relatives and strong sociocultural and commercial relations with the neighboring countries. This city has 96,143 residents aged 20 years or older. A total of 1427 (685 male, 742 female) subjects were included in this study. These subjects were chosen from the registry of primary care health centers of three different town centers and their villages in the Antakya district and the city center. All families living in the city, towns or villages had been registered by these health centers using a basic form called the Household Health Registry Form (HHRF). Volunteers who accepted to participate in the study and gave their written informed consent were enrolled. Ten ml of blood was drawn from each volunteer. Blood drawn from the volunteers was centrifuged at 3500 round per minute (min) for 5 min. The blood sample was deep-frozen at -70°C. The sample was then defrosted, mixed and centrifuged at 10,000 g relative centrifugal force (RCF) for 10 min.

Anti-HCV antibody was analyzed twice by a third-generation microparticle enzyme immunoassay (MEIA) containing HCV antigens from the viral core and from areas of the nonstructural NS3, NS4 and NS5 regions (Abbott Laboratories, Abbott Park, IL, USA).

The study protocol was reviewed and approved by the health administrator and also by the governor of Antakya. The protocol was approved by the human research ethics committee of the university. A structured interview was conducted by two trained interviewers to collect information about sociodemographic data, age, education (illiterate, primary school graduate, and secondary school graduate-plus), marital status, a previous history of drug injection ever, hepatitis ever, transfusion ever, previous surgical procedure, previous dental procedure, alcohol consumption, sexual risk factors, and pregnancy and delivery status (spontaneous delivery, section or abortus).

Statistical Analyses

A sample size was calculated with an expected parameter estimate based on a previous study performed in this region (12). Assuming a 3.9% ratio of HCV with a ±1% error, the minimum sample size thus required was approximately 1440 in the study group within a 95% confidence and 80% power. The sample size was calculated with *Epi-Info*. The categorical variables between the groups were analyzed by using the chi-square test or Fisher's exact test. Odds Ratio (OR) and 95% Confidence Interval (CI) were calculated. A p value <0.05 was considered as significant. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) v 18.0. Results were presented as n (%) and mean±SD.

RESULTS

The overall anti-HCV prevalence was 3.1% (44/1427). The sample mean age was 39.6±13.3 years (50.9±8.9 for anti-HCV (+) group and 39.2±13.4 for anti-HCV (-) group). Demographic characteristics of the population are shown in Table 1. The age range of the study population was 20-75 years, with 50.7% aged 20-39 years,

41.8% aged 40-59 years, and 7.5% aged over 60 years. Of the whole patient population, 83.8% were married, 22.4% were illiterate, 45.2% were graduated from primary school, and 32.4% were graduated from secondary school or higher.

Table 1. Demographic features of the study group

Variable name	Number	Percentage
Sex		
Male	685	48
Female	742	52
Age		
<30	372	26.1
30-39	351	24.6
40-49	321	22.5
50-59	276	19.3
≥60	107	7.5
Education		
Illiterate	319	22.4
Primary	646	45.2
Secondary plus	462	32.4
Birth Place		
Urban	418	29.3
Rural	1009	70.7
Marital Status		
Married	1197	83.9
Single	230	16.1
Total	1427	100

Distribution of the population in the Antakya region and the frequencies of HCV positivity in the study sample by sex and age groups are shown in Table 2. There was a steady rise in the prevalence of anti-HCV positivity with age.

Risk factors associated with HCV seropositivity are shown in Table 3. The anti-HCV prevalence was slightly higher in men (3.6%) than women (2.6%). The prevalence of anti-HCV positivity was significantly higher in subjects who were primary school graduates (3.4%) (OR: 4.1, 95% CI: 1.5-11.6, p=0.0001) and in subjects who were illiterate (5.0%) (OR: 2.7, 95% CI: 1.1-7.4, p=0.021) compared to subjects who were secondary school graduates-plus. The prevalence was 3.7% in married subjects (OR: 8.7, 95% CI: 1.2-63.7, p=0.003) compared to single subjects (0%). Having dental procedure, delivery at home, provocative abortion, partner living abroad, hypertension, and diabetes mellitus were all found to increase the anti-HCV positivity significantly. However, multivariate analysis showed that none of the variables found to be statistically significant in univariate analysis was an independent risk factor for HCV positivity.

Table 2. Demographic features of the population and relation of anti-HCV positivity in study sample by sex and age groups in Antakya region

Sex	Age groups	Population n	Sample n	HCV (+) n	HCV (+) %
Male					
	<30	21807	172	0	0
	30-39	11762	163	0	0
	40-49	8224	149	9	6.0
	50-59	4330	148	10	6.8
	≥60	4462	53	6	11.3
	Total	50585	685	25	3.6
Female					
	<30	18498	200	0	0
	30-39	10412	188	5	2.7
	40-49	8121	172	5	2.9
	50-59	4156	128	7	5.5
	≥60	4371	54	2	3.7
	Total	45558	742	19	2.6
Total					
	<30	40305	372	0	0
	30-39	22174	351	5	1.4
	40-49	16345	321	14	4.4
	50-59	8486	276	17	6.2
	≥60	8833	107	8	7.5
	Total	96143	1427	44	3.1

Table 3. Univariate analysis of risk factors for anti-HCV positivity

	HCV		
	Positive n (%)	**Negative n**	**OR (95% CI), p**
Sex			
Male	25 (3.6)	660	1.4 (0.8-2.6) 0.234
Female	19 (2.6)	723	
Birth place			
Urban	8 (1.9)	410	0.5 (0.2-1.1) 0.100
Rural	36 (3.6)	973	
Education			
Illiterate	16 (5.0)	303	4.1 (1.5-11.6) 0.0001
Primary (5 years)	22 (3.4)	624	2.7 (1.1-7.4) 0.021
Secondary-plus	6 (1.3)	456	Ref.
Surgery			
Yes	19 (2.3)	818	1.9 (1.1-3.5) 0.034
No	25 (4.2)	565	
Dental procedure			
No	0 (0.0)	276	10.9 (1.5-80.6) 0.001
Yes	44 (3.8)	1107	
Hospitalization			
No	19 (2.9)	626	1.1 (0.6-1.9) 0.785
Yes	25 (3.2)	757	
Marital Status			
Married	44 (3.7)	1153	8.7 (1.2-63.7) 0.003
Single	0 (0.0)	230	
Pregnancy			
Absent	1 (0.7)	139	Ref.
1-2	5 (1.6)	300	2.3 (0.3-52.5) 0.737
3+	13 (4.4)	284	6.3 (0.9-130.5) 0.084
Delivery			
Hospital	4 (1.6)	251	Ref.
In-house or hospital	6 (3.2)	183	2.1 (0.6-7.4) 0.259
In-house	8 (5.1)	150	3.5 (1.1-7.4) 0.032
Abortion			
Absent	7 (1.8)	394	Ref.
Spontaneous	6 (2.3)	252	1.3 (0.4-4.5) 0.876
Provocative	6 (7.2)	77	4.7 (1.3-14.9) 0.014
Section			
No	17 (2.6)	627	0.8 (0.2-3.3) 0.725
Yes	2 (2.0)	96	
Working Abroad			
No	25 (2.3)	1073	2.6 (1.4-4.8) 0.001
Yes	19 (5.8)	310	
Partner Living Abroad			
No	7 (1.7)	395	3.4 (1.3-8.8) 0.007
Yes	12 (5.7)	197	
Hypertension			
No	29 (2.3)	1232	4.2 (2.2-8.0) 0.0001
Yes	15 (9.0)	151	
Diabetes Mellitus			
No	36 (2.7)	1300	3.4 (1.6-7.7) 0.001
Yes	8 (8.8)	83	
Alcohol			
Non-user	41 (3.2)	1227	0.5 (0.2-1.7) 0.295
User	3 (1.8)	156	
Smoking			
No	37 (3.4)	1058	0.6 (0.3-1.3) 0.241
Yes	7 (2.1)	325	

DISCUSSION

These data show that HCV infection is highly prevalent in this area of southern Anatolia. Healthy individuals have an extremely variable HCV infection prevalence rate worldwide (13,14). While most countries have prevalence rates from 1%-2%, several countries have relatively high prevalence rates, including Egypt (15%), Pakistan (4.7%) and Saudi Arabia (5.6%) (15). Anti-HCV seroprevalence of narrow groups in different parts of Turkey was shown to range between 0% and 3.9% (6). This ratio was found to be 1% in the TURKHEP study that was executed in the whole country (16). Total HCV seroprevalence was determined as 3.1% in the province in which we worked. This ratio was higher than the overall country rate. The citizens of the province generally worked or visited neighboring countries such as Syria, Saudi Arabia and Egypt, where these people had kinship, together with historical and sociocultural relations. In Syria, there are no published data on HCV seroprevalence in the general population, but a positivity of 60.5% for intravenous drug users, 3% for health workers, and 6% for hemodialysis workers was reported (17,18). There are also no sufficient data on general HCV prevalence in Saudi Arabia, but high values like 5.8% were reported (19). Egypt perhaps has one of the highest HCV prevalences at approximately 15%. Prevalence increased with age, with the highest value being 39.4% between 55-59 years (20). Higher anti-HCV positivity in subjects who had visited a neighboring country than in those who had not (5.8% vs. 2.3%) was probably due to the high anti-HCV positivity in these countries.

The rate of acquiring HCV from the community is 15x100000 per person per year in the US and the route of transmission can be determined in only 60% (21). Among some potential exposures, nonparenteral drug use, sexually transmitted diseases, and low socioeconomic level can be considered (22,23). Twenty-eight percent of HCV- positive individuals were reported to have low socioeconomic level (23). In fact, in our study, 81% of subjects with anti-HCV positivity had rural origin and 36% of them were illiterate.

In our study, HCV infection increased with age, as supported by previous studies (24,25). This rate reached 6.2% in subjects between 50-59 years of age and 7.5% in subjects older than 60 years of age. This finding may reflect the improvement in health care and sanitation with improved socioeconomic level. Recently, the quality of health services has improved and novel therapeutic drugs have been accessible. Until a few previous decades, antibiotics and analgesics were administered to patients mostly through the parenteral route. During such therapies, non-disposable injectors were often used. Use of non-disposable injection equipment may increase the rate of HCV infections (26,27). In fact, all 44 subjects who were anti-HCV-positive in our study had a history of a dental procedure. In the Antakya region, dental therapies have been performed generally by dental technicians and not by medical dentists. Considering the common use of non-disposable injectors and poor sterilization conditions in previous decades, dental procedures might have played a crucial role in transmission of the disease. Similarly, in the current study population, 18 anti-HCV-positive women out of 19 had a history of childbearing, which may support a parenteral transmission. However, the age of the subject also increased as the number of deliveries increased in the population group. Additionally, 43.1% of anti-HCV- positive subjects had a history of a surgical intervention. The low rate of transmission in surgical interventions over dental procedures might be explained by the better sterilization conditions in hospitals. The prevalence of anti-HCV was higher in men. This might be a result of the men working abroad. All the anti-HCV-positive individuals in our study were married. Prevalence of anti-HCV positivity was also higher in patients with diabetes and hypertension. The probable explanation for higher prevalences in these groups is that these patients may require health care more frequently and that some patients with diabetes need everyday subcutaneous injection of insulin.

The prevalence of anti-HCV among the sexual and household contacts of chronic hepatitis C patients ranged from 0% to 27% (5,28). By using third-generation enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) techniques, they found the incidence of anti-HCV to be 7.3% (15.6% spouses, 3.2% in other relatives) (29). Guadagnino et al. (30) showed that spouses who had been married to the index cases longer than 20 years had a 7.5-fold risk of HCV seropositivity as compared with those married less than 20 years. Therefore, household transmission may contribute to anti-HCV-positive cases. Having no anti-HCV-positive individual in the population under 30 years of age can be explained both by the

improved health conditions and improved precautionary measures.

Recently, some Italian researchers have shown that HCV infection presented a geographic pattern in northern Italy, and they started to investigate a nontraditional route of transmission like biologic vectors (31). Since HCV was considered a flavivirus (32), in the past, some biologic vectors might have been implicated in HCV transmission. The Antakya region was quite rich for mosquito reservoir previously. The contribution of this vector in the elderly and in rural areas for anti-HCV positivity can be questioned. However, there is not enough evidence to support the hypothesis of the potency of mosquitoes for HCV transmission (33,34).

As a result, in the region of the current study, the anti-HCV seroprevalence was higher when compared to the whole country. Higher rates of anti-HCV positivity seem to be influenced by low education status, previous dental procedures, working in the neighboring countries with very high rates of anti-HCV positivity, and chronic medical conditions such as diabetes and hypertension.

REFERENCES

1. Choo QL, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood borne non-A non-B viral hepatitis genome. Science 1989; 244: 359-62.
2. Ebeling F. Epidemiology of the hepatitis C virus. Vox Sang 1998; 74(Suppl 2): 143-6.
3. Anonymous. Hepatitis C: global prevalence. Weekly Epidemiol Record 1997; 72: 341-4.
4. Mast EE, Alter MJ, Margolis HS. Strategies to prevent and control hepatitis B and C virus infections: a global perspective. Vaccine 1999; 17: 1730-3.
5. Memon MI, Memon MA. Hepatitis C: an epidemiological review. J Viral Hepat 2002; 9: 84-100.
6. Mıstık R and Balık I. Türkiye'de viral hepatitlerin epidemiyolojik analizi. In: Tekeli E and Balık I, ed. Viral Hepatit 2003. 1st ed. Ankara: 2003; 9-55.
7. Di Bisceglie AM, Goodman ZD, Ishak KG, et al. Long-term clinical and histopathological follow-up of chronic posttransfusion hepatitis. Hepatology 1991; 14: 969-74.
8. Saito I, Miyamura T, Ohbayashi A, et al. Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. Proc Natl Acad Sci USA 1990; 87: 6547-9.
9. Van Damme P, Vellinga A. Epidemiology of hepatitis B and C in Europe. Acta Gastroenterol Belg 1998; 61: 175-82.
10. Fried MW, Shindo M, Fong TL, at al. Absence of hepatitis C viral RNA from saliva and semen of patients with chronic hepatitis C. Gastroenterology 1992; 102: 1306-8.
11. Kotwal GJ, Rustgi VK, Baroudy BM. Detection of hepatitis C virus-specific antigens in semen from non-A, non-B hepatitis patients. Dig Dis Sci 1992; 37: 641-4.
12. Delialioğlu N, Öztürk C, Aslan G. Mersin ilinde HBsAg, Anti-HBs, Anti-HCV ve anti-HDV seroprevalansı. Viral Hepatit Dergisi 2001; 7: 416-9.
13. Alter MJ. Epidemiology of hepatitis C in the West. Semin Liver Dis 1995; 15: 5-14.
14. Mansel CJ, Locarnini SA. Epidemiology of hepatitis C in the East. Semin Liver Dis 1995; 15: 15-32.
15. Sievert W, Altraif I, Razavi HA, et al. A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. Liver Int 2011; 31: 61-80.
16. Tozun N, Ozdogan OC, Cakaloglu Y, et al. Nationwide prevalence study and risk factors for hepatitis A, B, C and D infections in Turkey. Hepatology 2010; 52(Suppl 1): 697A.
17. Othman BM, Monem FS. Prevalence of hepatitis C virus antibodies among intravenous drug abusers and prostitutes in Damascus, Syria. Saudi Med J 2002; 23: 393-5.
18. Othman BM, Monem FS. Prevalence of hepatitis C virus antibodies among hemodialysis patients in Damascus, Syria. Infection 2001; 29: 262-5.
19. Fakeeh M, Zaki AM. Hepatitis C: prevalence and common genotypes among ethnic groups in Jeddah, Saudi Arabia. Am J Trop Med Hyg 1999; 61: 889-92.
20. El-Zanaty F, Way A. Egypt Demographic and Health Survey, 2008. Cairo, Egypt: Ministry of Health and Population, 2009.
21. Alter MJ. The detection, transmission and outcome of hepatitis C virus infection. Infect Agents Dis 1993; 2: 155-6.
22. Alter MJ, Hadler SC, Judson FN, et al. Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C virus infection. JAMA 1990; 264: 2231-5.
23. Alter MJ, Margolis HS, Krawczynski K, et al. The natural history of community-acquired hepatitis C in the United States. The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. N Engl J Med 1992; 327: 1899-905.
24. Calabrese G, Vagelli G, Guaschino R, et al. Transmission of anti-HCV within the household of hemodialysis patients. Letter. Lancet 1991; 338: 1466.
25. Mortimer PP, Cohen BJ, Litton PA, et al. Hepatitis C virus antibody. Lancet 1989; 30: 798.
26. Chen TZ, Wu JC, Yen FS, et al. Injection with nondisposable needles as an important route for transmission of acute community-acquired hepatitis C virus infection in Taiwan. J Med Virol 1995; 46: 247-51.
27. Chiaramonte M, Stroffolini T, Lorenzoni U, et al. Risk factors in community-acquired chronic hepatitis C virus infection: a case-control study in Italy. J Hepatol 1996; 24: 129-34.
28. Hess G, Massing A, Rossol S, et al. Hepatitis C virus and sexual transmission. Lancet 1989; 2: 987.
29. Caporaso N, Ascione A, Stroffolini T. Spread of hepatitis C virus infection within families. Investigators of an Italian multicenter group. J Viral Hepat 1998; 5: 67-72.
30. Guadagnino V, Stroffolini T, Foca A, et al. Hepatitis C virus infection in family setting. Eur J Epidemiol 1998; 14: 229-32.
31. Bellavita P, Celega E, Misiani R. Hepatitis C virus infection. An endemic area in North Italy. Vox Sang 1994; 66: 81.
32. Choo Q-L, Richman KH, Han JH, et al. Genetic organization and diversity of the hepatitis C virus. Proc Natl Acad Sci USA 1991; 88: 2451-5.
33. Silverman AL, McCray DG, Gordon SC, Walker ED. Do mosquitoes carry and transmit hepatitis C? IASL Abstracts Hepatology 1994; 19: 124.
34. Silverman AL, McCray DG, Gordon SC, et al. Experimental evidence against replication or dissemination of hepatitis C virus in mosquitoes (Diptera: Culicidae) using detection by reverse transcriptase polymerase chain reaction. J Med Entomol 1996; 33: 398-401.