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一般的名称			研究報告の公表状況	Correlates of hepatitis C virus (HCV) RNA negativity among HCV-seropositive blood donors Busch, M.P. et al, Transfusion. 46, 469 - 475 (2006)	公表国 米国	
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研究報告の概要	使用上の注意記載状況・その他参考事項等
C 型肝炎ウイルス (以下, HCV) 感染した約 20%の人はウイルス血症が消滅するが, 理由は明確ではない。一方, HCV 初感染者の大多数は無症候であるが, 数十年後に臨床症状を発症する。すなわちこれは, 供血者の中に臨床上の潜在 HCV 感染者がいるということである。本研究では, 1999 年～2001 年 12 月に NAT を実施した合衆国の 5 つの血液センターで集められた 257 万 9290 名の同種供血者における HCV 抗体と核酸増幅検査 (以下, NAT) データ, 肝酵素アラニン・アミノトランスフェラーゼ (ALT) を調査すると同時に人口学的特性 (年齢, 性別, 人種及び教育レベル) も調査した。 NAT による HCV RNA 陰性患者は, セロコンバージョンまでの期間にかかわらず, 初回供血者 (19.6%) より反復供血者 (55.9%) の方が多かった。初回供血の HCV RNA 陰性者と RNA 陽性者の ALT レベルを比較すると, ALT が 60IU/L 未満の患者の比率はそれぞれ 98.51%, 83.78%, ALT が 120IU/L 以上ではそれぞれ 0.75%, 13.38%であり, ALT レベルは初回供血の HCV RNA 陰性者の方がより正常に近い傾向を示した。また, 初回供血者の HCV RNA 陰性の比率は, アジア系, 非ヒスパニック黒人系よりも (非ヒスパニック) 白人系で有意に高かった。 さらに有意ではないが, 男性より女性のほうが NAT 陰性の比率が高かった。 なお, この調査は, HCV 罹患率・有病率の高い合衆国南東部を除いた血液センターで実施されており, NAT は個々の供血者ではなくミニプールに対して行われた。	BYL-2006-0214

報告企業の意見	今後の対応
弊社の血漿分画製剤においても, 1988 年よりミニプール及びプール血漿での HCV に対する NAT を実施しているが, 無症候セロコンバージョンが起きた供血者の血漿が使用される可能性は否定できない。しかしながら, 製造工程におけるウイルス除去工程により, 血漿分画製剤の HCV に関する安全性は確保されていると考えられる。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

Correlates of hepatitis C virus (HCV) RNA negativity among HCV-seropositive blood donors

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BACKGROUND: Approximately 20 percent of persons infected with hepatitis C virus (HCV) clear viremia. Factors associated with resolution of viremia are not well defined. Implementation of routine nucleic acid testing (NAT) of blood donors has yielded a large data set for analysis of demographic correlates of resolved viremia.

STUDY DESIGN AND METHODS: HCV antibody and NAT data, liver enzyme (alanine aminotransferase [ALT]) results, and donor demographic characteristics were compiled for 2,579,290 allogeneic donations given at five large blood centers after NAT implementation in 1999 through December 2001. Donation HCV RNA status was compared between first-time donors categorized by ALT levels, sex, age, race and/or ethnicity, country of birth, level of education, blood center location, and blood group, with chi-square tests and multivariable logistic regression methods.

RESULTS: Of 35 confirmed-seropositive repeat donors, 19 (54.3%) tested negative for the presence of HCV RNA; there was no association between RNA status and preseroconversion intervals ($p = 0.74$). Of 2105 RIBA-positive, first-time donors, 402 (19.1%) tested negative for the presence of HCV RNA by NAT (presumptive resolved infections). There were significant differences in the frequency of RNA negativity among first-time donors categorized by ALT levels and by race and/or ethnicity. ALT levels were more likely to be elevated in RNA-positive, first-time donors ($p < 0.0001$). Viremia was less likely to resolve in Asian (8.2%) and black non-Hispanic (14.4%) donors than in white non-Hispanic (20.7%), Hispanic (22.1%), and other race and/or ethnicity (22.1%) donors ($p = 0.02$). No significant associations were found for age, sex, country of origin, level of education, blood type, and donor center location.

CONCLUSION: These results confirm that the frequency of HCV RNA negativity among seropositive persons differs by race and/or ethnicity. Follow-up studies of donors with resolved viremia are warranted to further elucidate viral, immunologic, and genetic factors underlying spontaneous viral clearance.

Only about 15 percent of persons recently infected with hepatitis C virus (HCV) develop a clinically overt hepatitis syndrome, which is generally mild, occurs within 5 to 12 weeks of exposure (mean, 8 weeks), and lasts from 2 to 12 weeks.[1,2] Although 85 percent of primary HCV infections are asymptomatic, most studies indicate that 70 to 80 percent of these infections become chronic with asymptomatic viremia generally persisting for decades before later disease manifestations.[1,3] This results in a reservoir of clinically occult HCV infections in persons presenting to donate blood.[3,4] HCV seroprevalence (confirmed by

ABBREVIATIONS: ARC = American Red Cross; BCP = Blood Centers of the Pacific; IDU(s) = injection drug use(rs); MP(s) = minipool(s); REDS = Retrovirus Epidemiology Donor Study; TMA = transcription-mediated amplification.

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recombinant immunoblot assay [RIBA]) among first-time donors in the United States ranges from 0.2 to 0.3 percent (reflecting a steady decrease from 0.6% in 1992 owing to increasingly effective education and deferral procedures). With approximately 2.6 million first-time donations per year in the United States, we estimated that 6000 to 9000 HCV-seropositive infections are diagnosed annually as a result of donor screening (data obtained from the American Red Cross [ARC] national database). Since introduction of nucleic acid amplification testing (NAT) in 1999, the viremia status of seropositive donors is routinely available. In addition, NAT screening detects approximately 60 additional HCV-infected donors in the acute viremic, seronegative phase of infection each year (data obtained from the ARC national database).[5,6] Results from interview studies of seropositive US blood donors have demonstrated that injection drug use (IDU) many years before the HCV-positive donation is the likely source of infection for the majority of cases, with transfusions before implementation of HCV screening and other parenteral and sexual risk factors implicated to a lesser extent.[7,8]

Evidence from human and chimpanzee studies indicates that long-term viremia status (i.e., clearance vs. persistence) is generally determined within 6 months but occasionally as long as 12 to 24 months after infection.[1,9-11] The mechanisms and determinants of apparent viral clearance after seroconversion (as demonstrated by RNA negativity but HCV seropositivity) are not well understood but likely involve a combination of host and viral factors, including mode of acquisition; dose and quasispecies complexity of HCV in the source inoculum; viral genotype and/or subtype; patient race and/or ethnicity, HLA type, sex, and age at infection; and numerous host immune response variables.[12-21] Of note, symptomatic acute infections have been associated with an increased rate of viral clearance (up to 50%) compared to asymptomatic infections, probably reflecting the dual effect of a more robust immune response that eradicates infected hepatocytes and results in manifestations of clinical hepatitis.[1,2]

Owing to the large numbers, diversity, and low-risk characteristics of HCV-infected blood donors, this population may offer unique insights into correlates of HCV resolution, relative to those from studies of patients with symptomatic acute infections, historically identified transfusion recipients, or prospectively followed high-risk populations (primarily active IDUs).[1,3] To investigate laboratory and demographic correlates of HCV RNA negativity in HCV-seropositive blood donors, we compiled data on first-time allogeneic (whole-blood community, directed, and apheresis) donations collected at five blood centers participating in the National Heart, Lung, and Blood Institute Retrovirus Epidemiology Donor Study (REDS) that tested HCV-seropositive between the start of HCV RNA NAT in mid-1999 through December 31, 2001. We also evaluated the NAT status of repeat allogeneic donors who gave an HCV antibody–positive donation in the NAT period.

MATERIALS AND METHODS

Five blood centers collecting approximately 8 percent of all US allogeneic donations participated in REDS including three ARC Blood Services regions (Greater Chesapeake and Potomac Region [Baltimore, MD, and Washington, DC], Southeastern Michigan Region [Detroit, MI], and Southern California Region [Los Angeles, CA]); the Blood Centers of the Pacific (BCP, San Francisco, CA); and the Sylvan N. Goldman Oklahoma Blood Institute ([Oklahoma City, OK]). Information on donation type, donation date, demographic characteristics (age, sex, race and/or ethnicity, education level, country of birth), first-time and repeat donor status, and screening and confirmatory test results have been compiled in a database by the REDS coordinating center (Westat, Inc., Rockville, MD) since 1991. The REDS protocol was approved by the institutional review board at each center.

We identified first-time allogeneic (whole-blood community or directed and apheresis) donations that were confirmed HCV antibody–positive and were collected between April 1999, the approximate start of HCV NAT screening at REDS centers, and December 31, 2001 (the study period). The data set for the three ARC centers was subsequently limited to HCV-seropositive donations given subsequent to September 8, 1999, because before that date seroreactive donations were frequently identified before pooling and consequently not subjected to NAT screening. During the study period, screening for antibodies to HCV was conducted with a third generation enzyme-linked immunosorbent assay (Ortho HCV Version 3.0 ELISA test system, Ortho-Clinical Diagnostics, Inc., Raritan NJ). A third-generation (Chiron RIBA HCV 3.0 strip immunoblot assay, Chiron Corp., Emeryville, CA) was used as the confirmatory test by all centers, except for one center (BCP), which used either second-generation RIBA HCV or third-generation HCV assay versions between April and July 1999, before switching exclusively to the third-generation version on July 15, 1999. NAT was conducted with a human immunodeficiency virus-1 (HIV-1) and HCV transcription-mediated amplification (TMA) system (Procleix, Gen-Probe, Inc., San Diego, CA) at four centers (ARC centers and BCP) and a second-generation HCV screening test (COBAS AmpliScreen, Roche Molecular Systems, Inc., Pleasanton CA) at one center (Oklahoma Blood Institute).[6,22] For the Procleix TMA system, minipools (MPs) consisting of 16 individual donations were screened by the multiplex HIV-1 and HCV assay (except for BCP where donations were screened with MPs of 24 for the initial 14 months of NAT screening).[6,22,23] Once a reactive MP was identified, the corresponding individual donations were tested by the multiplex HIV-1 and HCV

assay, followed, if reactive, by discriminatory HCV and HIV-1 TMA assays to identify the viremic donation. For this analysis, only those specimens that tested reactive by the discriminatory HCV TMA were categorized as HCV RNA–positive. The COBAS AmpliScreen HCV assay was performed on MPs of 24 donations, with two-step resolution with six-member intermediate pools, followed by individual donation testing to identify the HCV RNA–positive donation(s). Both the Procleix and the AmpliScreen systems are highly sensitive with 50 percent detection limits of 12 HCV copies per mL on neat samples based on probit analyses and projected sensitivities of MP NAT screening of fewer than 250 copies per mL.[22,23]

We also identified repeat allogeneic donors who had given at least one third-generation HCV ELISA–nonreactive donation since January through June 1996 (time of third-generation HCV ELISA implementation) followed by an HCV ELISA–reactive and third-generation RIBA–positive donation in the NAT study period (April-September 1999, to December 31, 2001). We evaluated the proportions of third-generation RIBA–positive donations that were RNA-positive or -negative as a function of estimated time since infection by evaluating NAT status as a function of the length of the seroconversion interval or time between the HCV antibody–negative and HCV antibody–positive donations.

To evaluate if HCV RNA negativity in seropositive donors was associated with demographic characteristics, we evaluated the number of HCV RNA-negative (presumptive resolved infections) and RNA-positive (presumptive chronic infections) donations from first-time seropositive donors in various demographic groups and used chi-square tests to compare the proportions of HCV RNA-negative donations between demographic groups. The demographic characteristics evaluated included age (<25, 25-34, 35-44, 45-54, 55-65, and ≥65 years old), sex, race and/or ethnicity (white non-Hispanic, black non-Hispanic, Hispanic, Asian, other non-Hispanic), country of birth (US; non-US), education level (less than high school degree, high school degree, some college or an associate degree, college degree, graduate or professional degree), and blood center location. We also assessed if the distribution of ABO/Rh blood groups and alanine aminotransferase (ALT) levels (normal, <60 IU/L; low-elevated, 60-120 IU/L; high-elevated, >120 IU/L) differed between the RNA-negative and RNA-positive groups. Missing data were excluded from the analysis (NAT results were not available for 2.4% of donations from HCV antibody–positive, first-time donors; race and/or ethnicity, level of education, and country of birth information were missing for 11.3, 9.3, and 3.8 percent of donations from RIBA-positive first-time donors, respectively; and information on age, sex, blood center, ABO/Rh, and ALT was missing for 0.1% of donations from first-time donors). Logistic regression models were conducted to obtain odds ratios (ORs) that compared the odds of being HCV RNA-negative between demographic groups. We included as independent variables in the adjusted models only those demographic characteristics with a chi-square p value of 0.1. The models were conducted both unadjusted (one demographic characteristic at a time) and adjusted (all demographic variables entered into the model). The adjusted model permitted us to evaluate whether the association between a particular demographic variable and NAT status was confounded by another demographic variable, that is, whether the apparent effect of one demographic variable on NAT nonreactivity could be attributed to the effect of another demographic variable on NAT status.

RESULTS

The five participating blood centers collected 2,579,290 allogeneic donations during the study period (start of routine MP-NAT of seroreactive donations in 1999 through December 31, 2001), including 616,228 first-time (23.9%) and 1,963,062 (76.1%) repeat donations. There were 2105 donations from HCV ELISA–reactive and RIBA-positive first-time donors (seroprevalence, 0.34%); of these 402 (19.6%) tested nonreactive and 1653 (78.5%) reactive by MP-NAT (50 donations [2.4%] from RIBA-positive, first-time donors had missing NAT results and were subsequently excluded from the analysis; Table 1). HCV seroprevalence rates were similar among the five REDS centers; the number of confirmed seropositive donors contributed per center varied from 227 (ARC Southeastern Michigan Region) to 729 (ARC Southern California Region).

We also identified 35 HCV seroconverters who gave at least one HCV ELISA–nonreactive donation subsequent to implementation of the third-generation version of the anti-HCV screening assay in 1996, which was followed by an ELISA-reactive and RIBA-positive donation during the current study period. Of these 35 repeat donors, 19 (54.3%) were HCV MP-NAT–nonreactive on their RIBA-positive donation (MP-NAT was not available for 1 positive donation [2.9%]). The median seroconversion interval length observed for the 34 seroconverters with a NAT result was 22.6 months (range, 3.4 to 60.4 months); 24 of the 34 seroconverters (70.6%) had an interval of at least 1 year. We hypothesized that repeat donors with shorter seroconversion intervals would have higher RNA positivity rates than donors with longer seroconversion intervals, since on average they should have been infected more recently and consequently had less time to clear the HCV virus. As shown in Table 1, however, there was no significant difference in HCV RNA rates in seropositive donors with shorter or longer seroconversion intervals ($p = 0.74$).

Further analyses of correlates of RNA negativity were limited to the 2055 first-time confirmed-seropositive

donors for whom NAT status was available. Significant associations ($p < 0.05$) were observed between HCV RNA negativity, and ALT levels (Table 2) and race and/or ethnicity (Table 3). ALT levels were more likely to be normal in RNA-negative donors than in RNA-positive donors, and high-elevated ALT levels (≥120 IU/L) were much more frequently observed in RNA-positive donors than RNA-negative donors ($p < 0.0001$; Table 2). Asian and black non-Hispanic donors were less likely to be RNA-negative than Hispanic, white non-Hispanic, or other non-Hispanic first-time donors ($p = 0.02$; Table 3). The odds of HCV RNA negativity were significantly lower in Asian donors (unadjusted OR, 0.34; 95% confidence interval [CI], 0.12-0.96) and in black non-Hispanic donors (unadjusted OR, 0.64; 95% CI, 0.44-0.93) than in white non-Hispanic donors (Table 4). These racial and/or ethnic differences were not explained by age or sex differences between race and/or ethnic groups because unadjusted and adjusted (for age and sex) ORs remained similar (Table 4).

Associations between HCV RNA negativity and sex ($p = 0.10$), age ($p = 0.12$), blood center location ($p = 0.17$), country of origin ($p = 0.64$), education ($p = 0.80$), and ABO/Rh status ($p = 0.72$) were all nonsignificant (Table 3).

TABLE 1. Proportion of RIBA-positive donations by first-time and repeat donors who tested HCV RNA–negative by MP-NAT and relationship of preseroconversion intervals for repeat donors by RNA status

Donor status	Number of RIBA-positive donations	HCV RNA–negative*	p Value
First-time	2055	402 (19.6)	
Repeat: length of preseroconversion interval (months)	34	19 (55.9)	
≤6	4	3 (75.0)	
>6-9	2	2 (100.0)	
>9-12	4	2 (50.00)	
>12-24	9	4 (44.4)	
>24-60	15	8 (53.3)	0.74

* Data are reported as number (%). MP-NAT results were not available for 50 RIBA-positive, first-time donors (2.4%) and for 1 RIBA-positive, repeat donor (2.9%).

TABLE 2. Proportion of first-time seropositive donations who were HCV RNA-negative or -positive (by MP-NAT) with normal, low-elevated, or high-elevated ALT levels

	ALT level (IU/L)			
HCV status	Normal (<60)	Low-elevated (60-120)	High-elevated (≥120)	p Value
RNA-negative	396 (98.51)	3 (0.75)	3 (0.75)	
RNA-positive	1384 (83.78)	47 (2.85)	221 (13.38)	<0.0001

* Data are reported as number (%).

DISCUSSION

Investigations in various populations at high risk for HCV infection have identified correlates of HCV RNA clearance, including host factors such as age, sex, and race and/or ethnicity. The effectiveness of the immune response (which is largely determined by host genetics but influenced by age, health status, and previous antigenic exposures) and the corresponding evolution of the virus during the preseroconversion and early postseroconversion phases of infection are probably the major determinants of successful resolution of HCV viremia.[12-21,24-26]

Thomas and coworkers[18] first reported that race and/or ethnicity is significantly associated with clearance of HCV RNA in a study of seropositive IDU in Baltimore, Maryland. These investigators found that 9.3 percent of 729 seropositive black non-Hispanic IDUs resolved HCV infection, compared to 36 percent of 44 nonblack donors. Although our findings confirm a significant association between HCV RNA negativity in seropositive first-time blood donors and race and/or ethnicity, the differences in frequencies between black non-Hispanic blood donors (14.4%) and nonblack donors (e.g., 20.7% for white non-Hispanic donors) are smaller than seen with the Baltimore IDU cohort. This difference may relate to the high rate of HIV coinfection in the IDU cohort (33.4% of HCV-seropositive IDUs were HIV-seropositive, and HIV coinfection was associated with lower rates of HCV clearance) and to the relatively small number of nonblack subjects in that study compared to our study (44 vs. 1153, respectively). We also found that the odds of Asian first-time seropositive donors being RNA-positive was approximately three times that of white non-Hispanic donors. To elucidate the basis for these race and/or ethnicity differences, investigators have begun to correlate HLA and other immune response polymorphisms with HCV clearance status. Strong associations with several HLA class I and II alleles have been detected.[19,20] Persons who have cleared HCV are also more likely than chronic carriers to be homozygous for specific inhibitory killer immunoglobulin-like receptors (KIR) combinations (KIR2DL3) and to harbor specific polymorphisms related to cytotoxic lymphocyte antigen 4 (CTLA-4), interleukin 10, and CD4+ CD25+ regulatory T-cells (T-reg; Thio and colleagues, submitted for publication). These genetic studies have been hampered by insufficient power owing to limited numbers of persons with resolved HCV infections from various race and/or ethnicity groups. Access to seropositive blood donors who have tested RNA-negative offers a solution to this problem, and we are now contributing specimens from donors with presumptive resolved infection to these studies.

TABLE 3. Proportion of donations from first-time seropositive donors in a demographic group testing HCV RNA (MP-NAT)-negative or -positive

Demographic group	HCV RNA status* Negative	Positive	p Value
Age (years)			
<25	28 (14.89)	160 (85.11)	
25-34	64 (23.70)	206 (76.30)	
35-44	180 (20.93)	680 (79.07)	
45-54	110 (17.89)	505 (82.11)	
55-64	15 (15.63)	81 (84.38)	
≥65	5 (20.83)	19 (79.17)	0.12
Sex			
Male	232 (18.40)	1029 (81.60)	
Female	170 (21.41)	624 (78.59)	0.10
Race and/or ethnicity			
Asian	4 (8.16)	45 (91.84)	
Black non-Hispanic	38 (14.39)	226 (85.61)	
Hispanic	62 (22.06)	219 (77.94)	
Other non-Hispanic	16 (21.05)	60 (78.95)	
White non-Hispanic	239 (20.73)	914 (79.27)	0.02

* Data are reported as number (%).

TABLE 4. OR comparing odds of being HCV-seropositive and RNA (MP-NAT)-negative by demographic characteristic

Demographic characteristic	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Age (years)		
<25	1.0	1.0
25-34	1.78 (1.09-2.90)	2.08 (1.20-3.60)
35-44	1.51 (0.98-2.33)	1.75 (1.07-2.87)
45-54	1.24 (0.79-1.95)	1.48 (0.88-2.46)
55-64	1.06 (0.54-2.09)	1.30 (0.62-2.73)
≥65	1.50 (0.52-4.36)	1.98 (0.66-5.96)
Sex		
Male	1.0	1.0
Female	1.21 (0.97-1.51)	1.14 (0.90-1.45)
Race and/or ethnicity		
White non-Hispanic	1.0	1.0
Asian	0.34 (0.12-0.96)	0.36 (0.13-1.01)
Black non-Hispanic	0.64 (0.44-0.93)	0.66 (0.45-0.96)
Hispanic	1.08 (0.79-1.49)	1.08 (0.78-1.48)
Other	1.02 (0.58-1.80)	1.03 (0.58-1.83)

* Adjusted for age, sex, and race and/or ethnicity.

Several groups have reported that HCV clearance rates correlate with sex (more frequent clearance in women than men) and age at time of infection (young donors resolve infection more frequently than older donors). In our analysis, although nonsignificant, the odds for women to clear viremia appeared 21 percent greater than for men ($p > 0.05$), similar to the findings in several other studies (reviewed in Orland et al.[1]). The association with age was not significant, and no clear trend was observed.

Our results confirm that HCV RNA positivity is associated with elevation of ALT in RIBA-positive donors. Sixteen percent of viremic seropositive donors had ALT elevations, including 13 percent with elevations greater than the high donor screening cutoff of 120 IU per L. This indicates that a substantial subset of viremic donors have active liver disease at the time of donation. Because liver disease in HCV infection is intermittent and follow-up studies of HCV-infected persons with normal ALT have demonstrated low rates of progression to clinical disease, viremic donors without ALT elevation at the time of donation also warrant clinical follow-up to evaluate prognosis and available therapeutic options.[27] In other analyses, we have shown that HCV RNA-negative, RIBA-positive donors (first-time and repeat donors) have a distribution of ALT levels that is essentially identical to that of HCV ELISA–nonreactive donors.[6] This supports the conclusion that the large majority of RNA-negative, anti-HCV–positive donors have no residual hepatic inflammation and have likely eradicated HCV infection. In this data set, the frequency of elevated ALT among RIBA-positive, first-time donors who tested RNA-negative was 1.5 percent, which is slightly higher than rates of ALT elevation among all HCV ELISA–nonreactive donors. This likely relates to the possibility that a minority of these donors still have active HCV infection that is undetectable by a single RNA determination performed in a MP format of 16 to 24 donations.

Our findings of a lower frequency of HCV RNA positivity in seroconverting repeat donors than in seropositive first-time donors was unexpected. We had hypothesized that seroconverters, on average, would have been more recently infected than first-time donors, and if sampled within the first 6 months after the identification of their RNA positivity, would have retained higher rates of viremia not having adequate time for clearance. We observed that 56 percent of seroconverters tested RNA-negative on their first antibody-positive donation, in contrast to 20 percent in first-time donors, with no apparent relationship between viral clearance and interdonation intervals. It is unknown whether these donors have resolved infection or represent individuals with transient low-level viremia that may only be detected intermittently. Of the 34 seroconverting donors identified in our study, 24 (71%) had interdonation intervals exceeding 12 months so that these donors would not have been expected to have different HCV RNA-positive frequencies than first-time donors. The lower percentage of viremia in repeat seropositive donors could relate to recent observations in chimps and humans that in early infection, as the immune system tries to control the infection, RNA levels fluctuate and may be intermittently nondetectable by MP NAT despite eventual development of chronic infection.[9,11] With time, either the immune system is able to eradicate the infection or the virus escapes. Also, demographic differences in first-time and repeat donors may explain the different RNA-positive frequencies observed in these two groups.

This study has several limitations. We used routine donor screening MP-NAT results, resolved to the individual donation level for NAT-reactive pools, to classify RIBA-

positive donors as RNA-positive or -negative. Owing to the 16- to 24-fold dilution factor inherent in MP-NAT, we may have failed to detect viremia in a proportion of seropositive donors with chronic infection (whether these donors were first-time or repeat donors or with interdonation intervals exceeding 12 months). The MP-NAT assays employed in donor screening, however, have 50 percent detection limits of approximately 200 gEq to mL, equivalent to that of commercially available quantitative HCV RNA assays that are widely used clinically to define viremia status of seropositive persons. We have retested specimens from MP-NAT-nonreactive, RIBA-positive donors individually and have identified HCV RNA positivity in 2 to 5 percent of cases; results from follow-up studies of such donors indicate that they have normal ALT levels and intermittent low-level viremia detectable when samples are tested without dilution.[28]

Finally, our current analysis was limited to demographic and laboratory data available from routine donor screening on approximately 8 percent of the US blood supply during the study period; the REDS centers do not include centers from the southeastern United States where HCV incidence and prevalence are higher than in the rest of the country (data from the ARC national database). We also did not recall donors to investigate risk factors or probable dates of infection, nor did we perform additional laboratory studies to confirm viremia status or further characterize the donors (e.g., HLA typing, cellular immune responses) and the virus (subtype, quasispecies diversity). We have recently begun a study that involves enrollment and follow-up of seropositive donors with resolved infections and matched control donors with persistent infections, as well as NAT-positive antibody-negative donors who are being followed prospectively through seroconversion to establish their resolution status. This study will hopefully contribute to the understanding of determinants of HCV clearance, as well as yield information to assist in counseling and clinical management of the several thousand HCV-infected donors identified annually by US blood centers.[29]

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医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2006年9月4日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	乾燥抗D（Rho）人免疫グロブリン		研究報告の公表状況	肝臓 2006;47(8):384-391	公表国 日本	
販売名（企業名）	抗D人免疫グロブリン-Wf（ベネシス）					

研究報告の概要

わが国のE型肝炎の実態を明らかにする目的で、全国から総数254例のE型肝炎ウイルス感染例を集め、これを解析した。
その結果、以下の知見を得た。
1)HEVは全国に浸透している。
2)感染者の多くは中高年（平均年齢約50歳）で、男性に多い(男女比約3.5対1)。
3)我国に土着のHEVの遺伝型は3型と4型であり、後者は北海道に多い。
4)年齢と肝炎重症度との間に相関がある。
5)遺伝型3型に比べて、4型は顕在化率も重症化率も高い。
6)発症時期は無季節性である。
7)感染経路は、動物由来食感染が約30%、輸入感染が8%、輸血感染が2%、不明が約60%であった。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から抗D（Rho）人免疫グロブリンを濃縮・精製した製剤であり、ウイルス除去を目的として、製造工程において濾過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
日本におけるE型肝炎ウイルス感染の統計学的･疫学的･ウイルス学的特徴を求めた調査の解析結果報告である。 万一原料血漿にHEVが混入したとしても、EMCをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

肝臓　47巻8号　384-391（2006）

＜原　著＞

本邦に於けるE型肝炎ウイルス感染の統計学的・疫学的・ウイルス学的特徴：全国集計254例に基づく解析

阿部　敏紀[1)]　相川　達也[2)]　赤羽　賢浩[3)]　新井　雅裕[4)]　朝比奈靖浩[5)]
新敷　吉成[6)]　茶山　一彰[7)]　原田　英治[8)]　橋本　直明[9)]　堀　亜希子[10)]
市田　隆文[11)]　池田　広記[12)]　石川　晶久[13)]　伊藤　敬義[14)]　姜　貞憲[15)]
狩野　吉康[16)]　加藤　秀章[17)]　加藤　将[18)]　川上　万里[19)]　北嶋　直人[20)]
北村　庸雄[21)]　正木　尚彦[22)]　松林　圭二[23)]　松田　裕之[24)]　松井　淳[25)]
道堯浩二郎[26)]　三原　弘[27)]　宮地　克彦[28)]　宮川　浩[29)]　水尾　仁志[30)]
持田　智[31)]　森山　光彦[32)]　西口　修平[33)]　岡田　克夫[34)]　齋藤　英胤[35)]
佐久川　廣[36)]　柴田　実[37)]　鈴木　一幸[38)]　高橋　和明[4)]　山田剛太郎[39)]
山本　和秀[40)]　山中　太郎[41)]　大和　弘明[42)]　矢野　公士[43)]　三代　俊治[4)*]

要旨：極く最近まで殆んど不明状態にあった我国のE型肝炎の実態を明らかにする目的で，我々は全国から総数254例のE型肝炎ウイルス（HEV）感染例を集め，統計学的・疫学的・ウイルス学的特徴を求めてこれを解析した．その結果，[i] HEV感染は北海道から沖縄まで全国津々浦々に浸透していること；[ii] 感染者の多くは中高年（平均年齢約50歳）で，且つ男性優位（男女比約3.5対1）であること；[iii] 我国に土着しているHEVはgenotype 3とgenotype 4であるが，後者は主に北海道に偏在していること；[iv] 年齢と肝炎重症度との間に相関があること；[v] Genotype 3よりはgenotype 4による感染の方が顕性化率も重症化率も高いこと；[vi] 発生時期が無季節性であること；[vii] 集積症例全体の約30%は動物由来食感染，8%は輸入感染，2%は輸血を介する感染に帰せしめ得たものの，過半の症例（約60%）に於いては感染経路が不明のままであること；等の知見を得た．

索引用語：　E型肝炎　　E型肝炎ウイルス　　疫学　　日本

1）一心病院，2）相川内科病院，3）甲府市立甲府病院，4）東芝病院，5）武蔵野赤十字病院，6）富山大学三内，7）広島大学分子病態制御内科，8）国立療養所東京病院，9）東京逓信病院，10）国保中央病院，11）順天堂大学静岡病院，12）関西医大三内，13）日立総合病院，14）昭和大学二内，15）手稲渓仁会病院，16）札幌厚生病院，17）豊川市民病院，18）北海道大学二内，19）鳥取大学二内，20）市立加西病院，21）順天堂大学浦安病院，22）国立国際医療センター，23）北海道赤十字血液センター，24）松田内科医院，25）至聖病院，26）愛媛大学三内，27）高岡市民病院，28）大阪医大二内，29）帝京大学溝口，30）勤医協中央病院，31）埼玉医大消化器肝臓内科，32）日本大学消化器肝臓内科，33）兵庫医大肝胆膵科，34）鳥取県立中央病院，35）慶應大学消化器内科，36）ハートライフ病院，37）NTT東日本関東病院，38）岩手医大一内，39）川崎医大川崎病院，40）岡山済生会総合病院，41）板橋中央総合病院，42）北見赤十字病院，43）国立病院機構長崎医療センター
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＜受付日2006年5月10日＞＜採択日2006年6月26日＞

Table 1 Remarkable predominance of male over female, irrespective of severity of the disease.

Gender	Total n = 243	Disease categories			
		Subclinical n = 71	AH[a] n = 135	ASH[a] n = 21	FH[a] n = 16
Female	55(23%)	18(34%)	29(21%)	4(19%)	4(25%)
Male	188(77%)	53(66%)	106(79%)	17(81%)	12(75%)
F/M ratio	1 / 3.4	1 / 2.9	1 / 3.7	1 / 4.3	1 / 3

[a]Abbreviations : AH, acute hepatitis ; ASH, acute severe hepatitis (defined by prolonged prothrombin time, i.e., PT value < 40%) ; FH, fulminant hepatitis.

Table 2 Age of the subjects, possibly influencing clinical manifestations.

Age in yrs	Total n = 242	Disease categories			
		Subclinical n = 70	AH n = 135	ASH n = 21	FH n = 16
Less than 40	63(26%)	38(54%)[a]	21(16%)	3(14%)	1(6%)
40 to 59	105(43%)	20(29%)	70(52%)	11(53%)	4(25%)
60 or more	74(31%)	12(17%)	44(32%)	7(33%)	11(69%)[b]
Mean ± SD	50.1 ± 15.6	42.3 ± 15.9[c]	52.8 ± 14.4	52.8 ± 15.6	58.9 ± 10.1[d]

[a] $P < 0.001, 0.003, < 0.001$ against "AH", "ASH", "FH" respectively; [b] $P = 0.010, < 0.001$ against "AH", "Subclinical" (Chi square test). [c] $P < 0.001, 0.009, < 0.001$ against "AH", "ASH", "FH" (t test) ; [d] $P = 0.047$ against "AH" (Welch test).

緒　言

我国や西欧諸国は，アジア・アフリカの熱帯亜熱帯地域諸国と異なり，E型肝炎が頻発する地域ではないから，相当数の症例を集積するには時間と手間がかかる故，100例以上の症例を纏めて解析した報告は，我々の知る限り英文であれ和文であれ一報だに存在しない．我々は，約3年の歳月をかけて，共著者の夫々が過去およびリアルタイムに経験した症例の情報と検体を持ち寄り，更にはこれに我国から学会や論文で発表された症例の情報をも追加し，2006年1月末までに総数254例の，国内で経験されたHEVヒト感染例を集積することを得た．かほどの多数例を纏めて解析した仕事は未見であるし，聊か興味深い知見も得られたので，以下にそれを報告する．

方　法

症例の任意登録

共著者の夫々が，過去及び現在進行形で経験したHEV感染例について，地域，年齢，性，発病年，発病月，病型診断(Subclinical, Acute Hepatitis, Acute Severe Hepatitis, Fulminant Hepatitis のいずれか)，経過中最高ALT値，経過中最高総ビリルビン値，経過中最延長プロトロンビン時間値，ウイルス学的診断根拠 (HEV RNA陽性，あるいはIgM抗体・IgG抗体共陽性)，HEV genotype，推定あるいは確定された感染経路，海外渡航歴の有無，等の情報を任意登録した．2006年1月末の時点で，この『任意登録』によって集積し得た症例数はn＝206である．尚，HEV RNAが陽性でありながらgenotypingが未施行であった症例については，可能な限り検体の入手に努力し，sequencingを行った (方法後出)．

既報告例の引用登録

国内学会での過去の報告例については，抄録から上記調査項目に相当するデータを拾い集めた．論文発表例[1)-16)]については，一部は，当該論文著者自身から上記調査項目に相当するデータを任意登録して貰ったが，それが不可能であった場合には論文中の記載から該当データを引用登録した．この『引用登録』によって集積し得た症例数はn＝48であり，そのうち最古の症例

Table 3 Geographical distribution of HEV genotypes, showing a significant predominance of type-3 over type-4 in the areas other than Hokkaido.

Areas[a]	Total n = 228	HEV genotype			
		1	2	3	4
Hokkaido	123	—	—	58(47%)	65(53%)[b]
Tohoku	18	—	—	17(94%)	1(6%)
Kanto-Koshin'etsu	48	5(10%)[c]	—	31(65%)	12(25%)
Chuhbu-Hokuriku	8	1(9%)[c]	—	7(91%)	—
Kinki	10	1(10%)[c]	—	9(90%)	—
Chuh-Shikoku	10	1(10%)[c]	—	7(70%)	2(20%)
Kyushu-Okinawa	11	—	—	9(82%)	2(18%)

[a] Japan was divided, from northeast to southwest, into 7 areas, each of which includes the following prefectures. "Hokkaido" : Hokkaido alone. "Tohoku" : Aomori, Iwate, Miyagi, Akita, Yamagata, and Fukushima. "Kanto-Koshin'etsu" : Ibaraki, Tochigi, Gunma, Saitama, Chiba, Tokyo, Kanagawa, Shizuoka, Yamanashi, Nagano, and Niigata. "Chuhbu-Hokuriku" : Toyama, Ishikawa, Fukui, Gifu, Aichi, and Mie. "Kinki" : Shiga, Kyoto, Osaka, Hyogo, Nara, and Wakayama. "Chuh-Shikoku" : Tottori, Shimane, Okayama, Hiroshima, Yamaguchi, Kagawa, Ehime, Tokushima, and Kochi. "Kyushu-Okinawa" : Fukuoka, Saga, Nagasaki, Kumamoto, Oita, Miyazaki, Kagoshima, and Okinawa.

[b] $P < 0.001$ against other areas (Chi square test).

[c] All but one were from cases of imported infection.

Table 4 HEV genotype and clinical manifestation : the severer the disease the higher the frequency of genotype 4.

HEV genotype		Disease categories		
		Subclinical	AH	ASH + FH
1	(n = 7)	—	6	1
2	(n = 0)	—	—	—
3	(n = 135)	52	76	7
4	(n = 78)	7	48	23
	Rate of type-4[a]	7/59(12%)	48/130(37%)	23/31(74%)

[a] $P < 0.001$ between "Subclinical" and "AH" as well as between "AH" and "ASH+FH" (Chi square test).

は1979年に発生したものであった[13]．

HEV genome 塩基配列解析

ORF1内の異なる3領域の，それぞれ69 nt[17]，326 nt[18]，821 nt[19]の全てあるいは少なくとも一つの断片をPCRで増幅し，direct sequencingすることによりgenotypeを決定した．

統計学的有意差検定

群間の比率の差や平均値の差の有意性検定の為に用いた統計学的方法は各々のTableの脚注の中に記す．

結　果

HEV感染者の居住地

居住地情報が得られた症例の地域別内訳（括弧内は例数）は，北海道（n＝130），岩手（15），宮城（1），山形（1），福島（1），茨城（4），栃木（3），群馬（1），埼玉（6），千葉（6），東京（23），神奈川（5），静岡（1），山梨（1），長野（1），新潟（2），富山（3），石川（1），愛知（3），京都（1），大阪（2），奈良（2），兵庫（5），鳥取（4），岡山（4），広島（2），愛媛（2），福岡（1），長崎（12），熊本（1），大分（2），沖縄（3），であった．

HEV感染者の性と年齢

性別情報不明あるいは病型情報不明であった11名を除いた243名に基づく，性差の成績をTable 1に示す．同様に，年齢不詳あるいは病型情報不明の12名を除外

Table 5 Liver function test levels differed by HEV genotype.

Parameters[a]	Genotype 3			Genotype 4			P
	n	mean	SD	n	mean	SD	
peak ALT (IU/L)	101	1676	1390	75	3048	2501	< 0.001[b]
peak T.B. (mg/dL)	80	7.1	8.6	71	11.8	8.9	0.01[c]
nadir P.T. (%)	74	79.6	26.3	67	63.3	27.7	< 0.001[c]

[a] Abbreviations : ALT, alanine amino transferase ; T.B., total bilirubin ; P.T., prothrombin time. [b] By Welch. [c] By *t* test.

Table 6 Month when the infection occurred, suggesting that there was no seasonality.

Month	Number of cases	Adjusted number
January	20	
February	16	
March	21	20[a]
April	37	24[b]
May	11	
June	17	
July	20	
August	22	
September	26	21[c]
October	17	
November	23	
December	18	

[a] Of the 21 cases in March, 2 were infected simultaneously by eating the same *namagimo* (raw liver) of wild boar[21] while the other 19 were exposed to respective infection-sources, and hence the number of independent infections should be 20, not 21 ; [b] Similarly, of the 37 cases in April, 11 were from a mini-outbreak that occurred after wild boar barbecue party[20] and 4 were from deer-*sashimi* sharing[22] ; [c] Of the 26 in September also included 6 individuals from a mini-outbreak[10].

した242名に基づく，年齢分布の成績をTable 2に示す．

男性優位，中高年優位が一見して顕著であるのみならず，不顕性感染群と顕性感染群（特に劇症肝炎群）との間に，年齢分布の顕著な有意差が認められた．即ち，高齢になるほど重症化率が高かった．逆に云えば，不顕性感染群には若年者が多く存在した．

Genotype 分布の地域差

HEV genotype が判明した228例について，居住地（全国を北海道，東北，関東甲信越，中部北陸，近畿，中国四国，九州沖縄の7ブロックに分割）ごとの genotype 分布を Table 3 に示す．

北海道以外の地域では genotype 3 が圧倒的多数を占めたが，北海道に於いては genotype 3 と 4 がほぼ同数存在した．Genotype 1 が検出された8名中7名はインド（n＝4），バングラデシュ（2），ネパール（1）への渡航歴を有していた．

HEV genotype と肝炎重症度との相関

Genotype 情報及び病型診断情報の両方が得られた220例について，病型ごとの genotype 分布を Table 4 に示す．

Genotype 4 の頻度が，不顕性感染群から急性肝炎群へ，更には重症肝炎群（急性肝炎重症型＋劇症肝炎）へと有意差を以て上昇（12%→37%→74%）していた．同様に，Table 5 に見る如く，経過中最高 ALT 値，経過中最高総ビリルビン値，経過中最延長プロトロンビン時間値のいずれもが，genotype 4 の相対的高病原性を示唆する所見を示した．

季節性

発生月が判明した247例の集計結果を Table 6 に示す．

4月が突出して高い発生例数（n＝37）を示したが，そのうちの11例（於長崎）[20]及び2例（於鳥取）[21]は夫々同一感染源による小規模集団感染に属するものであった故，11 cases→1 incidence，2 cases→1 incidence として，互いに独立する感染発生件数をカウントし直すと，4月のそれは n＝24 に減少した．同様に，3月と9月にも夫々1件ずつの小規模集団感染事例[10][22]が含まれていた．かくて，互いに独立する感染発生件数（Table 6 に於ける"adjusted number"）で比較する限り，顕著な月別変動は存在しなかった．

Table 7　Routes of transmission.

Routes	Number of cases (%)	With direct evidence	With indirect evidence
Contact with animal	1(0.5%)	—	1[a]
Blood transfusion	5(2.3%)	5[b]	—
Travel and Import	17(7.9%)	—	13[c]
Zoonotic food-borne	68(31%)	5[d]	26[e]
Unknown	125(58%)	—	—

[a] Patient's pet cat was anti-HEV positive [8]. [b] Complete matching of HEV sequences between donor and recipient was observed in each case [11),13)]. [c] Nucleotide sequences of HEV from the patients were more homologous to those in the visited countries (India, Thailand, Nepal, Pakistan, Bangladesh, China) than those in Japan. [d] Complete matching of HEV sequences between patients and left-over animal meats [16),22)]. [e] Shown in literature [7),10),20),21)].

感染経路

感染経路を確定あるいは推定し得た症例は，全体の約40%でしかなかった（Table 7）.

5例の輸血感染（1例は愛知県，1例は東京，3例は北海道）は全て，ドナーと受血者の間でHEV RNA sequenceの一致が確認された直接証明例である．一方，他の感染経路（animal contact, travel and import, zoonotic food-borne）に於いては，感染源と感染者のHEV塩基配列が一致するとの直接証拠が得られたのは，シカからの感染[22]とイノシシからの感染[16]の2事例5名のみであって，その他は全て間接証拠からの推定である．感染源であると確定あるいは推定された動物種は，ブタ（症例数n＝44），イノシシ（15），シカ（5），動物種不明（2）であった.

北海道と本州以南で感染経路を比較すると，輸入感染の頻度に顕著な差が認められた：北海道1/130(0.8%) vs 本州以南16/124（13%）.

考　察

本邦を含む先進工業地域諸国からは初出と思われる，この200例を越えるHEV感染例の解析から得られた成績の中には，幾つかの興味深い知見が含まれている.

先ず，感染者のデモグラフィーに関しては，従来の教科書におしなべて"a disease of young adults"と記載されていたのに反し，本研究の成績は「中高年男性の病気」であることを強く示唆した．少数例ではあるが同様の成績が我国からも（A型肝炎に比較してE型肝炎患者は高齢で男性優位）[23]フランスからも（男女比約4対1，平均年齢約50歳）[24]も報告されているので，従来の教科書や常識が依拠していた流行地に於ける疫学と，これから明らかにされるであろう非流行地に於ける疫学の間に，相当の差異があるものと考えられる.

肝炎重症化の因子についても然りである．流行地に於ける観察から，妊娠第三期に於ける感染が従前唯一の重症化因子として認識されて来たが，非流行地である日本に於ける本研究の集計例の中に妊婦例は一例だに存在せず，寧ろ，加齢とHEV genotype 4が，新たな重症化因子として浮き彫りになった.

特に，HEV genotype 4とdisease severityとの間の有意な相関は，従前未報告の新知見であり，本研究の成果の中で最も特筆に値するものである．即ち，重症肝炎例にgenotype 4が多く見られるとの報告は従来から存在したが[15),24)]，病原性の強弱に関するgenotype 3と4との間の差異を統計学的有意差を以て示したのは，本報告が初めてである．両genotypesの間にはゲノム構造上も若干の差異がある（genotypes 1, 2, 3ではORF1とORF3が別フレーム上にあるが，genotype 4に於いては同一フレーム）し，増殖速度の差異（genotype 3<genotype 4）を示唆する所見（姜貞憙，松林圭二他，unpublished results）も得られているから，本研究が示唆したgenotype 4の相対的高病原性について，今後その機序が次第に明らかにされて行くと思われる.

HEV感染に於けるzoonotic transmissionの重要性は，特に我国からの多数の報告[7),8),10),14),16),20)-22),25)]により広く認識されるところとなった．その所為もあり，今回の全国集計に任意登録された症例の多くに於いては，動物由来感染を疑うための問診が相当積極的に為されていたが，それでもなお，zoonotic transmissionで説明し得る症例は全体の約30%でしかなかった．輸入感染例の約3倍もの頻度で動物由来感染が存在するということ自体が，新しく且つ刮目すべき知見ではあったものの，もっと重要な知見は，集計症例全体の約60%もが感染経路不明のまま残されたという事実の方だったかもし

れない．何故なら，それにより，我々が未だ把握していない感染経路の存在をも念頭に置いた今後の研究の必要性が示されたからである．そして，その目的の為には，特に北海道に於いて一層積極的な調査を行うことが望まれる．北海道で経験される輸入感染の頻度（0.8%）は本州以南でのそれ（13%）の10分の1以下でしかないという事実が，道内の感染源の重要性を何よりも雄弁に物語っているからである．

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Demographic, epidemiological, and virological characteristics of hepatitis E virus infections in Japan based on 254 human cases collected nationwide

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To know the reality of hepatitis E virus (HEV) infections in Japan, quite obscure until a few years ago, we have collected a total of 254 human cases of HEV infection, and analyzed for demographic, epidemiological, and virological characteristics. As a result, we now know [i] HEV has penetrated nationwide from Hokkaido to Okinawa; [ii] hepatitis E is a disease of middle-aged people (approx. 50 years old in average) with a predominance of male over female (approx. 3.5 vs 1); [iii] HEV strains of genotype 3 and 4 are autochthonous in Japan, but the latter is present almost exclusively in Hokkaido; [iv] the older the age the severer the disease; [v] HEV genotype 4 is associated with more obvious and severer clinical manifestations than genotype 3; [vi] no seasonality in its incidence; and [vii] transmission routes remain obscure in most cases (approx. 60%), whereas about 30%, 8%, and 2% are ascribable to zoonotic food-borne transmission, imported infection, and via blood transfusion, respectively.

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