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研究報告の概要	○日本における、オカルトB型肝炎ウイルス(HBV)感染供血者由来の血液製剤に起因する輸血後HBV感染の残存リスク背景: 血液スクリーニング検査で行われるHBV NATは、日本で輸血後HBV感染(TT-HBV)の予防に貢献しているが、それでも年間4～13件のTT-HBVが発生している。 研究デザインと方法: 日本赤十字社はヘモビジランスの一環として、TT-HBVが疑われた献血の保管検体を解析している。TT-HBVに関与した献血はウィンドウ期(WP)あるいはオカルトHBV感染(OBI)に分類された。さらに、HBc抗体低力価(S/CO 1.0以上12.0未満)かつHBs抗体200 IU/L未満である4,742人の献血者の血液を個別NAT(ID-NAT)によって分析し、HBc抗体価とウイルス血症の関連性について調べた。 結果: より感度の高い現行のNAT試験による20供血ミニプールNATの導入で、OBIの検出率が100万件当たり3.9件から15.2件に、また確定OBI感染率も、100万件当たり0.67件から1.49件に上昇した。現行の20プールNAT導入後におけるWTPとOBI供血が原因であるTT-HBVは、それぞれ75%及び85%がID-NATによって防ぐことが可能であったと考えられる。ID-NAT試験の結果、HBc抗体低力価かつHBs抗体200 IU/L未満である検体の1.94%がウイルス血症であり、低力価陽性時においてHBc抗体価とウイルス血症の頻度は相関していないことが明らかとなった。 結論: 日本赤十字社は、全献血の1.3%を占める、HBc抗体低力価かつHBs抗体低値の血液を全て廃棄することにより、最大限の安全策を講じることを選択した。			
報告企業の意見	輸血後HBV感染が疑われた献血の保管検体を個別NATにより解析したところ、HBc抗体低力価かつHBs抗体200 IU/L未満である検体の1.94%がウイルス血症であり、またHBc抗体価とウイルス血症の頻度は相関していないことが明らかとなったとの報告である。			
今後の対応	日本赤十字社では、化学発光酵素免疫測定法(CLEIA)によりHBs抗原、HBc抗体検査を実施することに加えて、精度を向上させたNATシステムを導入し、20プールでスクリーニングNATを行い、陽性血液を排除している。なお、2012年8月より抗体検査の判定基準を強化し、HBc抗体(C.O.I.)1.0以上かつHBs抗体200 IU/L未満の感染既往を示す血液を不適としている。また、個別スクリーニングNATの導入についても検討を行っている。HBV感染に関する新たな知見等について、今後情報収集に努める。			
使用上の注意記載状況・その他参考事項等	新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、細菌、原虫等の感染、vCJD等の伝播のリスク			

TRANSFUSION COMPLICATIONS

Residual risk of transfusion-transmitted hepatitis B virus (HBV) infection caused by blood components derived from donors with occult HBV infection in Japan

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BACKGROUND: Nucleic acid amplification testing (NAT) for hepatitis B virus (HBV) during blood screening has helped to prevent transfusion-transmitted HBV infection (TT-HBV) in Japan. Nevertheless, 4 to 13 TT-HBV infections arise annually.

STUDY DESIGN AND METHODS: The Japanese Red Cross (JRC) analyzed repository samples of donated blood for TT-HBV that was suspected through hemovigilance. Blood donations implicated in TT-HBV infections were categorized as either window period (WP) or occult HBV infection (OBI) related. In addition, we analyzed blood from 4742 donors with low antibody to hepatitis B core antigen (anti-HBc) and antibody to hepatitis B surface antigen (anti-HBs) titers using individual-donation NAT (ID-NAT) to investigate the relationship between anti-HBc titer and proportion of viremic donors.

RESULTS: Introduction of a more sensitive NAT method for screening minipools of 20 donations increased the OBI detection rate from 3.9 to 15.2 per million, while also the confirmed OBI transmission rate increased from 0.67 to 1.49 per million. By contrast the WP transmission rate decreased from 0.92 to 0.46 per million. Testing repository samples of donations missed by minipools of 20 donations NAT showed that 75 and 85% of TT-HBV that arose from WP and OBI donations, respectively, would have been interdicted by ID-NAT. The ID-NAT trial revealed that 1.94% of donations with low anti-HBc and anti-HBs titers were viremic and that anti-HBc titers and the frequency of viremia did not correlate.

CONCLUSIONS: The JRC has elected to achieve maximal safety by discarding all units with low anti-HBc and anti-HBs titers that account for 1.3% of the total donations.

The prevalence of hepatitis B virus (HBV) surface antigen (HBsAg) in Japan is slightly higher than the average for developed countries. A recent screening of blood donors, local residents, and school pupils found an estimated national prevalence of HBsAg of 0.71%.¹ However, the prevalence was higher during the 1990s, being 1.5% among first-time blood donors aged in their 40s.² Taking into account horizontal transmission and a birth cohort effect, a relatively large cohort with historical HBV infection might persist among older individuals in Japan.

To prevent transfusion-transmitted HBV (TT-HBV) infection, Japanese Red Cross (JRC) blood centers introduced HBsAg screening for all blood donations in 1972. In 1989, antibody to hepatitis B core antigen (anti-HBc) testing was introduced to exclude donations by people with prior HBV infection. Because total elimination of anti-HBc-reactive donations might have seriously reduced the blood supply, donations with high antibody to hepatitis B surface antigen (anti-HBs) titers and those

ABBREVIATIONS: CLEIA(s) = chemiluminescence enzyme immunoassay(s); ID = individual donation; JRC = Japanese Red Cross; LOD = limit of detection; OBI = occult hepatitis B virus infection; PC(s) = platelet concentrate(s); S/CO = signal-to-cutoff ratio; TT-HBV = transfusion-transmitted hepatitis B virus infection; TTI = transfusion-transmitted infection; WP = window period.

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with low anti-HBc titers have been accepted, and only donations with low anti-HBs and high anti-HBc titers were excluded.

In addition to this serologic screening algorithm, the JRC implemented multiplex nucleic acid amplification testing (NAT) for HBV, hepatitis C virus (HCV), and human immunodeficiency virus Type 1 (HIV-1) in 1999.³ Although NAT has greatly reinforced blood safety regarding TT-HBV infection, 4 to 13 TT-HBV infections continue to arise annually. While some occur as a result of transfusion with blood components obtained during the window period (WP), others arise due to components being derived from donors with occult HBV infection (OBI) defined as detectable HBV DNA in peripheral blood but no detectable HBsAg.^{4,5} Although donations from donors with OBI have helped to maintain an adequate blood supply, such donations have also raised a concern about the risk of TT-HBV. Here, we describe the current status of TT-HBV under the NAT screening system as well as problems inherent in the current HBV screening algorithm, especially with regard to OBI-derived blood donations. We also discuss the feasibility of strategies that could increase HBV safety in countries such as Japan with a slightly elevated prevalence of HBV.

MATERIALS AND METHODS

Screening donated blood at JRC blood centers

The JRC blood centers are the only facilities authorized to handle blood collection, processing, testing, and delivery in Japan. Donated blood is screened at these centers for HBsAg, anti-HCV, anti-HIV-1 and -2, anti-human T-lymphotropic virus type 1, anti-*Treponema pallidum*, and human parvovirus B19 antigen. Whereas HBsAg-positive blood is rejected, HBsAg-negative samples are further tested for anti-HBc and anti-HBs (Table 1). Blood

with a high anti-HBs titer (≥ 200 IU/L) is accepted irrespective of the anti-HBc titer and that with a low anti-HBc titer is also accepted irrespective of the anti-HBs titer. Blood with high anti-HBc and low anti-HBs titers (< 200 IU/L) is disqualified. All blood had been serologically tested before 2008 using the agglutination method with the initial cutoff for a high anti-HBc titer being a dilution factor of 2^6 , which was later revised to 2^5 . All agglutination tests were replaced with chemiluminescence enzyme immunoassays (CLEIAs, CL4800 testing system, Fujirebio, Tokyo, Japan) in 2008 and the threshold for anti-HBc positivity is currently a signal-to-cutoff ratio (S/CO) of 12.0. This value was validated as being essentially equivalent to an agglutination titer of 2^5 . Blood donations with elevated serum alanine aminotransferase (> 60 IU/mL) are also rejected.

NAT

Samples that were qualified by the testing algorithm for anti-HBc and anti-HBs described above as well as by HBsAg testing are then screened using NAT. The JRC started NAT in 1999 using a real-time multiplex polymerase chain reaction system with a minipool format that originally comprised 500 samples (Ampli-NAT MPX system, Roche, Indianapolis, IN).⁶ The pool size was decreased to 50 in 2000 and to 20 in 2004. The JRC implemented the Roche TaqScreen MPX system for NAT in 2008 with a pool size of 20, but with an approximately threefold increase in sensitivity because of the increased sample volume required for nucleic acid extraction and improvements in reagents. The screening sensitivity of HBV is 650, 260, and 76 copies/mL (50% limit of detection [LOD]; JRC data) for 50-, (50p) and 20-, (20p) sample pools using AmpliNAT and 20p using TaqScreen, respectively.

A trial screening using individual-donation NAT (ID-NAT) proceeded at the Tokyo Blood Center between December 2010 and May 2011 to verify the distribution of the rate of donations containing HBV DNA relative to anti-HBc titers and the residual TT-HBV risk that could arise from transfusion with blood donations that have low anti-HBc and anti-HBs titers. All available donations with both an anti-HBc titer between 1.0 and 12.0 S/CO and an anti-HBs titer of less than 200 IU/L were screened by ID-NAT using the Roche TaqScreen MPX system with a 50% LOD of 3.8 copies/mL (JRC data). The sensitivity of ID-NAT used in lookback studies (described below) was 13 copies/mL (50% LOD) using AmpliNAT until July 2008 and 3.8 copies/mL (50% LOD) using TaqScreen from August 2008.

Hemovigilance system

The JRC established a hemovigilance system in 1993 and has since collected reports on adverse effects caused by blood transfusion. Through blood screening the JRC obtains information about repeat donors who have

TABLE 1. HBV screening algorithm applied at JRC blood centers*

	Anti-HBc titer	
	Low $< 2^5(2^5)$ or S/CO ≥ 1.0 but < 12.0	High $\geq 2^5(2^5)$ or S/CO ≥ 12.0
Anti-HBc reactive 4.9% 261,000, 49,000		
Anti-HBs ≥ 200 IU/L	Accepted 2.04% 108,000, 20,000	Accepted 1.38% 73,000, 14,000
Anti-HBs < 200 IU/L	Accepted 1.31% 69,000, 13,000	Rejected 0.19% 10,000, 2,000

* HBsAg-negative donations are tested for both anti-HBc and anti-HBs. Dilution factors for anti-HBc titers were applied for agglutination testing. Dilution factors in parentheses were applied between 1997 and 2007. The S/CO ranges are currently used for CLEIA. Ratios (%) of donations for each category are shown (2010 data). Observed number and number per million (italics) of donations are also included.

recently acquired infection.⁷ Their previous donations are evaluated for transfusion-transmitted infection (TTI) risk by considering donation timing and performing ID-NAT on repository samples (lookback studies). If they are judged as harboring a TTI risk, the JRC notifies the relevant facilities that used the component at risk and requests that physicians investigate whether any patient who received a transfusion of the component has acquired the corresponding infection.

The JRC also obtains information about TTI in transfused patients through voluntary reports by physicians who are involved in blood transfusion at medical facilities.⁷ Upon receiving such information, the JRC analyzes repository blood samples obtained from implicated donations using ID-NAT. The TTI risk of cocomponents derived from the implicated and previous donations provided by implicated donors is assessed. The JRC notifies the relevant medical facilities of the findings. Implicated blood components are interdicted if they have not yet been used for transfusion.

The JRC headquarters and central laboratory determine the causal relationship between the implicated donation and posttransfusion infection considering patient clinical course, results of virologic analysis including ID-NAT and sequence analysis, serologic viral markers, and donation timing. Even if all repository samples implicated for TTI are verified as being ID-NAT-negative, implicated donors are followed up for repeat donation thereafter for sero- or NAT conversion, because the possibility that the index donation was provided during the ID-NAT WP persists. All processes for lookback studies are defined in national guidelines⁸ that describe in detail the test items and timing of testing for donated blood and at-risk patients in addition to the roles of the relevant physicians, blood centers, and blood authority.

Sequence analysis

The HBV genome sequence identity is assessed between implicated repository blood samples and patient samples by sequencing 1550 bp of the alpha region within the HBV pre-S and S regions using a genetic analyzer (ABI 3130XL, Life Technologies Japan, Tokyo, Japan). When the viral load is too low to sequence, viral nucleic acid is further extracted from larger plasma volumes if the accompanying plasma bag is available. When findings are ambiguous, HBV obtained from donor or patient samples is cloned, amplified, and sequenced.

Estimation of current risk of TT-HBV

Although universal pre- and posttransfusion testing of patient samples for TTI has been recommended, the likelihood that all transfused patients undergo this evaluation is low. Moreover, the JRC hemovigilance system described

above is voluntary. Therefore, TTI might be underreported to JRC blood centers. The exact amount of TT-HBV infections that could occur under the current screening system must be defined to assess novel TT-HBV-mitigating strategies. This study therefore reevaluated the current risk of TT-HBV infection based on data obtained under current system.

The projected number of ID-NAT-positive donations derived from OBI donors was calculated using the ID-NAT positivity rate obtained in the ID-NAT trial screening described above and the number of donations with low anti-HBc and anti-HBs titers. The additional WP yield in donations determined by ID-NAT was calculated based on rates of detection of recently infected donors.^{9,10} Assuming that the frequency of donation is constant at any time during the presymptomatic phase of acute infection, the yields by tests for an infection marker are in direct proportion to the length of time during which each test gives a yield. The potential ID-NAT yield (screening NAT negative) was calculated herein by multiplying the screening NAT yield by the ratio of the interval between ID-NAT detection and 20p-NAT detection (11.2 days) to that between 20p-NAT detection and HBsAg detection (9.7 days). The interval covered by each NAT strategy (11.2 and 9.7 days) was calculated using the value for the detection limit of each test (3.8, 76, and 1000 copies/mL for ID-NAT, 20p-NAT, and CLEIA detection, respectively) and the doubling time of HBV in human peripheral blood (2.6 days).^{9,11} The number of donations that could appear in the ID-NAT-negative WP was similarly calculated separately for each component type taking into account both the interval between 1 copy/bag and ID-NAT detection deduced from the mean plasma volume of each component type and the number of each component issued to hospitals.

We estimated the number of TT-HBV infections with reference to our previous systematic lookback study.⁷ The infectivity of ID-NAT-positive and screening NAT-negative components was calculated in that study as being 3% (95% confidence interval [CI], 0%-17.2%, $n = 33$) and 50% (95% CI, 28.2%-71.8%, $n = 22$) for OBI- and WP-derived components, respectively. The incidence rate for TT-HBV infections was thus obtained by multiplying the number of estimated at-risk donations deduced using the above method by the infection rates (0.03 or 0.5).

Statistical analysis

Data were statistically analyzed using computer software (SSRI for Windows, Excel Statistics Version 8, Social Survey Research Information Co. Ltd, Tokyo, Japan). Significance was determined using the chi-square test except for associations between total viral load in the components and alanine aminotransferase (ALT) levels in patients that were evaluated using the Mann-Whitney U test.

RESULTS

Reports of possible TT-HBV infections

The JRC blood centers received 789 reports of possible TT-HBV infections between 2001 and 2010 (Fig. 1). The number of such reports obviously increased in 2004 and 2005 because a nationwide systematic retrospective study started in 2004 that also identified patients with TT-HBV infection that would have previously been unrecognized. Causality was investigated in all but two of these possible TT-HBV infections. The possibility of TT-HBV infection was precluded in 97 (12.3%) of the 789 reported patients without testing repository samples based on evaluation of the patient's clinical course and the transfusion setting for each. For all of the remaining patients, repository samples were tested serologically and by ID-NAT to detect the HBV genome. Of the 789 initial reports, 98 (12.4%) were

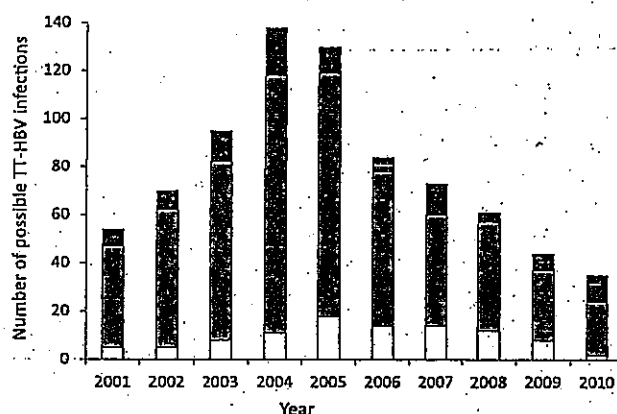


Fig. 1. Annual number of potential TT-HBV infections. (□) Patients in which possibility of TT-HBV was excluded (n = 97, 12.3%) without testing repository samples. (■, ▨) Patients in whom HBV DNA was identified or not, respectively, in the repository samples corresponding to a donation from the donor of the implicated blood components. Four patients are not included as HBV DNA sequence identity was not established.

determined to be TT-HBV infections after the introduction of 50p-NAT. The HBV sequence identity was established between donor and recipient in 88 of these cases, and TT-HBV was determined considering other HBV markers and the clinical setting in the remaining 10. An HBV genome was not detected in repository samples for 587 (74.4%) potential TT-HBV infections. Although HBV was detected in four repository samples, HBV sequence identity was not confirmed between donors and recipients. Forty-two (43%) of the established TT-HBV infections were discovered through lookback studies that were started based on risk information provided by JRC blood centers. The remaining 56 (57%) were initially recognized by physicians at medical facilities. The number of established TT-HBV infections ranged from 4 to 13 per year between 2006 and 2010.

Infection status of donors implicated in TT-HBV infection

The sensitivity of NAT screening improved through the three phases described above (50p-AmpliNAT, 20p-AmpliNAT, and 20p-TaqScreen). With the increased sensitivity of 20p-TaqScreen, the NAT yield of OBI donations increased from 3.9/million to 15.2/million, whereas the yield of WP donation decreased from 13.2/million to 5.7/million (Table 2). This was caused by the simultaneous introduction of CLEIA in 2008 for serologic screening including HBsAg detection, which effectively shortened the period that could be covered by 20p-NAT.

The established TT-HBV infections that occurred during each period were categorized based on the presence or absence of the HBV genome in the implicated component (that is, ID-NAT positive or negative) and the infection status of the donation (WP related [anti-HBc nonreactive] or OBI related [anti-HBc reactive]). Table 2 also shows the numbers of established TT-HBV infections associated with each group during each period. Figure 2 shows the incidence (per million donations) of estab-

TABLE 2. NAT yield and number of TT-HBV infections relative to three phases of screening NAT*

Screening system	50p-AmpliNAT	20p-AmpliNAT	20p-TaqScreen
Duration of screening period	Feb. 2000– Jul. 2004 (4.5 year)	Aug. 2004– Jul. 2008 (4.0 year)	Aug. 2008– Mar. 2010 (1.67 year)
Sensitivity of screening NAT (copies/mL)†	650	260	76
Sensitivity of ID-NAT used for lookback study (copies/mL)†	13	13	3.8
Number of donations tested	24,702,784	19,513,054	8,746,037
Confirmed WP donations (/million)		258 (13.2)	50 (5.7)
Confirmed OBI donations (/million)	473 (19.1)	76 (3.9)	133 (15.2)
Number of donations causing established HBV transmission			
ID-NAT-negative WP	5	6	1
ID-NAT-positive WP	28	12	3
ID-NAT-negative OBI	4 (1)‡	1 (0)‡	2 (1)‡
ID-NAT-positive OBI	13 (1)‡	12 (1)‡	11 (5)‡

* Yields by ID-NAT trial conducted from December 2010 are not included in the table.

† 50% LOD.

‡ Numbers of donations with anti-HBs of greater than 10 mIU/mL.

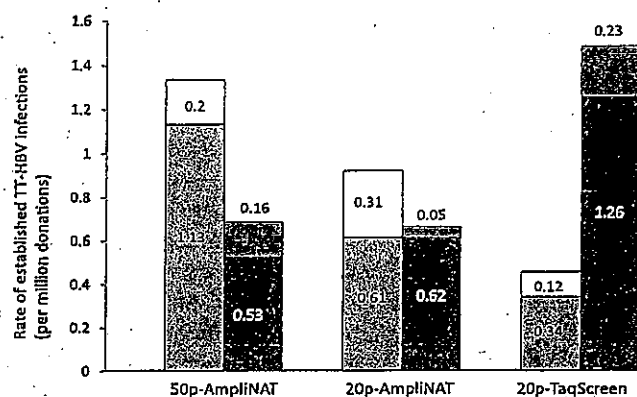


Fig. 2. Number of established TT-HBV infections grouped according to pool-based NAT screening systems. See Table 2 for intervals when indicated NAT systems were applied, sensitivities of NAT systems used, and actual yields for each category at each interval. (□, ▨) Infections caused by transfusion with ID-NAT-negative and -positive WP-derived components, respectively. (■, ▩) Infections caused by transfusion with ID-NAT-negative and -positive OBI-derived components, respectively.

lished TT-HBV infections relative to the three periods. The rate of infections caused by transfusion with a WP-derived component notably decreased with increasing NAT sensitivity, but that caused by transfusion with OBI-derived components rather increased despite the increased NAT sensitivity. Current NAT screening protocols indicated that TT-HBV infections occur more frequently due to transfusion with OBI- than with WP-related components (1.49/million vs. 0.46/million donations; Fig. 2). Nine TT-HBV infections occurred as a result of transfusion with blood components containing more than 10 mIU/mL anti-HBs during the past decade (Table 2). Two of them were caused by donations with negative ID-NAT.

The number of TT-HBV infections caused by transfusion with ID-NAT-negative components accounts for 15% (2/13) and 25% (1/4) of OBI- and WP-related TT-HBV infections, respectively, according to the current NAT system (Table 2). These infections involving ID-NAT-negative donations were determined as TTI by analyzing repository blood samples obtained before the index donation and/or by following up with the implicated donors after the index donation. Details of the clinical course of a typical TT-HBV infection caused by ID-NAT-negative OBI-related blood components are shown in Tables 3 and 4.

Impact of blood product on transmission rate

Table 5 shows the numbers of implicated donations by either ID-NAT negative or positive for groups categorized by the type of component and WP/OBI status. During the past decade, ID-NAT-positive donations have caused 79 TT-HBV infections. Transfusion with red blood cells (RBC),

fresh-frozen plasma (FFP), and platelet concentrate (PC) was associated with infections in 42, 22, and 15 of them, respectively. Of 19 TT-HBV infections associated with ID-NAT-negative donations, 2, 4, and 13 were caused by transfusion with RBCs, FFP, and PC, respectively. Transfusion with blood components containing a larger plasma volume (FFP and PC, but not RBCs) caused more frequent TT-HBV infections among patients who received ID-NAT-negative donations (17/19, 89%) than among those who received ID-NAT-positive donations (37/79, 47%; $p < 0.01$), which could be a reflection of the plasma volume effect on infectivity.

Table 5 also shows that if ID-NAT had been implemented during the screening, 81% of established TT-HBV infections would have been avoided. The introduction of ID-NAT would have been the most (95%) and least (54%) effective for preventing TTI caused by RBC- and PC-related transfusions, respectively. Under the current 20p-TaqScreen system, 75 and 85% of TT-HBV infections arising from WP and OBI donations, respectively, are ID-NAT positive and will be interdicted by ID-NAT. In particular, the effect of ID-NAT will be 100% for OBI-related infections caused by RBC transfusion.

Outcomes of patients with TT-HBV infection

ALT levels during TT-HBV infection were determined in 68 transfusion recipients who developed TT-HBV infection. Table 6 shows the maximal ALT values relative to WP or OBI donations and ID-NAT-positive or -negative donations. Almost half (47%, 32/68) of the patients had maximal ALT values of more than 1000 IU/L. The proportion of patients with maximal ALT of more than 1000 IU/L was greater in OBI-related (61%, 19/31) than in WP-related (35%, 13/37; $p < 0.05$) infections. Total viral load in the implicated components did not significantly differ between patients with ALT values above and below 1000, which was true for both WP- and OBI-related infections. Although barely insignificant, total infused viral load tended to be lower in OBI- than in WP-related patients among groups with maximal ALT values of more than 1000.

Three patients with TT-HBV infection died of fulminant hepatitis after the introduction of NAT. One was caused by transfusion with PC derived from an ID-NAT-negative, WP-related donation (Genotype A with wild type precore region). The other two developed hepatitis after transfusion with RBCs derived from ID-NAT-positive, OBI-related donations (Genotype B with a G1898A precore mutation and Genotype C with a G1896A precore mutation).

ID-NAT screening trial in donations with low anti-HBc and anti-HBs titers

During a 6-month ID-NAT trial, 4742 (0.74%) of 640,628 blood donations at the Tokyo Blood Center with low anti-

HBc and anti-HBs titers were analyzed by ID-NAT. The number of donations analyzed by ID-NAT decreased as the anti-HBc titer increased (Fig. 3). HBV DNA was detected in 92 (1.94%) of the 4742 donations. Figure 4 shows the frequency of ID-NAT-positive donations relative to the anti-HBc titer. The frequency of ID-NAT positivity for HBV did not correlate with the anti-HBc titer and did not tend to increase with an increasing anti-HBc titer. The proportions of anti-HBs-positive (>10 mIU/mL) donations among those that were ID-NAT positive and

negative were 77 and 75%, respectively, and did not significantly differ. The proportion of anti-HBs-positive donations increased with increasing anti-HBc S/CO values among ID-NAT-negative donations (67.5, 82.0, and 87.5% for anti-HBc S/CO 1.0-3.9, 4.0-7.9, and 8.0-11.9, respectively; $p < 0.01$ between any two groups). The frequency of ID-NAT positivity between males (1.8%) and females (2.4%) did not significantly differ. Eighty-three (90.2%) of the 92 ID-NAT-positive donors were at least 50 years of age. Fifteen had a viral load of less than 100 copies/mL, whereas quantitative NAT could not detect HBV DNA loads in samples from the remaining 77. The distribution of HBV genotypes among the ID-NAT-positive donations did not differ from that among the general Japanese population: Genotypes A, B, C, and D, $n = 1, 24, 45$, and 1 , respectively (21 were undetermined).

TABLE 3. Representative TT-HBV infection caused by OBI-derived, ID-NAT-negative blood component: clinical course of a patient who received an implicated blood component.

Date	Clinical events and test results
Nov. 10, 2008	Surgery to treat head injury* HBsAg negative, anti-HBc negative, HBV DNA negative, preoperatively Transfused until Nov. 20 with 21 RBC units, 5 PCs, and 11 FFP† including one derived from the donation of Mar. 27, 2008, shown in Table 4
Mar. 05, 2009	AST 15, ALT 32
Mar. 25, 2009	AST 517, ALT 1273
Mar. 30, 2009	AST 1312, ALT 3110, HBsAg positive, IgM-anti-HBc positive Reported to JRC blood center
Mar. 31, 2009	AST 695, ALT 2396
Apr. 01, 2009	HBsAg negative, anti-HBs positive, HBV DNA positive

* Recipient was a teenage boy who was injured in a traffic accident.
† HBV DNA was not detected based on ID-NAT for the repository samples from these 37 blood components transfused. These results were obtained in the first lookback study performed in April 2009.

Estimation of current TT-HBV risk in Japan

From the frequency of ID-NAT-positive (1.94%) donations among those with low anti-HBc and anti-HBs titers (69,000/year or 13,000/million; see Table 1 and below), we calculated that 1339/year or 252/million donations should be ID-NAT positive among screening NAT-negative donations with low anti-HBc and anti-HBs titers. Using an infectivity rate of 3%⁷ among components derived from OBI donations that were screening NAT negative and ID-NAT positive, we calculated that 40/year or 7.6/million OBI-related TT-HBV infections should arise. If TT-HBV infections related to OBI-derived ID-NAT-negative donations are taken into account, then the total number of TT-HBV infections should be 47/year or 8.9/million. This estimate was based on the observation that TT-HBV infection caused by ID-NAT-negative components during the

TABLE 4. Representative TT-HBV infection caused by OBI-derived, ID-NAT-negative blood component: HBV marker profile of blood donor responsible for the outcome shown in Table 3

Date of donation	Date of testing	Test results
Oct. 17, 2007*	Oct. 17, 2007 (screening)	Pool NAT negative, anti-HBc 2* (negative), anti-HBs negative ID-NAT negative
	Feb. 24, 2010 (repository sample tested in second lookback study)	
Mar. 27, 2008† (index donation)	Mar. 27, 2008 (screening)	Pool NAT negative, anti-HBc 2* (negative), anti-HBs negative ID-NAT negative (negative result reported to corresponding facility)
	Apr. 7, 2009 (repository sample tested in first lookback study)	
Feb. 05, 2010‡	Feb. 05, 2010 (screening)	Pool NAT negative, anti-HBc 15.4 S/CO§ (positive), anti-HBs negative ID-NAT positive (high probability of TT-HBV infection in Patient A reported to corresponding facility)
	Feb. 10, 2010 (donated blood sample tested in second lookback study)	

* RBCs derived from this donation were transfused to an HBsAg-negative patient. Patient continued to be HBsAg-negative until May 2008 when he died.

† FFP derived from this donation was transfused to patient shown in Table 3. Cocomponent (RBCs) processed from this donation was transfused to a patient who died of the primary disease soon after transfusion. Whether TT-HBV occurred remains unknown.

‡ This donation was rejected due to anti-HBc seroconversion and a second lookback study was conducted on the donation of October 17, 2007.

§ Because of very low HBV load in donated blood sample of February 5, 2010, HBV sequence was assessed in donor blood only at 193 bp (Nucleotides 475-667) of S region. HBV sequence in that region was identical except for nt. 654 between the blood samples from donor and patient on April 01, 2009.

TABLE 5. Blood components implicated in established TT-HBV infection*

Screening period	ID-NAT+/ID-NAT-					
	WP transmissions established			OBI transmissions established		
	RBCs	FFP	PC	RBCs	FFP	PC
50p-AmpliNAT	15/0	6/1	7/4	5/0	5/1	3/3
20p-AmpliNAT	8/2	0	4/4	7/0	5/1	0
20p-TaqScreen	2/0	0	1/1	5/0	6/1	0/1
Total	25/2	6/1	12/9	17/0	16/3	3/4
	WP plus OBI				All components	
	RBCs	FFP	PC	FFP + PC	WP	OBI
50p-AmpliNAT	20/0	11/2	10/7		28/5	13/4
20p-AmpliNAT	15/2	5/1	4/4		12/6	12/1
20p-TaqScreen	7/0	6/1	1/2		3/1	11/2
	100%	86%	33%		75%	85%
Total	42/2	22/4	15/13	37/17	43/12	36/7
	95%	85%	54%		78%	84%

* Ratios (%) in the two bottom rows represent rates of ID-NAT-positive events or effectiveness of ID-NAT implementation.

TABLE 6. Maximal values for ALT in patients with TT-HBV infection and total viral load contained in implicated components

	ALT	
	<1000	>1000
WP* (n‡)	24 (7)§	13
OBI† (n‡)	12 (2)§	19
Total viral load (copies/bag)		
WP		
n‡	21	10
Min	40	100
Max	260,000	560,000
Median	1,400	9,100
Mean	20,460	74,790
OBI		
n‡	8	16
Min	60	40
Max	6,240	19,200
Median	630	1,470
Mean	1,440	3,750
ID-NAT status		
Positive‡	30	28
Negative‡	6	4
Component types		
RBCs‡	19	16
FFP‡	7	13
PC‡	10	3

* Patients transfused with WP-related components include 11, 8, and 18 patients with malignant hematologic disorder, solid tumor, and others, respectively.

† Patients transfused with OBI-related components include 7, 11, and 13 patients with malignant hematologic disorder, solid tumor, and others, respectively.

‡ Numbers of patients.

§ Numbers in parentheses, patients with maximal ALT values of less than 100 IU/L.

|| Total viral load was calculated using viral concentrations in implicated donations and average plasma volume of each component type. When viral load was less than 100 copies/mL, total viral load in the component was calculated assuming that viral concentration is logarithmically distributed between 1 and 100 copies/mL.

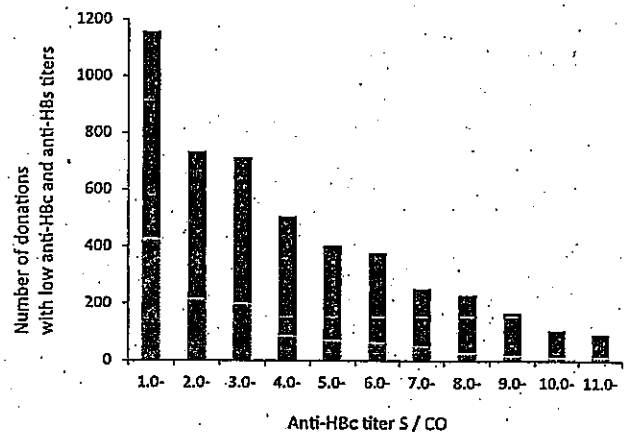


Fig. 3. Number of donations screened by ID-NAT trial categorized by anti-HBc titer. All donations tested had low anti-HBc (S/CO 1.0-11.9) and anti-HBs (<200 IU/L) titers and were qualified serologically based on algorithm applied at JRC blood centers. Donations verified to be ID-NAT-positive were disqualified. (■, □) donations with anti-HBs titers of more than and not more than 10 mIU/mL, respectively.

20p-TaqScreen period accounted for 15% (2/13) of all OBI-related infections (Table 2).

We estimated how many more WP-related TT-HBV infections would be prevented by introducing ID-NAT. The current screening NAT yield (30 donations/year or 5.7/million, Table 2) was multiplied by the ratio of the interval between ID-NAT and 20p NAT detection (11.2 days) to that between 20p NAT and HBsAg detection (9.7 days). We then deduced that 34.6/year or 6.6/million more viremic donations would be captured by ID-NAT. The number of ID-NAT-negative WP donations was calculated separately for each component type. Based on the plasma volume of each component (20, 200, 240, 450, and 120 mL for RBCs, PC, FFP-3, FFP-5, and FFP1.5,

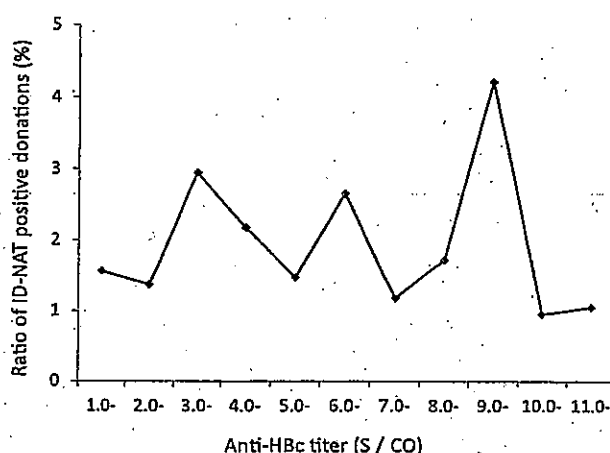


Fig. 4. Ratios (%) of ID-NAT-positive donations with low anti-HBc and anti-HBs titers relative to anti-HBc titer.

respectively), the deduced intervals between 1 copy/bag and ID-NAT detection were 16.3, 24.9, 25.5, 28, and 23 days, respectively. The ratio of the number of those components issued to hospitals is 6.3:2.2:2.1:0.7:0.1. The incidence of ID-NAT-negative WP donations calculated from these data was 59.5/year or 11.4/million. Adding ID-NAT-positive WP donations (34.6/year or 6.6/million), current risk related to WP donations amounts to 94.1/year or 18.0/million. The effect of ID-NAT on the reduction of all WP donation would be 37% (6.6/18.0). If the infectious risk (50%) of ID-NAT-positive, screening NAT-negative WP-related components is also applied to ID-NAT-negative WP-related components, the total number of WP-related TT-HBV infections would be 47.1/year or 9.0/million. Together, these estimates for WP- and OBI-related TT-HBV infections indicate that 94.1/year or 17.9/million TT-HBV infections are likely to occur in Japan.

DISCUSSION

Infection with HBV results in a wide spectrum of clinical manifestations ranging from asymptomatic liver dysfunction with only slightly elevated transaminase levels or acute self-limiting hepatitis to chronic hepatitis that in some patients progresses to cirrhosis, liver failure, or hepatic cell carcinoma. In rare cases, HBV infection can cause fulminant hepatitis that is associated with high mortality. Fulminant hepatitis in Japan is frequently associated with primary infection by HBV carrying precore or core-promoter mutations.^{12,13} These HBV mutants are frequently found among chronic HBV carriers^{14,15} who typically have an anti-HBc-positive serostatus. To prevent fulminant hepatitis arising as a result of blood transfusion,¹⁶ the JRC incorporated anti-HBc testing into blood screening in 1989.

The agglutination method had been used for all serologic testing at JRC blood centers before 2008. Although this method was somewhat insensitive to HBsAg, it could semiquantify anti-HBc. Thus, the cutoff point for the anti-HBc titer had been set at 2^6 , and donations with an anti-HBc titer of at least 2^6 and an anti-HBs titer of less than 200 mIU/mL were disqualified.¹⁷ Although this anti-HBc testing had essentially prevented transfusion-transmitted fulminant hepatitis since 1989,¹⁸ reports of fulminant or acute severe hepatitis continued for an additional 7 years. These conditions were attributed to transfusion with components with a 2^5 anti-HBc titer.¹⁸ Consequently, the JRC lowered the anti-HBc cutoff from 2^6 to 2^5 in 1997. The agglutination method for serologic screening was replaced in 2008 with CLEIAs, which can also semiquantify anti-HBc. The policy described above is maintained in the algorithm for HBV screening with CLEIA; the range defined as a low anti-HBc titer includes S/CO values between 1.0 and 11.9, and donations with anti-HBc S/CO values within this range are currently accepted.

The highly sophisticated strategy of multiplex NAT was designed to decrease the incidence of TTI. Implementing HBV NAT into blood screening was important in Japan mainly because of the unsatisfactory sensitivity of the standard agglutination method to HBsAg. The JRC implemented multiplex NAT targeting HBV DNA, HIV RNA, and HCV RNA during 1999⁶ and improved the sensitivity of the test at three points. The 98 infections described herein had been confirmed as TT-HBV since the introduction of 50p-AmpliNAT in 2000. The HBV genome was not detected in donor repository samples of 587 (74.4%) suspected TT-HBV infections. The JRC has informed the appropriate physicians of the ID-NAT results of viral detection that imply a low or high probability of TT-HBV infection.

In parallel with the increase in the screening NAT sensitivity, the incidence of WP-related TT-HBV infection has decreased as predicted, whereas that of OBI-related TT-HBV infection has not decreased (Fig. 2). To explain the increasing number of OBI-related TT-HBV infections, the increase in the sensitivity of NAT used in JRC laboratories for retrospective studies might have helped to identify TT-HBV infections, thus sustaining the number of OBI-related TT-HBV infections despite improvements in screening NAT sensitivity.⁴

This consideration could encourage the speculation that most of the 587 infection reports that had been excluded from established TTI (Fig. 1) based on negative results from repository samples might have been confirmed as TT-HBV had more sensitive NAT and a larger sample volume been analyzed. With regard to this notion, the outcomes of recent hemovigilance for TT-HBV are described below. During the 20p-Iaqscreen period, the JRC received 61 clinical reports of possible HBV-TTI. Seventeen were determined as TTI, among which, three

repository samples were ID-NAT negative. Historical HBV infection was confirmed in 10 patients by retesting pretransfusion samples. Results from HBV tests of posttransfusion samples from five patients were false positive. The possibility of TT-HBV was ruled out in two patients related to ID-NAT-negative donations because repeated blood donations from two of two and three of three implicated donors were not sero- or NAT-converted. The remaining 27 patients related to ID-NAT-negative donations are inconclusive for TTI as follow-up studies have not yet been completed. Some of the 587 reported infections had been confirmed to be associated with passive anti-HBc transfer from infused components. Thus, it is unlikely that a considerable proportion of the infections excluded from the TTI category were real TT-HBV infections.

Among 19 patients with TT-HBV infections associated with ID-NAT-negative donations, 17 (89%) of them were caused by transfusion with FFP or PC that contained a larger plasma volume (120 to 450 mL) than RBCs (20 mL). In contrast, 37 (47%) were caused by FFP or PC among 79 infections associated with ID-NAT-positive donations (Table 5). This finding suggests that because HBV infectivity is extremely high, the relationship between infectivity and plasma volumes contaminated with HBV could only be established in the era of ID-NAT screening when the viral load in the donation is low enough to escape ID-NAT screening. This might explain why we could not previously establish such a relationship using viral loads around the sensitivity of the pool-based NAT system or serology.⁷ If ID-NAT is introduced as routine screening, it will prevent 75 and 85% of WP- and OBI-related infections. In particular, all RBC-related TT-HBV could be prevented because of the small plasma volume involved. The finding also suggests that novel viral reduction technologies^{20,21} could be an attractive strategy to decrease the incidence of TT-HBV because these technologies are presently more applicable to FFP or PC than to RBC.

The maximal ALT levels of patients with TT-HBV infection showed that transfusion with components harboring an extremely low HBV load that escaped NAT screening is not necessarily associated with mild clinical illness. This seems particularly true for OBI-related infection (Table 6). The frequency with which transfusion causes severe hepatitis (i.e., ALT > 1000) is significantly higher for OBI- than for WP-derived components. Moreover, OBI-derived components tend to cause severe hepatitis despite lower total viral loads compared with those in the WP-derived components. These findings should be further substantiated by analyzing samples from patients that are regularly obtained after transfusion because most of the maximal ALT values described in this article were found after occasional sampling.

Three patients died of TT-HBV fulminant hepatitis caused by transfusion with blood that had escaped NAT

screening. Two of them were notably caused by transfusion of OBI-derived RBCs, and the other was caused by an ID-NAT-negative WP donation. Although a larger plasma volume might generally be required to establish TT-HBV infection under the NAT screening system, plasma volume or the total infused viral load might not be determining factors in fulminant hepatitis. Although viral genome mutations such as those in precore or core-promoter regions are frequently associated with the development of fulminant hepatitis in Japan,^{12,13} other crucial factors have not clearly been demonstrated despite considerable investigation.

The JRC accepted 5.3 million donations in 2010, of which 4.9% (261,000) was anti-HBc reactive (Table 1), 0.19% (10,000) was rejected because of high anti-HBc and low anti-HBs titers. Another 3.4% (182,000) was accepted because of high anti-HBs titers (≥ 200 IU/L). The notion that blood components with an anti-HBs titer of more than 100 IU/L are not infectious is generally accepted.²²⁻²⁵ The relationship between anti-HBs titer and TT-HBV infection will be discussed elsewhere (manuscript in preparation). Importantly, 1.3% of donations (69,000) with low anti-HBc and anti-HBs titers were accepted, and this category included all donations to which OBI-related TT-HBV infections were attributed. Our ID-NAT trial verified that 1.94% of the donations with low anti-HBc and anti-HBs titers were HBV DNA positive.²⁶ Accordingly, an estimated 1339/year or 252/million viremic OBI donors and 47/year or 8.9/million TT-HBV infections caused by OBI-derived components would be missed by the current screening algorithm. When estimates for WP-related TT-HBV infections are included, the calculated number of TT-HBV infections was 94.1/year or 17.9/million. Whole blood withdrawn from donors in Japan is split into RBCs and FFP, and the total number of components processed averages 23% more than the number of donations. However, because of outdated and rejection by testing or processing problems, the number of components finally issued by JRC becomes almost the same as the number of donations. Therefore, the calculated number of TT-HBV infections was not significantly influenced by the issue of splitting.

The considerable discrepancy between the estimated and established TT-HBV incidence per million (8.9 to 1.49 and 9.0 to 0.46 for OBI-related and WP-related infections, respectively) might be due to the following factors. A clinical manifestation of HBV infection is often unclear in patients transfused with blood components harboring a low viral load and low proliferative ability. Physicians might thus be likely to overlook infection under such circumstances. Medical practitioners are not compliant with national guidelines for lookback investigations. Indeed, only 30% to 40% of transfused patients were reportedly traced for TTI even after the guidelines were established.²⁷ A considerable proportion of patients who receive blood

transfusions die before TTI evaluation.²⁸ In fact, when we inquired about the outcomes of transfusions with components containing verified HBV at medical facilities, 99 (42%) of 238 patients who had been transfused with such components had already died (JRC data from 2009 to 2010). The transmissibility of ID-NAT-positive donations might require reevaluation because of the low numbers of patients analyzed in the previous study⁷ (30 and 22 for OBI- and WP-related cases, respectively). The fact that a large proportion of elderly patients are immune to HBV due to prior infection might also contribute to the low figure for established TT-HBV and, finally, anti-HBs in cotransfused components neutralizes HBV. Classified WP donation that is anti-HBs positive and could be attributed to possible vaccine breakthrough infection or anti-HBc-negative chronic OBI could also be a factor influencing infectivity. However, we have not encountered any implicated WP donations with anti-HBs among established TT-HBV infections.

Because of the high probability of a residual risk of TT-HBV, novel strategies that reinforce the safety of blood components but do not damage the blood supply should be implemented. Transfusion with ID-NAT-negative infectious components currently cause 15 and 25% of OBI- and WP-related TT-HBV infections, respectively (Table 2), and screening with ID-NAT would interdict 85 and 75% of these infections, respectively (Table 5). With respect to this, the ID-NAT screening of only donations with low anti-HBc and anti-HBs titers that are currently qualified has been suggested.²⁹ However, screening with ID-NAT might not be as effective as expected. For example, the variability in viral load in individuals with OBI might allow persistent OBI-related TT-HBV infection; some individuals might have an intermittently elevated viral load.³⁰⁻³³ Such donations could be identified as HBV positive only when the viral load exceeds the detection threshold of ID-NAT screening. Alternatively, the detection of intermittent viremia might reflect the stochastic phenomenon inherent in NAT technology, particularly at very low viral concentrations. Moreover, one report describes a donor in whom viral load increased in blood samples over a period of several years.³⁴ Nine among 48 blood donations from this donor were ID-NAT positive, and two of four ID-NAT-positive and three ID-NAT-negative blood transfusions had caused TT-HBV infections. The diverse fluctuation of viremia described above has supposedly hindered the efficient detection of viremic donations by pool-based NAT screening,³⁵ which is predictable even in the event of ID-NAT screening. Table 5 shows that ID-NAT is not sensitive enough in 16% of established OBI-related transmission events although most of those events are caused by FFP or PC transfusions and ID-NAT screened RBC transfusions are relatively safe. Moreover, although viremia is considered undetectable in most individuals with OBI, this assumption might be

dependent on the sensitivity of the NAT used; a considerable number of donations might have viremia with a viral load below the ID-NAT detection limit.

Another strategy that might increase the safety of OBI-derived donations could be to accept only those OBI-derived donations with a profile that is safer than the current standards, if such a profile can be found and systematically applied. We initially expected to find that OBI donations with a very low anti-HBc titer would be safer based on ID-NAT. However, the finding from the ID-NAT trial was that the frequency of viremia does not correlate with anti-HBc titers in the range of S/CO 1.0 to 11.9. Therefore, we concluded that the risk of TT-HBV infection will not be mitigated by implementing a strategy that qualifies only donations with very low anti-HBc titers such as S/CO between 1.0 and 3.0.

We speculated during 2003 that more than 4% of donations would be disqualified if the anti-HBc cutoff were set at 2¹, that is, if all donations with low anti-HBc and anti-HBs titers are rejected. We thought that the loss of so many donations would cause catastrophic damage to the blood inventory and thus that cutoff was not implemented. However, based on current data, the number of donations received in 2010 with low anti-HBc and anti-HBs titers was 69,000, which accounts for 1.31% of all donations in Japan. Given this ratio, we consider that to eliminate all donations with low anti-HBc and anti-HBs titers is feasible. We verified that severe hepatitis is caused more often by OBI- than WP-derived blood. The fact that two patients died of fulminant hepatitis related to OBI-related donations is also serious. Rejecting this category of donations would eliminate nearly all those harboring a risk of OBI-related infection.²⁶ However, a slight, but distinct risk of TT-HBV infection might persist because a small fraction of OBI donors have an anti-HBc titer of less than 1.0 S/CO, and these donors as well as NAT WP donors present a TT-HBV risk.³⁶ A committee of the Ministry of Health, Labour and Welfare of the Japanese government has just discussed and authorized the implementation of a new policy in which all donations with low anti-HBc and anti-HBs titers would be rejected.

In conclusion, ID-NAT screening of donations with low anti-HBc and anti-HBs titers revealed that nearly 2% of these donations were associated with low-level viremia and that viremia was identified over the entire range of anti-HBc titers. Importantly, anti-HBc titer did not correlate with the frequency of viremia. The elimination of all donations with low anti-HBc and anti-HBs titers would be important to any strategy aimed at preventing OBI-related TT-HBV infections in countries such as Japan that have a slightly elevated HBV prevalence in blood donations. If this strategy is implemented, the only acceptable donors with OBI in Japan will be those with high anti-HBs titers (≥ 200 IU/L).

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CONFLICT OF INTEREST

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医薬品 研究報告 調査報告書

識別番号・報告回数	報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
		2013. 8. 12	該当なし	
一般的名称	新鮮凍結人血漿	Allain JP, Mihaljevic I, Gonzalez-Fraile MI, et al. Transfusion. 2013 Jul;53(7):1405-15. doi: 10.1111/trf.12096. Epub 2013 Jan 30.	公表国 英国	
販売名(企業名)	新鮮凍結血漿-LR〔日赤〕(日本赤十字社) 新鮮凍結血漿-LR〔日赤〕成分採血(日本赤十字社) 新鮮凍結血漿-LR〔日赤〕J120〔日本赤十字社〕 新鮮凍結血漿-LR〔日赤〕J240〔日本赤十字社〕 新鮮凍結血漿-LR〔日赤〕J480〔日本赤十字社〕	研究報告の公表状況		
研究報告の概要	○オカルトB型肝炎ウイルス(HBV)感染供血者由来の血液製剤の感染性 背景:オカルトHBV感染(OBI)は、欧州において供血の1,000~50,000例に1例の割合で確認されている。OBI供血者由来の血液製剤の感染性を調べることを目的として研究を行った。 研究デザインと方法: OBI供血者の過去の供血の受血者について遡及または追跡調査を行った。同意が得られた供血者と受血者を対象に、血清学的及びゲノム研究を実施し、感染性に影響があると考えられる複数の変数の変数を調べた。 結果: 供血者-受血者のペア105組中45組(42.9%)は、過去のHBV感染の証拠として、HBc抗体を保有していた。HBc抗体保有率15%(ドイツとスペインの65歳以上)を差し引くと、補正感染率は28%であった。HBc抗体保有率は、HBs抗体陰性OBI血液製剤の受血者(ワクチン未接種)では44人中28人(63.8%)に上昇した。対照的に、HBs抗体陽性OBI血液製剤の受血者は、26人中4人(15.4%)のみがHBc抗体陽性であった。HBs抗体陽性の製剤による感染率は、輸血された血漿量に依存していた(新鮮凍結血漿[200mL]の感染率は85~100%、血小板製剤[血漿50mL]は51%、赤血球製剤[血漿20mL]は24%)。OBI 供血のHBV DNAの50%最小感染量は1,049(117~3,441)コピーと推定された。11組の供血者-受血者ペアから得られたpre-S/S領域のゲノムを解析したところ、ウイルス株の配列相同性は99%とした場合、10例が輸血感染と確認され、1例が否定された。 結論: OBI供血者由来の血液製剤によるHBV感染リスクは高く、このリスクはHBs抗体の存在とウイルス量によって異なる。そのため、疫学的状況に応じて、HBc抗体及びHBV NATスクリーニング等の安全対策を講じることが正当化されるだろう。			
報告企業の意見	オカルトHBV感染供血者からの血液製剤の感染性を調査した結果、感染リスクは高く、このリスクは製剤中のHBs抗体の存在及びウイルス量によって異なったとの報告である。			
今後の対応	日本赤十字社では、化学発光酵素免疫測定法(CLEIA)によりHBs抗原、HBc抗体検査を実施することに加えて、精度を向上させたNATシステムを導入し、20プールでスクリーニングNATを行い、陽性血液を排除している。なお、2012年8月より抗体検査の判定基準を強化し、HBc抗体(C.O.I.)1.0以上かつHBs抗体200 IU/L未満の感染既往を示す血液を不適としている。また、個別スクリーニングNATの導入についても検討を行っている。HBV感染に関する新たな知見等について、今後も情報の収集に努める。			
	使用上の注意記載状況・その他参考事項等 新鮮凍結血漿-LR〔日赤〕 新鮮凍結血漿-LR〔日赤〕成分採血 新鮮凍結血漿-LR〔日赤〕J120 新鮮凍結血漿-LR〔日赤〕J240 新鮮凍結血漿-LR〔日赤〕J480 血液を介するウイルス、細菌、原虫等の感染、vCJD等の伝播のリスク			

TRANSFUSION COMPLICATIONS

Infectivity of blood products from donors with occult hepatitis B virus infection

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BACKGROUND: Occult hepatitis B virus (HBV) infection (OBI) is identified in 1:1000 to 1:50,000 European blood donations. This study intended to determine the infectivity of blood products from OBI donors.

STUDY DESIGN AND METHODS: Recipients of previous donations from OBI donors were investigated through lookback (systematic retrieval of recipients) or traceback (triggered by clinical cases). Serologic and genomic studies were undertaken on consenting donors and recipients. Multiple variables potentially affecting infectivity were examined.

RESULTS: A total of 45 of 105 (42.9%) donor-recipient pairs carried antibodies to HBV core (anti-HBc) as evidence of previous HBV infection. Subtracting 15% of anti-HBc population background, the adjusted transmission rate was 28%. Anti-HBc prevalence increased to 28 of 44 (63.8%) in unvaccinated recipients receiving anti-HBs-negative OBI blood products. In contrast, four of 26 (15.4%) recipients of anti-HBs-positive products were anti-HBc positive.

Transmission with anti-HBs-negative products depended on volume of plasma transfused (85%-100% with 200 mL of fresh frozen plasma [FFP], 51% with 50 mL in platelet concentrates [PCs], and 24% with 20 mL in red blood cells [RBCs], $p < 0.0001$ FFP vs. RBCs). The 50% minimum infectious dose of OBI HBV DNA was estimated at 1049 (117-3441) copies. Donor and recipient strains sequence homology of at least 99% confirmed transfusion-transmitted infection in 10 cases and excluded it in one case.

CONCLUSION: Blood products from donors with OBI carry a high risk of HBV transmission by transfusion. This risk is dependent on presence of anti-HBs and viral dose. This may justify safety measures such as anti-HBc and HBV nucleic acid test screening depending on epidemiology.

In the past 5 years, nucleic acid testing (NAT) of hepatitis B virus (HBV) genome has been implemented in most countries of Europe applied either in plasma pools of various sizes (6-96) or in individual donations.¹ Preliminary data collected in Europe, South Africa, and South East Asia indicated that, to be effective, HBV NAT needed maximum sensitivity since for the majority of both window period infection and late stage occult HBV infection (OBI), levels of circulating viral

ABBREVIATIONS: ID₅₀ = 50% minimum infectious dose; OBI = occult hepatitis B virus infection; PC(s) = platelet concentrate(s); VL = viral load.

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genome were very low (<100 IU/mL).²⁻⁴ OBI is defined as the presence of circulating HBV DNA in the absence of detectable HBV surface antigen (HBsAg) excluding the window period. Little data are available on the clinical relevance of these HBV DNA-positive donations in the presence of antibodies to HBV. In a context of anti-HBc screening associated with NAT in minipools of 50, the Japanese Red Cross estimated OBI infectivity by transfusion at 3%.⁵ In Western Europe, a few cases of clinical transmission of HBV led to traceback studies suggesting infectivity of anti-HBc only-positive donors⁶ and in one case with anti-HBs.⁷ In high endemic areas where both HBV natural contact and systematic HBV vaccination are frequent,⁸⁻¹⁰ several studies reported the difficulties of lookback exercises (identification and testing of recipients of donations preceding an identified seronegative HBV NAT-positive donation).

In this study, lookback and traceback cases from five European countries were assembled to evaluate the still uncertain level of infectivity of blood products prepared from OBI blood donations. Factors potentially influencing infectivity and pathogenicity such as viral load (VL) transfused, the presence of anti-HBs in donor or recipient blood, and the potential role of chemotherapeutic or immunosuppressive drugs taken by recipient were evaluated.

MATERIALS AND METHODS

Testing sites

The collaboration was established between seven blood centers from Croatia, Denmark, Germany, Poland, and Spain and three virology laboratories in Giessen and Langen, Germany, and in Cambridge, UK. HBV DNA testing was implemented in 2005 in Poland, 2008 in Spain, and 2009 in Denmark. Croatia and Germany traceback cases were identified before implementation of HBV DNA testing between 2003 and 2010. Prevalence of OBI in blood donations was previously reported.²

Diagnosis of OBI

Blood donor samples were screened for HBsAg using the PRISM system or Architect (Abbott Laboratories, Delkenheim, Germany) or Vitros (Ortho Clinical Diagnostics, Johnson & Johnson, Sollentuna, Sweden) and for HBV DNA with Ultrio on Tigris (Novartis, Emeryville, CA) applied to individual-donor samples, the Cobas s201 (Roche, Basel, Switzerland) as previously described,¹¹ or validated in-house NAT applied to pools of 6 to 96 samples. Reactive samples were retested with virus-specific probes identifying the virus responsible for the initial signal. HBV-reactive samples were confirmed with real-time polymerase chain reaction (PCR), single-round or nested PCR assays. Donor samples carrying HBV DNA

but HBsAg negative were further tested for antibodies to HBV core (anti-HBc) and anti-surface antigen (anti-HBs). Antibody-negative donors carrying HBV DNA were not included in this study because the infectivity of window period cases has been previously demonstrated in humans.⁵

Selection of blood product recipients for infectivity studies

All studies conducted had received local ethical approval. In lookback, donations from repeat donors preceding the OBI index donation were identified as well as the recipients of the corresponding products. The index blood identified as carrying OBI was discarded and the donor deferred from donation. Blood centers contacted the hospitals and collected information regarding the identity, records, and personal details of the patients. Living patients were contacted and informed of a potential HBV transmission by transfusion. They were informed and asked to sign an informed consent to collect a blood sample to test for HBV markers.

In traceback, hospitals contacted the blood centers when a clinical case of acute HBV infection was identified in a transfused patient. Archived samples of implicated blood donations were tested for HBV markers. If a donor was suspected, complete HBV work-up was conducted and recipients of other donations from the suspected donor were also tested for the presence of HBV markers. The recipients of all identified blood components from these donations were traced and tested if alive and consenting.

Donor and recipient sample supplemental testing

Recipient samples were tested for HBV markers: HBsAg, anti-HBc, anti-HBs, and HBV DNA and, when reactive, were confirmed in Giessen, Langen, or Cambridge laboratories as described.^{2,12} Whenever possible, VL was obtained by real-time PCR assay or in-house PCR and estimated in copies or international unit (IU)/mL (ratio 5 to 1). Standard or nested PCR was used to amplify full genome, basal core promoter/precore, pre-S/S region, or S region. Amplicons were sequenced to identify strain genotype and specific mutations, particularly in the S protein major hydrophilic region and determine the genetic relationship between donor and recipient strains as previously described.^{13,14}

Additional information collected from recipients

Hospitals were asked to provide additional information: date of transfusion and the type of product transfused, the date of blood sample collection within the lookback or traceback exercises, possible occurrence of clinical hepatitis, and the underlying disease. Drugs received were also recorded to evaluate the immune status of the patient and

TABLE 1. Overall scope of the study

Items	Number
OBI donors	24
Lookback donors	19
Traceback donors	5
Median (range) age (years)	46.5 (29-69)
Male donors	19
Female donors	5
Number of donations	251
Number of recipients identified	224*
Number of recipients tested	104 (46.9%)
Number of blood products transfused	105†
FFP	19
PC	12
RBCs	74

* In 73 cases, either the recipient or the product transfused was not identified. In most cases, when the recipient was identified as having died (46 patients), further search was not undertaken.

† In one case, one RBC unit and one PC from the same donor went to the same recipient.

classify them into immunocompetent or immunodeficient. Presence of anti-HBs without anti-HBc before transfusion was considered a marker of vaccination. Recipients older than 30 years without anti-HBs were assumed to be unvaccinated since HBV vaccine became common in exposed groups, newborn, or adolescents in the late 1980s unless explicit data on vaccination was available.

Statistical analysis

Categorical variables were compared using Fisher's exact test and, for continuous variables, the nonparametric Mann-Whitney test. A logistic regression was performed (using the glm function from the R statistical software, <http://www.r-project.org/>). The 50% minimum infectious dose (ID_{50}) was estimated by Probit analysis¹⁵ using a statistical package in Probit analysis (SPSS, SPSS, Inc., Chicago, IL), a statistical treatment of the sigmoid response curve. The log value of the estimated amount of virus infused is plotted on the X-axis and the infection rate on the Y-axis. The infection rate was assumed 1.0 or 100% in each of the anti-HBc-positive recipients and 0% in each of the anti-HBc-negative recipients. From the probability curve the ID_{50} (and 95% confidence interval [CI]) can be estimated.

RESULTS

Table 1 shows the distribution of OBI blood donors identified in the collaborating centers through either lookback or traceback process. Each donor gave a mean of 10.5 donations (range, 2-22 donations) that were transfused to 224 recipients. In a large proportion of cases, the plasma from these donations was directed toward fractionation rather than therapeutic fresh-frozen plasma (FFP). Red

blood cells (RBCs) were transfused in 79% of cases. Blood products from these donations were transfused to recipients of whom 46.4% were identified as alive and consenting to being tested for HBV markers. Forty-six recipients had died by the time the lookback or traceback exercise was initiated and no information on the cause of death was available, five declined participating, and 69 could not be found.

All 24 index donor samples were reactive with the NAT screening assay and identified as HBV DNA carriers by discriminatory assay. All index samples contained anti-HBc confirmed by two different assays in two independent laboratories and nine samples contained anti-HBs with levels ranging 20 to 160 IU/L. In 10 of the 24 donor strains, sequences were obtained in the pre-S/S region classifying four strains as genotype A2 and six strains as Genotype D1. In 11 donor samples, VL ranged between less than 10 and 198 IU/mL. In nine donors of whom multiple samples were available, HBV DNA was consistently detected in six donors and fluctuated in three donors ranging between negative and 75 IU/mL, negative and 12 IU/mL, and 1.8 and 56 IU/mL, respectively.

Analysis of HBV markers

The presence of anti-HBc with or without anti-HBs in recipients was taken as indicator of HBV transmission by transfusion, irrespective of potential recipient infection before transfusion or of otherwise acquired infection during the interval between transfusion and recipient sample collection. This interval ranged between 2 months and 8.5 years (median, 3 years). In the lookback group, 25 of 68 recipients (36.8%) carried anti-HBc with or without anti-HBs and 54.1% (20/37) in the traceback group (Table 2). Forty-five of 105 recipients (42.9%) of OBI products carried anti-HBc, 17 of 62 (27.4%) tested for HBV DNA carried this marker (five HBsAg positive), and 15 of 105 (14.3%) carried anti-HBs as only HBV marker. Information regarding HBV vaccination was available in seven of these 15 cases and they had all been vaccinated.

Factors potentially influencing HBV transmission

The number of HBV genome copies in the plasma transfused (viral dose) was a prime factor. The volume of plasma each recipient received with the transfused product was estimated according to European standards at 200 mL in FFP, 50 mL in platelet concentrate (PC) from whole blood, and 20 mL per RBC unit. The rate of possible HBV infection (anti-HBc with or without anti-HBs) increased from 23/74 (31.1%) to 6/12 (50%) to 16 of 19 (84.2%) for RBCs, PCs, and FFP, respectively (Table 3). The difference in transmission rate between RBCs and FFP was highly significant ($p \leq 0.0001$), even more when only anti-HBs-negative blood products were considered

TABLE 2. HBV serologic markers in recipient according to mode of presentation and product transfused

Mode	Product	Donor-recipient pairs (%)	Anti-HBc+, anti-HBs+	Anti-HBc+, anti-HBs-	Anti-HBc-, anti-HBs+	Anti-HBc-, anti-HBs-	Partial data	Possible infection (%)
Lookback	RBCs	46 (48.4)	8	2	7	24	5	10 (21.7)
	PC	6 (70.0)	1	1	1	3	0	2 (33.3)
	FFP	16 (73.9)	9	4	1	2	0	13 (81.3)
Total		68 (54.0)	18	7	9	29	5	25 (36.8)
Traceback	RBCs	28 (70.0)	11*	2	5	9	1†	13 (42.9)
	PC	6 (100.0)	4	0	1	1	0	4 (66.7)
	FFP	3 (60.0)	1	0	0	0	1†	3 (100.0)
Total		37 (72.5)	16	3	6	10	2	20 (54.1)‡
Grand total		105	34 (32.4)	10 (9.5)	15 (14.3)	39 (37.1)	7 (6.7)	45§ (42.9)

* One patient received both RBCs and PC from the same donor; possible infection was attributed to PC.

† One patient was anti-HBc positive but anti-HBs not tested.

‡ Considering 21 anti-HBc-positive recipients.

§ Includes 33 anti-HBc+/anti-HBs+, 10 anti-HBc only, and two anti-HBc- with untested anti-HBs.

(Table 4). Fifteen of 15 FFP (100%), four of six PCs (67%), and nine of 23 (39%) RBC unvaccinated recipients of anti-HBs-negative products carried HBV markers.

In 17 transfused products VL was estimated. In 13 products, although HBV DNA was detected, the quantitative assay was either negative or below the assay limit of quantification (5 IU/mL). In the corresponding products, VL was considered below the assay limit of quantification and assigned this value divided by two (between 2 and 200 IU/mL). When multiple index and archived samples from a donor were quantified the geometric mean value was utilized to estimate the VL of products lacking direct quantification. Considering only unvaccinated recipients ($n = 63$) of anti-HBs-negative products, the projected viral dose transfused was 1032 copies ($n = 28$) in anti-HBc-negative and 2694 copies ($n = 35$) in anti-HBc-positive recipients, respectively (Fig. 1; $p = 0.003$) and the estimated ID_{50} as calculated by probit analysis was 1049 (117-3441) copies. However, in 17 recipient samples with directly quantified infused amount of HBV the ID_{50} was 535 copies (95% CI cannot be calculated) and the difference between the viral dose of 1500 copies (range, 180-56,000) observed in nine infected and 900 copies (range, 200-19,800) in eight noninfected patients was not significant ($p = 0.29$).

The levels of anti-HBs in donors ranged between 20 and 160 IU/L (median, 40 IU/L). As shown in Table 4, four of 26 (15%) and 41 of 80 (51%) recipients of blood products from OBI donors with or without anti-HBs, respectively, carried anti-HBc ($p = 0.013$), and an even larger difference in potential transmission rate was found when only unvaccinated recipients were taken into account (15% vs. 64%, $p < 0.0001$). Unlike in recipients of anti-HBs-negative OBI blood the amount of transfused plasma had no impact on the anti-HBc prevalence in recipients of anti-HBs-positive OBI blood. Only three of 19 RBC recipients (17.6%) carried anti-HBc and, significantly, none of three FFP recipients were infected.

While 15 of 15 (100% of FFP recipients of anti-HBs-negative OBI blood) were infected, none of three FFP recipients of anti-HBs-positive OBI blood had markers of HBV infection. We therefore assumed that four of 26 (15.4%) recipients of anti-HBs-positive OBI blood products carried anti-HBc because of HBV infection unrelated to the OBI transfusion. When this percentage of 15.4% was taken as the background anti-HBc prevalence, the transmission rate of anti-HBs-negative OBI blood products given to the unvaccinated recipients (>30 years) could be estimated by subtraction of 15.4% from the anti-HBc prevalence. The 15% background prevalence in the older recipient population is in line with epidemiologic data (see discussion). With this assumption being compatible with our data assuming negligible contribution of transmission risk of anti-HBs-positive OBI blood, the transmission rate by anti-HBs-negative OBI blood products

TABLE 3. Serologic reaction patterns in recipients of blood products from donors with OBI

Product	Tested recipients	Possible transmission anti-HBc+, anti-HBs+/-	Probable immunity anti-HBc-, anti-HBs+	No infection anti-HBc-, anti-HBs-
RBCs	74	23 (31.5)*	12	39
PC	12	6 (50.0)	2	4
FFP	19	16 (84.2)†‡	1	2§
Total	105§	45 (42.9)	15 (14.3)	45 (42.9)¶

* This number includes one recipient with incomplete data but one recipient having received RBCs and PC from the same donor was subtracted.

† One recipient with incomplete serology but anti-HBc positive was considered possible transmission.

‡ Highly significant difference between infectivity of RBCs and FFP $\chi^2 = 21.7$; $p = 0.0003$.

§ One patient carried 13 IU/L anti-HBs and was probably vaccinated to HBV; the other received anti-HBs-positive FFP.

¶ Eight recipients with incomplete serologic data are not included but being either anti-HBc or anti-HBs negative are considered not infected.

TABLE 4. Impact of anti-HBs in transfused products or in vaccinated recipients on OBI infectivity

Category	Anti-HBc positive in recipients/total (%)			
	All products	RBCs	PC	FFP
All*	45/105 (42.9)	23/74 (31.5)†	6/12 (50.0)	16/19 (84.2)
All receiving anti-HBs-positive blood	4/26 (15.4)	3/19 (17.6)	1/4 (25.0)	0/3 (0)
All receiving anti-HBs-negative blood	41/80 (51.3)	20/55 (35.7)	5/8 (62.5)	16/17 (94.1)
Unvaccinated patients receiving anti-HBs-negative blood‡	28/44 (63.6)	9/23 (39.1)	4/6 (66.7)	15/15 (100)
Adjusted rate (%) of transmission after deduction of 15% background anti-HBc	48	24	51	85

* All donors identified as OBI carried anti-HBc and all recipients considered infected were anti-HBc positive.

† One RBC unit and PC unit were transfused to the same recipient; the RBCs were not counted.

‡ Recipients for whom no information regarding vaccination was available were excluded.

increases from 24, 51, and 85% in recipients of RBCs, PCs, and FFP, respectively (Table 4).

Immunodeficiency related to young age (premature infants or newborn) or to hematologic disorders and/or the drugs received for these conditions may cause susceptibility to lower infectious dose of HBV. Immune status was known for 61 of 105 recipients (58.1%) as estimated by the collaborators but very few details regarding the drug regimen were available. Of 36 recipients classified as "immunocompetent," 24 (66.7%) were anti-HBc positive after transfusion (six with anti-HBc only). In the "immunodeficient" group eight of 24 (33.3%) were anti-HBc positive after transfusion. This difference was significant ($p = 0.01$).

Prediction of OBI transfusion risk

A logistic regression was performed with presence of anti-HBc in the recipient as outcome. Potential predictors were presence or absence of anti-HBs in donors, vaccination, immunodeficiency (each with two levels), transfused product type (encoded as 1 for RBCs, 2 for PCs, and 3 for FFP), and donor HBV DNA (in IU/mL). Since copies of viral dose received is highly correlated with product, which is a much better predictor, it was dropped from further analysis and replaced by donor HBV DNA in the logistic regression model (Table 5). Only two variables are significant predictors of infection: donor anti-HBs ($p = 0.012$) and transfused product ($p = 0.00078$). As

shown in Table 5 the presence of donor anti-HBs reduces the risk of infection by approximately fivefold, while PC over RBCs or FFP over PC increases risk by approximately threefold (and consequently FFP over RBCs by approximately ninefold). Donor HBV DNA load, although not significant, contributes to improved prediction. The logistic regression can be used to predict whether a recipient will be positive for anti-HBc based on the above predictors. The performance of such predictor can be measured by the area under the receiver operator characteristic curve, which is 0.738. This means that the predictor score of an anti-HBc-positive individual will be above that of an anti-HBc-negative individual in 73.8% of cases making this a reasonably useful predictor in practice.

Genetic evidence of HBV infection by transfusion

In only 11 donor samples, sufficient length of the genome in the pre-S/S region was sequenced to determine HBV genotype. In 11 recipients from seven of these 11 donors, sufficient HBV DNA was present to amplify, sequence, and determine genetic homology between donor and recipient sequences (Table 6). In 10 of these paired sequences, nucleotide sequences were at least 99% homology. In one case, two donor and five recipient sequences were obtained (Fig. 2) forming a unique cluster with more than 99% homology. The 11th pair (D4569) donor and recipient strains were of different genotypes (A2 and C), excluding transfusion as the origin of the recipient infection.

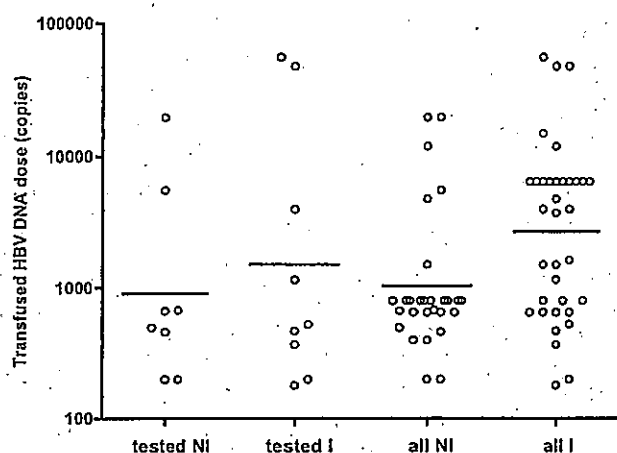


Fig. 1. Distribution of HBV DNA load transfused in noninfected (NI) and potentially infected (I) blood product recipients. Only recipients receiving anti-HBs-negative blood products and not vaccinated were included. The first group indicated as "tested" corresponded to donation samples involved in the case actually quantified by quantitative PCR. The second group indicated as "all" includes those of the first group and those where the VL of the involved donation was not actually quantified but extrapolated from other samples from the same donor that were quantified. Estimation of viral dose transfused is indicated under Materials and Methods. The horizontal bar indicates the geometric mean of the viral dose transfused for each subgroup. It is 1032 HBV DNA copies for noninfected recipients and 2694 copies for recipients carrying HBV markers (p test = 0.0033).

HBV mutants in OBI donors

Three of seven donors (G4-G6, Table 6) had mutations in the S gene, which were very relevant for the antigenicity of HBsAg. In G4, the mutation K160N changed one of the two HBsAg subtype determinants from wild type to an unknown specificity. In Case G5, four subsequent substitutions from Amino Acids 142 to 145 altered the group-specific determinant "a" profoundly. This caused false-negative results with some HBsAg assays, but most assays detected the transmitted mutant in the infected recipient who had a much higher viremia than the donor. The donor G6 had donated 42 times over 10 years. The five available FFPs from the past 10 months were analyzed for HBV quasi-species composition by cloning of the amplified DNA and sequencing eight to 20 clones. All clones coded for seven amino acid substitutions in the HBsAg loop, four of them altering determinant a and transiently up to four additional variable amino acid substitutions.

Clinical consequences of HBV transmission

The lookback procedure did not identify any of the 62 anti-HBc-positive recipients who had suffered from clini-

cally apparent hepatitis or chronic HBV infection with persistence of HBsAg over the average 2 years until the posttransfusion sample was collected. The only recipient who was acutely infected had a HBV genotype different from the Donor D4569 (Table 6). Thus, transmission of HBV from OBI leads in most cases to asymptomatic resolving infection with subsequent development of anti-HBc and anti-HBs. However, four index donor cases shown in Table 6 (G3-G5) were identified because recipients developed acute liver failure with all signs of acute HBV infection that was fatal in three of them. All three patients had some degree of immunologic disturbance. One patient had a sepsis soon after the transfusion of the infectious donation. The second patient had an unidentified type of hematologic disorder, and the third patient had autoimmune hepatitis. In two cases the interval between transfusion and fulminant hepatitis was 4 (G4) and 3 (G5) months after transfusion, strongly suggesting the connection between transfusion and hepatitis. Further, in all four cases transfusion transmission was confirmed by more than 99% identity between donor and recipient strain sequences.

DISCUSSION

Although HBV DNA was identified in 1:1000 to 1:50,000 donations depending on the epidemiology of HBV in different areas,^{1-4,9,11,16} the infectivity of blood units from OBI donors was not determined except in Japan where it seemed to be very low.⁵ In Europe clinical trace-back cases indicated a relatively high rate of transmission.^{6,12} Except for a preliminary report,⁶ no lookback data related to OBI infectivity have been reported in Europe. In high-prevalence Taiwan or Hong Kong, lookback studies emphasized the reliance on donor and recipient strain sequence homology to establish transfusion transmission when no pretransfusion sample was available.⁸⁻¹⁰

In this study, both lookback and traceback cases have been examined to compare the data collected in each situation. In relation with the large proportion of elderly patients and of patients with oncologic underlying conditions, nearly 40% of recipients identified died shortly after transfusion or did not accept to participate as previously reported (Table 1).^{17,18}

A pretransfusion sample being unavailable and molecular evidence of HBV donor-recipient strains identity being available in only 10% of cases, identification of possible HBV transfusion transmission relied on post-transfusion detection of anti-HBc. The concomitant presence of anti-HBs supported the likelihood of recent transmission, as this marker level tends to decline with time, particularly in older people.¹⁹ Such likelihood was lower when anti-HBc was the only marker of HBV infection (27% of cases). The sole reliance on the presence of anti-HBc in recipients without pretransfusion sample and tested

TABLE 5. Logistic regression analysis of factors influencing recipient infection status

Factor	Estimate	Relative risk (OR)	SE	p Value
Intercept*	-1.25	0.28 (0.10, 0.80)	0.53	0.018†
Anti-HBs (donor)	-1.51	0.22 (0.07, 0.72)	0.60	0.012†
Vaccination (recipient)	-0.65	0.52 (0.12, 2.23)	0.74	0.38
Product transfused	1.09	2.97 (1.57, 5.59)	0.32	0.0008‡
Viral dose (IU/mL)	-0.01	0.99 (0.97, 1.01)	0.01	0.20
Immunosuppressive drugs	0.04	1.04 (0.31, 3.52)	0.62	0.94

* The intercept indicates the baseline relative risk in this study, the OR of positive people in the study after taking account of predictors.

† $p < 0.05$.

‡ $p < 0.01$.

often several years after transfusion seems a major weakness in this study. However, it was the only feasible solution and several elements indicate that it has been a valid approach. 1) The study was conducted in populations where HBV infection is relatively low (<8% of anti-HBc and <1% of HBsAg prevalence); the risk of nosocomial infection is therefore limited. The recipient population being relatively old (median, 46 years) makes the risk of transmission through intravenous drug abuse or promiscuous sexual activity after hospitalization and transfusion relatively low. 2) The background anti-HBc prevalence in the population has been eliminated by the deduction of 15% anti-HBc originating from infection contracted before the implicated transfusion after correction for recipient age (see results and discussion below). 3) The data collected showing the close correlation between volume of plasma transfused and the presence of anti-HBc in recipients strongly supports the responsibility of infection by transfusion of anti-HBs-negative OBI blood. Further, this correlation did not exist in recipients of anti-HBs-positive OBI blood. 4) Finally in the limited number of cases where HBV DNA could be sequenced in donor and recipient more than 99% of homology clearly incriminated the OBI-carrying donor who, in all cases, was anti-HBs negative. The identification of one of 11 cases where sequence homology excluded transfusion as origin of the infection is in line with the 15% deduction applied to estimate the transmission risk of OBI blood in our study population.

For traceback cases infectivity of the transfused unit was not in doubt and this could have created a bias overestimating OBI infectivity. However, the rate of potential infection observed in a larger number of lookback cases was not considerably lower (36.8% vs. 54.1%) and the difference was not significant ($p = 0.16$, Table 2). Overall, the 42.9% rate of potential infection was unexpectedly high and infections from other sources were considered. No specific risk groups were identified. The background prevalence of anti-HBc in Croatia, Denmark, Germany, and Spain ranges between 5 and 8% in the general population.^{20,21} In Germany and Spain the prevalence of anti-HBc reaches 15% in the age group older than 65 years.²²

Since the majority of the patients in this study were of older age, a 15% lower adjustment seemed appropriate. Such adjustment was consistent with the prevalence of HBV markers in recipients of anti-HBs-containing blood components (15.4%). Therefore, the conservatively estimated rate of HBV OBI transmission is 28% overall and 22% for lookback cases. This is compatible with the 18.2% rate (two of 11 cases) identified in a Taiwanese study.⁸ The much lower transmission rate of 3% (one of 33) observed in Japan for transfusion of

blood components collected from OBI donors⁵ may be explained by the fact that in Japan donors with HBV DNA levels detectable by minipool NAT or with anti-HBc hemagglutination inhibition titer of greater than 16 were already excluded and many recipients were immune.

Three main specific elements potentially interfering with HBV OBI transmission are: 1) the viral dose (number of HBV genome copies/mL multiplied by the volume of plasma transfused); 2) the presence of anti-HBs either in the donor blood or passively acquired by simultaneously transfused blood components from anti-HBs-carrying donors or actively acquired in a vaccinated patient; or 3) the recipient immune status, which is variably impaired in neonates or patients receiving chemotherapy or immunosuppressive treatments.²³ For instance, cyclosporine or fludarabine in combination with anti-CD20 are known to cause severe immunodeficiency and HBV reactivation.^{24,25} These factors were examined overall.

The viral dose transfused to the recipient resulted in two independently significant factors: plasma volume in components ($p = 0.0003$) and VL. The projected geometric mean value of viral DNA received by transfusion in possibly infected and noninfected recipients was significantly higher in anti-HBc-positive recipients ($p = 0.003$). However, when only the actually measured viral dose infused in 17 recipients was compared, the difference in geometric mean viral dose between infected and noninfected recipients was not significant while the dose ranges overlapped (Fig. 1). The ID_{50} calculated by probit analysis (1049; 117-3441) was, however, not significantly different (Tables 3 and 4; Fig. 1). Several possible factors, both viral and host-related, might play a role in the infectivity of OBI HBV and for the not so clear distinction between infectious and noninfectious VL.

1) Fifteen percent of anti-HBc-positive recipients were considered to have acquired the infection from another source. These may be among the recipients who received a low VL and would in this case blur the data. 2) Not all infected recipients may develop anti-HBc for a sufficiently long period of time or at high enough amounts to be detected. In fact, seronegative or primary OBI has been

TABLE 6. Characterization of donor and recipient HBV infection.

Donor*		Recipient(s)						
ID	VL (IU/mL)	Genotype	HBsAg loop mutations	HBV DNA	VL	Genotype	Percent homology	Clinical liver disease
10 proven transmissions								
G3	8	D1	wt†	Pos	0.8E5	D1 S114P	99.6	Acute liver failure
G4	5.3	A2	M103I, K160N, Y161F	Pos	2E4	A2	100	Acute liver failure
G5	48	D1	P142L, S143L, D144E, G145K, I150T, F158L	Pos	3400	D1	100	Acute liver failure
O0118	<10	D2	wt	Pos	4.6E4	D2	99.8	Acute hepatitis
C1223	<10-75	D3	wt	Pos	19	D3	99.8	Asymptomatic
				Pos	5	D3	100	Asymptomatic
				Pos	<5	D3	100	Asymptomatic
				Pos	543	D3	100	Asymptomatic
				Pos	182	D3	99.8	Acute liver failure
				Pos	7	D1	100	Asymptomatic
D0283	<5	D1	wt	Pos	<10			Asymptomatic
Probable or possible transmissions‡								
V1320	<10	D	wt	Neg	ND			Nothing noted
G6	9-56	A2	M103I, L109H, P120Q, G130S/N, N131K, S132F					
No transmission								
D4569	69	A2	wt	Pos	4.8E6	C		Asymptomatic
O0121	10	D	No seq	Neg	Neg			Asymptomatic

* All donors were consistently anti-HBs and HBsAg negative.
† wt = wild type.
‡ Three more recipients of G6 donor without sequenced DNA were recorded.

* All donors were consistently anti-HBs and HBsAg negative.

† wt = wild type.

‡ Three more recipients of G6 donor without sequenced DNA were recorded.

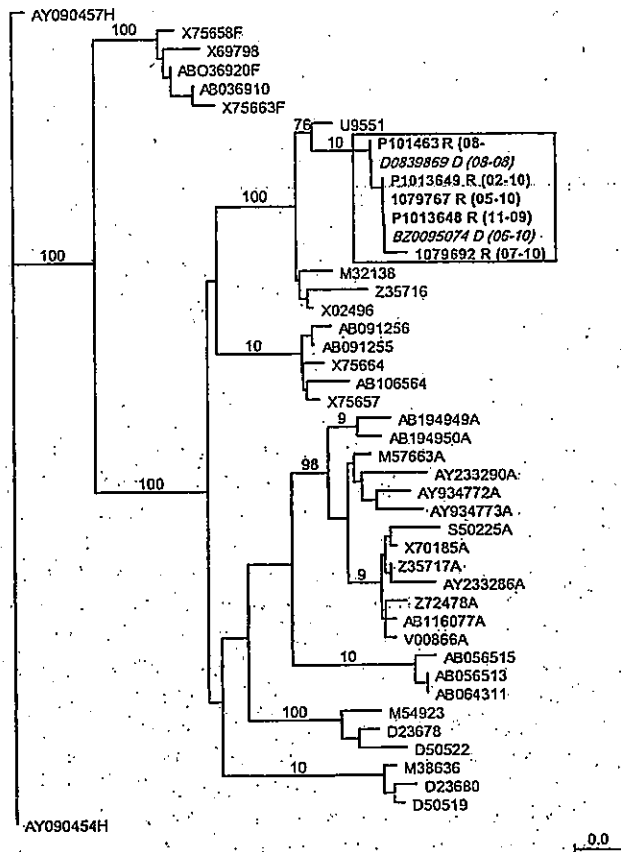


Fig. 2. Phylogenetic tree of OBI donor C1223-recipients pre-S/S sequences. Reference sequences were taken from GenBank and indicated by their accession code followed by the genotype. The two sequences from the donor (indicated by *D* in italics) collected at 2-year intervals (month and year in parentheses) and sequences from strains of five different recipients (indicated by *R*) form a specific cluster (boxed) separated by a bootstrap value of 100. Bootstrap values are indicated above tree main branches. The scale is indicated at the right bottom corner.

described²⁶ and occurs in approximately 15% of subjects with intrahepatic HBV DNA.²⁷ 3) The Poisson distribution of infectious particle estimated by probit analysis that the ID₅₀ (95% CI) was approximately 1000 (100-3500) virions, which may explain that some recipients receiving relatively high doses of viral genomes remained anti-HBc negative. 4) Viral fitness might play a role in the apparently lower infectivity of HBV in OBI, although in four traceback cases, the transmitted strains replicated well and caused fatal liver disease in three of them. 5) Most important the detection of several mutations in the HBsAg loop of infectious donors without detectable anti-HBs (Table 6) suggests that unrecognized antibodies in OBI donors drive mutant selection.^{2,28} Immune complexes frequent in chronic HBsAg carriers²⁹ may occur in OBI carriers.

Absence of such antibodies may be the major factor for greater than 100-fold higher infectivity of virus transfused during the window period.^{5,30}

OBI transmission experiments in chimpanzees showed that detectable anti-HBs was protective.³¹ In our study, anti-HBs in the OBI donor blood was exclusively found in lookback cases and in the presence of antibodies significantly less frequently transmission occurred ($p = 0.01$, Table 4) in agreement with previous data.³² Infectivity of anti-HBs-positive OBI was previously reported but only when viral dose was beyond the neutralizing capacity of levels of anti-HBs below 30 IU/L.⁷ The potential interference of components containing anti-HBs of natural or vaccine origin simultaneously transfused was not considered here since no systematic testing of archived samples was possible. Recent data have shown that the protective efficacy of vaccine-induced antibodies to Genotype A2 was relatively less protective against other genotypes such as Genotypes B, C, and D.³³ In this study, too few samples were genotyped to undertake verifying this aspect.

The significant lower risk of OBI infection in potentially immunodeficient recipients and lack of significance in the logistic regression analysis (Table 5) was unexpected but the small number of cases and the relative inaccuracy of the stratification may have played a role. In addition, some cases might have been unable to mount an anti-HBc response. The therapeutic drug-induced potential immunodeficiency of a recipient has rarely been reported in previous studies of HBV transfusion transmission of OBIs. In Hong Kong, five recipients were considered susceptible to HBV infection since no HBV markers were detected after transfusion but were not infected.⁹ These individuals had been exposed to fewer than 1000 to 19,000 copies of HBV DNA but may have been protected by anti-HBs present in other transfused components collected from recovered or vaccinated donors. A single presumably immunocompetent recipient of 49 considered confirmed transfusion infection by molecular homology (95%) received 1900 copies of HBV DNA.⁹

There were considerable fluctuations in donor VL over time (up to 100 times) in the follow-up samples as previously described.⁷ This may play a role in HBV infection as illustrated by a report from Japan of a series of recipients of apheresis PCs from an OBI carrier donor over a period of 7 years. The viral dose received by the 10 recipients available for testing ranged between fewer than 10,000 and 40,000 copies but four were not infected, two had an asymptomatic HBV infection and four an acute, symptomatic infection.³⁴

The multiple factors identified as potentially interfering with infectivity of transfused blood products from OBI donors were assembled to develop a matrix predictive of infectivity. Only two variables are significant predictors of infection: donor anti-HBs ($p = 0.016$) and transfused

product ($p = 0.0008$). The donation VL, although not significant in the model, contributes to improve prediction and the estimated HBV dose received by infected recipients tended to be higher than in uninfected patients (Fig. 1).

As pointed out in several previous articles on OBI infectivity, the ultimate evidence of transfusion transmission is the identity of a long enough informative sequence between donor and recipient HBV DNA.^{8,7,9,25} In one report, homology of at least 95% was considered sufficient evidence⁹ but in this and other reports, more than 99% was observed. This percentage, however, is dependent on the region of the genome and the genetic diversity of the sequenced amplicons. Typically more than 500 nucleotides in the S region including the major hydrophilic region are reliably informative. Such genetic evidence was particularly important in endemic regions where four of five donor-recipient paired strains were less than 90% homologous and excluded transfusion responsibility.⁹ In this study, the pre-S/S or S gene sequencing confirmed the identity of strains between donor and recipients in 10 cases but excluded this connection in one pair (D4569, Table 6). In several other cases (G4, G5, and G6) specific mutations leading to unusual amino acid substitutions in the HBsAg loop as frequently observed in OBIs from Europe² can be taken as unique flagging of the transmitted viral strains. This genetic variability in the HBsAg loop is particularly frequent in genotype D OBIs that are dominant in most of Europe.

In conclusion, anti-HBs-negative blood from OBIs in Europe is estimated at 25% up to 100% infectious by transfusion depending on the plasma volume transfused (Table 4). Since transfusion-transmitted infections are often a cause for legal procedures, the collection of a pre-transfusion recipient sample kept as archive as routinely done for donor samples would be critical in determining the transfusion origin of an HBV infection. The results may justify in European countries different blood safety measures, such as anti-HBc screening of blood donors where prevalence is less than 2% to 4% or, especially in higher anti-HBc prevalence countries, the introduction of sensitive HBV-NAT.

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JPA designed the study, analyzed the data, and wrote the manuscript; IM, MI, GE, KG, LHH, JMG, and EB collected data on donors and recipients, tested them for HBV markers, and reviewed the manuscript; CE collected data on donors and

recipients, tested them for HBV markers, and reviewed the manuscript; MS generated some of the virus quantification and molecular data on German samples and reviewed the manuscript; LW performed the statistical analysis, in particular the regression analysis of risks, and reviewed the manuscript; LB supervised the collection and testing of donor and patients from Valladolid and reviewed the manuscript; HU identified the donors and recipients, collected clinical data, and reviewed the manuscript; DC performed confirmation of OBI in samples, VL quantification, and molecular analysis of donors and recipients other than from Germany and revised the manuscript; NL contributed to the study design, analyzed some of the data, and contributed to the writing of the manuscript; WHG contributed to the study design, data analysis, and writing of the manuscript; and MC supervised the molecular testing and sequencing on the Paul Ehrlich Institute samples and reviewed the manuscript.

CONFLICT OF INTEREST

JPA is occasional speaker for Novartis and has received a research grant from Novartis; NL is consultant for Novartis; and WHG is consultant for Abbott Laboratories and occasional speaker for Abbott, Roche, and Diasorin. None of the other authors has any conflict of interest to declare regarding the subject of this study.

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医薬品 研究報告 調査報告書

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一般的名称	新鮮凍結人血漿	研究報告の公表状況	上村正巳、富樫和枝、青木寿成、大木直江、内田茂治、松村博雄、布施一郎、第61回日本輸血・細胞治療学会総会、2013年5月16日-18日；神奈川県、	公表国 日本	
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社） 新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）				
研究報告の概要	○HEVが検出された血液製剤輸血後、6ヶ月を経過してE型肝炎を発症した1症例 患者は27歳女性、疾患は急性前骨髄性白血病。入院当日より継続的に輸血を施行、2011年10月31日に寛解導入療法を開始し、12月2日には骨髄穿刺で寛解が認められ、原因検査目的で各種ウイルスDNAを確認するもすべて陰性であり造血回復遅延の原因は不明であった。4月末-5月に40℃台の発熱があり、フェリチン値の上昇を認め肝機能が徐々に悪化、6月4日には最大AST/ALT:698/972 IU/Lとなったが、その後ゆっくりと改善した。肝障害の原因としてHEV感染の可能性を考え、6月1日に採取した検体で検査したところHEV-RNAが検出された。患者の輸血前保管検体からはHEV-RNAは検出されなかった。 患者に輸血された血液製剤はFFP 117本、RCC 26本、PC 34本であり、全ての製剤を検査した結果、2011年10月に輸血されたFFP製剤1本よりHEV-RNAが検出された。原因製剤と患者血液中のウイルス塩基配列の解析結果は1箇所塩基相違があった以外はすべて一致しており、本症例は輸血用血液製剤によるHEV感染と特定された。 HEV感染は一過性で終息することが多いが、一部症例で2-9週の潜伏期を経て肝炎を発症する。本症例では患者が免疫抑制状態にあったためか、原因製剤輸血後6ヶ月を経て肝炎を発症した。文献的には健康成人のHEV感染既往は約5%とされているため、献血血液にHEVが混入するリスクは稀ではないと考えられる。輸血後肝炎が発症した際はHEV感染も考慮して原因調査を行うことが重要である。				
報告企業の意見	HEVが検出された血液製剤輸血後、6ヶ月を経過してE型肝炎を発症した症例の報告である。				
今後の対応	日本赤十字社では、厚生労働科学研究「経口感染によるウイルス性肝炎（A型及びE型）の感染防止、病態解明、遺伝的多様性及び治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。				
	使用上の注意記載状況・その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、細菌、原虫等の感染、vCJD等の伝播のリスク				

0-2-4 HEV が検出された血液製剤輸血後、6 ヶ月を経過して E 型肝炎を発症した 1 症例

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 上村正巳¹⁾、富樫和枝¹⁾、青木寿成¹⁾、大木直江¹⁾、内田茂治²⁾、松村博雄³⁾、布施一郎¹⁾
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【はじめに】輸血後感染症のリスクは未だゼロではなく、輸血前後の感染症検査や経過観察は輸血後感染症の早期発見に不可欠である。今回我々は、輸血後に E 型肝炎を発症し血液センターの週及調査から、およそ 6 ヶ月前に輸血された FFP の 1 バッグより感染原因となった E 型肝炎ウイルス (HEV) が検出された症例を経験したので報告する。【症例】患者は 27 歳女性、疾患は急性前骨髄性白血病。入院当日より継続的に輸血を施行、2011/10/31 に寛解導入療法を開始し、12/2 には骨髄穿刺で寛解を確認した。12/13 および翌年 1/12、3/23 より 3 度の地固め療法を行ったが、地固め療法中に造血回復の遅延が認められ、原因検索目的で各種ウイルス DNA を確認するもすべて陰性であり造血回復遅延の原因は不明であった。4 月末～5 月に 40℃ 台の発熱があり、フェリチン値の上昇を認め肝機能が徐々に悪化、6/4 には最大 AST/ALT:698/972 IU/L となったが、その後ゆっくりと改善した。肝障害の原因として HEV 感染の可能性を考え、6/1 採取した検体で検査したところ HEV-RNA が検出された。患者の輸血前保管検体からは HEV-RNA は検出されず、輸血による HEV 感染を疑い血液センターへ週及調査を依頼した。【週及調査結果】患者に輸血された血液製剤は FFP 117 本、RCC 26 本、PC 34 本であり、全ての製剤を検査した結果、2011 年 10 月に輸血された FFP 製剤 1 本より HEV-RNA が検出された。原因製剤と患者血液中のウイルス塩基配列の解析結果は 1 箇所塩基相違があった以外はすべて一致しており、本症例は輸血用血液製剤による HEV 感染と特定された。【考察】HEV 感染は一過性で終息することが多いが、一部症例で 2～9 週の潜伏期を経て肝炎を発症する。本症例では患者が免疫抑制状態にあったためか、原因製剤輸血後 6 ヶ月を経て肝炎を発症した。文献的には健康成人の HEV 感染既往は約 5% とされているため、献血血液に HEV が混入するリスクは稀ではないと考えられる。輸血後肝炎が発症した際は HEV 感染も考慮して原因調査を行うことが重要である。

0-2-5 輸血後肝炎との鑑別を必要とした de novo 肝炎と輸血後感染症検査データの重要性

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【はじめに】de novo B 型肝炎とは HBsAg 陰性患者が免疫抑制や化学療法により HBV が再活性化され引き起こされる肝炎である。今回我々は輸血後肝炎を疑われたが de novo 肝炎であった症例を経験し、輸血後感染症検査結果の把握の重要性を再認識したので報告する。【症例・結果】63 歳女性、IgG-κ 型多発性骨髄腫、多発性骨髄腫と診断され当院入院。導入化学療法前は AST24、ALT18、HBsAg(-)、HBcAb(-) であった。導入化学療法 3 コース後、自己末梢血幹細胞採取、約 1 ヶ月後、末梢血幹細胞移植を実施し、濃厚血小板-LR-10 を 3 回輸血した。約 8 ヶ月後、AST216、ALT306 と増悪したため輸血後肝炎が疑われ、輸血前保存血清にて HBV-DNA 検査を実施。結果が検出感度以下であったため、赤十字血液センターへ副作用検査として同一検体を提出したところ、個別 NAT 検査にて弱陽性であった。輸血された PC 製剤の 3 ドナーはともに個別 NAT で陰性であった。HBsAb は、検体の汚染を考慮し HBV-DNA 検査終了後に院内で検査した結果 5.6mIU/ml であった。以上より de novo 肝炎と診断された。なお、輸血後 3 ヶ月後検査として実施されていた HBV-DNA は 3log copy/ml であり、その結果が把握されていなかった。【まとめ】致死率の高い de novo 肝炎に対しては HBV-DNA が陽性化した時点で直ちに核酸アナログ投与を行う事が勧められているが、本症例は肝炎発症後からの投与となった。この症例を契機に輸血後感染症検査の結果の把握が早期の肝炎の診断・治療に繋がることを再認識し、輸血部門としては臨床側へ輸血後感染症検査を促すのみではなく検査結果も把握するように改善した。

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2013. 5. 16	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿	研究報告の公表状況	Ziemann M, Heuft HG, Frank K, Kraas S, Görg S, Hennig H. Transfusion. 2013 May;53(5):1088-94. doi: 10.1111/trf.12074. Epub 2013 Jan 16.	公表国 ドイツ	使用上の注意記載状況・ その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分 採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)	○サイトメガロウイルス(CMV)初感染のウィンドウ期中の供血と輸血感染リスク 背景：アフエレーシス供血者のような供血間隔が短い供血者は、ウィンドウ期供血のリスクが高い。CMVのウィンドウ期供血者の頻度については、輸血感染CMV(TT-CMV)のリスクがある患者において、CMV抗体陰性の供血者を選択することが有益であるかどうかを決めるために重要な情報である。 研究デザインと方法：直前の抗体陰性時から最大35日以内に、スクリーニング検査でCMV抗体検査で陽性となった供血者93人について確認検査を行った。さらに、セロンバーンジョンの検体のCMV DNA濃度を測定した。 結果：12人(13%)にセロンバーンジョンが確認された。このうち3人の直前の抗体陰性検体はCMV DNA陽性であった(25%、30 IU/mL未満)。また、10人の初回抗体陽性検体はCMV DNA陽性であった(83%、最高1,600 IU/mL)。CMV DNAの保有率及び濃度はいずれも直前の抗体陰性検体よりも初回抗体陽性検体で有意に高く(それぞれ$p=0.004$、$p=0.02$)、最高濃度はセロンバーンジョンの約2週間後に観察された。 結論：供血間隔の短い供血者においてCMVセロンバーンジョンが疑われる例の多くは、スクリーニング検査の偽陽性結果によるものである。ウィンドウ期供血は稀であるとともに、初回抗体陽性供血と比べてCMV DNA量は少ないことから、TT-CMVを防ぐためには、供血間隔が短い初回抗体陽性供血者からの血液製剤を使用しないことが特に有効である。			
報告企業の意見		今後の対応			
短い供血間隔の供血者におけるCMV感染ウィンドウ期中の供血及び輸血感染リスクについて調査したところ、ウィンドウ期供血は稀でありCMV DNAも低濃度であるが、初回抗体陽性供血はウィンドウ期に比べてCMV DNA保有率及び濃度が高いことが明らかとなり、TT-CMVを防ぐためには、初回抗体陽性供血由来の血液製剤を使用しないことが特に有効であるとの報告がある。		日本赤十字社では、CMVの感染防止に有効とされる、保存前白血球除去した血液製剤のみを供給している。さらに、必要に応じてCMV抗体が陰性であることを確認した血液製剤を供給している。今後CMV感染に関する新たな知見等について情報の収集に努める。			

研究報告の概要

TRANSFUSION COMPLICATIONS

Window period donations during primary cytomegalovirus infection and risk of transfusion-transmitted infections

Malte Ziemann, Hans-Gert Heuft, Kerstin Frank, Sabine Kraas, Siegfried Görg, and Holger Hennig

BACKGROUND: Donors with short interdonation intervals (e.g., apheresis donors) have an increased risk of window period donations. The frequency of cytomegalovirus (CMV) window period donations is important information to decide whether selection of seronegative donors might be advantageous for patients at risk for transfusion-transmitted CMV infections (TT-CMV).

STUDY DESIGN AND METHODS: CMV seroconversion in 93 donors with positive results in routine CMV antibody testing within at most 35 days after the last seronegative sample was evaluated by Western blot and/or a second antibody test. In donors with unconfirmed seroconversion, an additional later sample was tested. Concentration of CMV DNA was determined in pre- and postseroconversion samples.

RESULTS: CMV seroconversion was confirmed in 12 donors (13%). Among these, the last seronegative sample was CMV DNA positive in three donors (25%, below 30 IU/mL). The first seropositive sample was CMV DNA positive in 10 donors (83%, maximum 1600 IU/mL). Both prevalence and median concentration of CMV DNA were higher in the first seropositive sample ($p = 0.004$ and $p = 0.02$), with maximum concentrations being reached about 2 weeks after seroconversion. No CMV DNA was detected in samples from donors with unconfirmed seroconversion.

CONCLUSION: At least in donors with short interdonation intervals, most suspected CMV seroconversions are due to false-positive results of the screening test. As window period donations are rare and contain less CMV DNA than the first seropositive donation, avoidance of blood products from primarily seropositive donors is especially helpful to avoid TT-CMV if donors with short interdonation intervals are concerned.

Human cytomegalovirus (CMV) is usually transmitted by direct person-to-person contact and causes mostly asymptomatic or mild mononucleosis-like infections leading to lifelong latency of CMV infections with possible reactivations.¹ In risk groups like low-birth-weight newborns or patients receiving allogeneic stem cell transplants, however, CMV infections can be caused by blood transfusions and lead to increased morbidity and mortality.^{2,3} Such transfusion-transmitted CMV infections (TT-CMV) have traditionally been explained by transmission of latently infected cells, presumably of the myeloid lineage.⁴ The risk of TT-CMV can be reduced by approximately 92% by white blood cell (WBC) reduction of blood components,⁵ but even after implementation of leukoreduction, "breakthrough" infections persist with rates as high as 1% to 3% of transfused high-risk patients.⁵

This residual risk could be due to infectious virions, which may be associated with the plasmatic CMV DNA detected during primary CMV infections and reactivations of latent infections.^{6,7} Donors with latent CMV infections or during the late phase of primary CMV infections can be recognized by detection of CMV antibodies. Donations from those donors can be avoided by selection of

ABBREVIATIONS: gB = glycoprotein B; MEIA = microparticle enzyme immunoassay; TT = transfusion-transmitted cytomegalovirus infection.

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seronegative blood products for patients at risk for TT-CMV.² On the other hand, selection of seronegative blood products increases the proportion of viremic donations from donors with primary CMV infection before development of CMV antibodies (window period donations). Even if window periods of 6 to 8 weeks with prominent viremia have been suggested for CMV,⁸ to our knowledge neither the actual length of CMV window periods nor the course of viremia during window periods in healthy blood donors have been studied so far.

The key difficulty is that primary CMV infections routinely are diagnosed by detection of CMV antibodies, which means after completion of the window period. Information about the window period can then only be gathered from already existing samples, for example, drawn at previous donations. Therefore, the course of CMV DNA concentration during the window period could be monitored best in donors with short interdonation intervals. We conducted a multicenter study involving five blood donation services with together approximately 600,000 donations per year, examining pre- and postseroconversion plasma samples from donors with CMV seroconversion during an interdonation interval of at most 35 days.

MATERIALS AND METHODS

Donors

Donors with positive results in routine CMV antibody testing within at most 35 days after the last seronegative sample were included in the study. Thirty-nine cases of suspected CMV seroconversion were contributed by the Institute for Transfusion Medicine of Hannover Medical School, 29 by the German Red Cross Blood Donor Service East, 20 by the Institute of Transfusion Medicine of the University Hospital Schleswig-Holstein, four by the German Red Cross Blood Donor Service North, and one by the Institute of Transfusion Medicine of the University Hospital Leipzig, all located in Germany. None of these donors had been included in a previous publication about the course of primary CMV infections.⁹ Plasma samples were stored at -20°C or less until analysis.

Detection of CMV antibodies

As screening tests varied between institutions and during the study period, 64 samples were tested by the anti-CMV and IgG plus IgM test (Enzygnost, Dade Behring Marburg GmbH, Marburg, Germany), 12 by the Architect anti-CMV IgG assay (Abbott GmbH & Co. KG, Wiesbaden, Germany), 12 by the Biotest Anti-CMV recombinant IgG enzyme-linked immunoassay (Biotest AG, Dreieich, Germany), four by the CMV-IgG-ELA test (Medac GmbH, Wedel, Germany), and one by a CMV IgG test (Abbot AxSYM, Abbott GmbH & Co. KG).

Confirmation of CMV seroconversion

For confirmation of CMV seroconversion, both a micro-particle enzyme immunoassay (MEIA) against AD169-coated microparticles (Architect anti-CMV IgG assay, Abbott GmbH & Co. KG) and a CMV Western blot (recom-Blot, Mikrogen GmbH, Neuried, Germany) were used. The Western blot contained the recombinant antigens CM2 and IE1 (similar to the nonstructure proteins p52 and IE1 "immediate early protein"), p65 and p150 (similar to the tegument proteins pp65 and pp150), and glycoprotein B (gB)1 and gB2 (resembling the membrane gB). Band intensity was categorized into "very weak," "weak," "well visibly," and "very strong intensity" according to the manufacturer's instructions. Seroconversion was defined as switching from negative to positive results according to the manufacturer's instructions for the MEIA or for the Western blot as an increase in band intensity of at least two categories for at least one band or of at least one category for at least two bands.

To exclude an early stage of antibody development in donors with unconfirmed seroconversion, even a later sample was tested for CMV antibodies by a screening assay and—in case of positive results of the screening assay—the Western blot.

Detection of CMV DNA

DNA from 1-mL aliquots of plasma samples was isolated and tested for CMV gB-specific sequences in duplicate using a highly sensitive TaqMan PCR as described elsewhere.⁶ The 95% limit of detection was determined to be 12 international units CMV per mL (IU/mL) plasma using semilogarithmic dilutions of the First WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (NIBSC Code 09/162, National Institute for Biological Standards and Control, Potters Bar, UK) containing between $10^{2.5}$ and 10^4 IU/mL CMV strain AD169. Twenty-four samples of each concentration were processed in six consecutive TaqMan PCR runs. The 95% detection limit was calculated by means of probit analysis. Quantification of CMV DNA-positive samples was carried out by a standard curve derived from these validation experiments as well as eightfold measurements of semilogarithmic dilutions between 10^3 and 10^5 IU/mL. As CMV DNA concentrations below 30 IU/mL could not be quantitated reliably for single samples, those values were used only for statistical evaluations. For four donors, only plasma samples with 250 μL per sample were available for CMV PCR, corresponding to a detection limit of 48 IU/mL for these samples. Samples with invalid internal control or diverging results were retested twice. The presence of CMV DNA was confirmed by reproducibly positive PCR results. Samples with only one positive result for CMV DNA in the first PCR run and insufficient sample volume

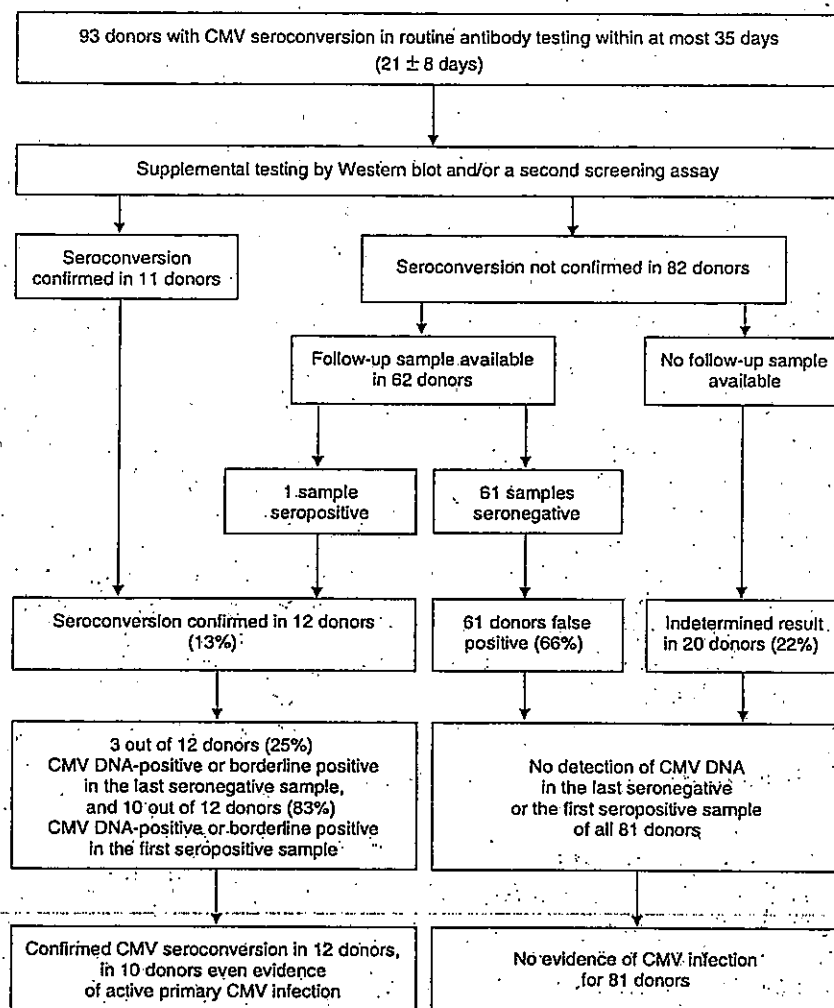


Fig. 1. Supplemental testing of donors with reactive CMV antibody tests.

for repeated testing were termed borderline positive for CMV DNA.

Statistical analysis

Unless stated otherwise, means are calculated as arithmetic means $\pm$ standard deviation (SD). Calculations were assisted by database and statistical programs (Excel, Microsoft Corp., Redmond, WA; SPSS, SPSS Inc., Chicago, IL). Differences between CMV DNA concentration in the last seronegative and the first seropositive sample were examined by Wilcoxon's rank-sum test; differences in the prevalence of CMV DNA in plasma were tested using the chi-square test.

RESULTS

Confirmation of CMV seroconversion

CMV seroconversion was confirmed in 12 of 93 donors (13%). These donors showed antibodies against nonstruc-

ture proteins or tegument proteins, but no or only weak antibodies against the membrane gB.

For 11 donors, confirmation of the suspected seroconversion was possible comparing the last seronegative and the first seropositive sample according to the routine screening assay. For 62 of 82 donors with unconfirmed seroconversion, an additional later sample was available for antibody testing. These samples were drawn 368 ± 500 days after the suspected seroconversion (range, 26-2078 days) and tested negative for all but one donor. This donor tested weak positive in both MEIA and Western blot 913 days after the suspected seroconversion. Further details are shown in Fig. 1.

A difference in the rate of false-positive results according to the screening assay used could not be shown.

Detection of CMV DNA

In donors with confirmed CMV seroconversion within at most 35 days, the last seronegative sample was positive for

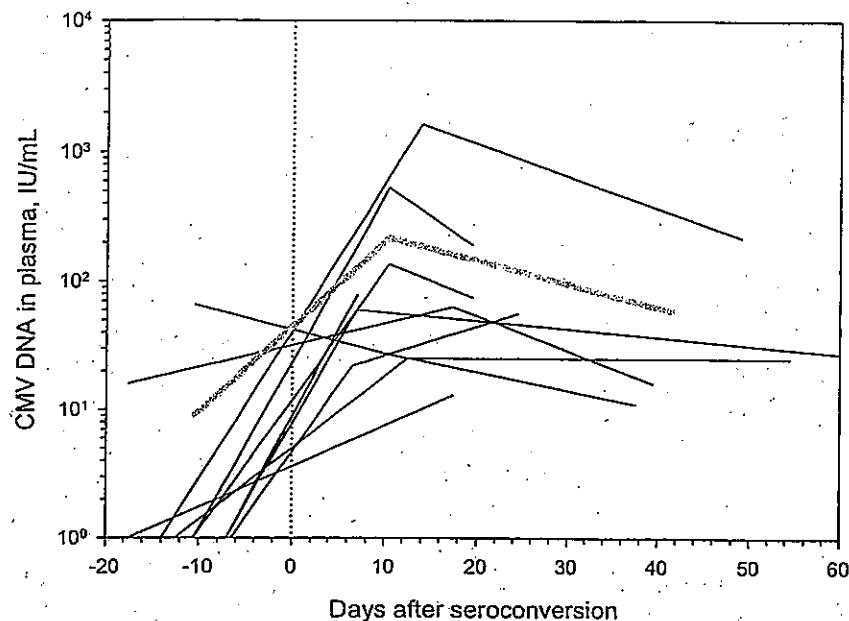


Fig. 2. Concentration of CMV DNA in plasma during seroconversion. Thin black lines represent CMV DNA concentrations in donors with confirmed CMV seroconversion relative to the assumed date of seroconversion (middle of the interval between the last seronegative and the first seropositive sample). Even if DNA concentrations below 30 IU/mL plasma cannot be determined reliably for single samples, those values are meaningful for statistical comparisons. Mean concentrations of CMV DNA during seroconversion are depicted by the gray line.

CMV DNA in one donor and borderline positive in two donors (together 25%). The concentration of CMV DNA in all three samples was below 30 IU/mL. In the last seronegative sample of one of these donors, antibodies against CMV (CM2 and p65) were detectable by Western blot despite a negative result in the routine screening test. The second donor was only weak positive for antibodies against p65, and the third donor showed no reactions in the Western blot.

The first seropositive sample was CMV DNA positive in eight donors and borderline positive in two donors (together 83%, median less than 30 IU/mL, maximum 1600 IU/mL). No CMV DNA was detected in samples from donors with unconfirmed seroconversion. The higher rate of detection of CMV DNA in the first seropositive compared to the last seronegative sample was highly significant ($p = 0.004$). Even the median CMV DNA concentration in the first seropositive sample was higher than in the last seronegative sample ($p = 0.02$). The interval between the last seronegative and the first seropositive sample of the only donor without detection of CMV DNA despite confirmed seroconversion was 28 days.

The course of the CMV DNA concentration in plasma during the seroconversion period of donors with confirmed seroconversion is summarized in Fig. 2. As the actual seroconversion date could be any date between the last seronegative and the first seropositive sample, the middle of the interval between the last seronegative and

the first seropositive sample has been assumed to be the seroconversion date. CMV DNA concentrations are depicted as small circles and all measurements for a given donor connected by a thin black line. The thick gray line represents the mean CMV DNA concentration for the last seronegative and the first and second seropositive samples. It shows only low concentrations during the seronegative window period, and maximum concentrations approximately 2 weeks after the development of CMV antibodies.

In Fig. 3, the incidence of CMV DNA in plasma in the last seronegative and the first seropositive sample is shown for the current study (interdonation interval: no more than 35 days) and compared to an earlier report for donors with longer interdonation intervals.⁶

DISCUSSION

Contrary to common assumptions, this study demonstrates the highest CMV DNA concentrations during primary CMV infections being detected shortly after seroconversion and only low concentrations found during the window period. Even in donors with interdonation intervals of at most 35 days, CMV window period donations occurred in only 25% of donors with primary CMV infection and contained less than 30 IU CMV DNA/mL. Significantly more donors (83%) were CMV DNA positive or borderline positive in their first seropositive sample

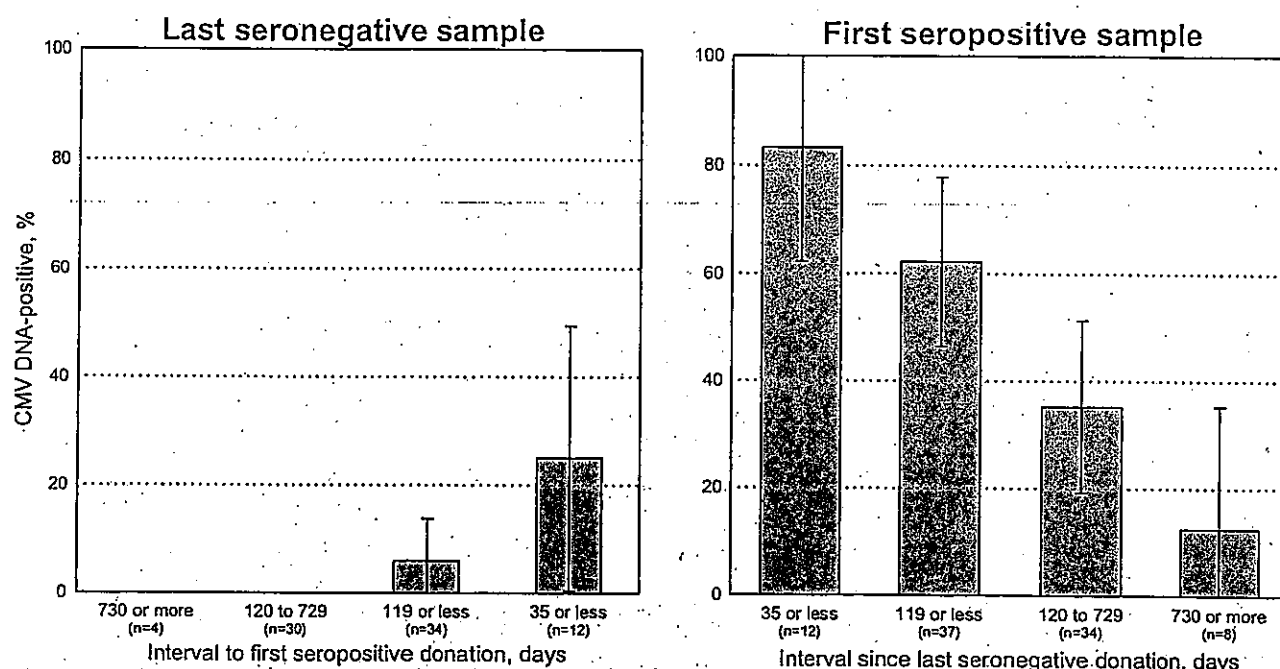


Fig. 3. Detection of CMV DNA before and after seroconversion. The figure summarizes data from the current study and a previous publication of our group.⁶ Donors were categorized according to the interval between the last seronegative and the first seropositive sample. The rate of detection of CMV DNA in plasma is given with its 95% CI.

($p = 0.004$), which also showed higher CMV DNA concentrations up to 1600 IU/mL.

Continuation or even intensification of primary CMV infections, after primary detection of CMV antibodies is facilitated by delayed production of antibodies against the gB, which is an important target for neutralizing antibodies.⁹ Generic tests for CMV antibodies also detect antibodies against nonstructure proteins or tegument proteins, which are produced early in primary CMV infections and can precede production of antibodies against gB by several weeks.⁹ In the current study, no CMV DNA-positive sample tested more than borderline positive for antibodies against gB. Even if there are further neutralizing epitopes of CMV, these results are concordant with an earlier observation that clearance of CMV DNA from whole blood correlates with development of antibodies against gB in blood donors with primary CMV infection.⁹

CMV DNA in plasma as surrogate marker for systemic viremia was detected in all donors with confirmed seroconversion with the exception of only two donors. The interval between the last seronegative and the first seropositive sample was 14 and 28 days in these donors, so that a short period of systemic viremia might have been missed. The presence of CMV DNA in plasma samples of almost all donors with CMV seroconversion contradicts earlier reports,¹⁰ but is consistent with the previously published finding, that the frequency of detection of CMV DNA is dependent on the interval between the last serone-

gative and the first seropositive sample.⁶ Furthermore, the highly sensitive PCR allowed detection of low concentrations of CMV DNA, which would have been missed by others (the method used by Drew and coworkers,¹⁰ for example, had a distinctly higher limit of detection).

The finding of delayed detection of CMV DNA in plasma in newly seropositive donors is compatible with the hypothesis that primary CMV infections caused by direct person-to-person contact first led to a localized infection, for example, in the nasopharyngeal mucosa, and concomitant production of antibodies against nonstructure and tegument proteins. After an interval of days to several weeks, this local infection is followed by viremia during which the virus spreads to other organs, like kidneys. Concurrently, antibodies against gB are induced, which probably contribute to termination of the viremic phase. The duration of viremia in immunocompetent subjects seems to be short in most subjects, impairing the detection of CMV DNA in donors with longer interdonation intervals. If only primarily infected donors with evidence of CMV DNA in plasma are studied, however, even cases with prolonged viremia up to several months have been reported.⁹

In a study of CMV-vaccinated and nonvaccinated subjects, Zhang and coworkers¹¹ noticed a medium interval of 2 weeks between the first seropositive sample and the first detection of CMV in body fluids by viral culture. As the study was still blinded at the time of publication, the

effect of CMV vaccination on antibody response in this report remains to be determined. Nevertheless, the data of Zhang and colleagues, as well as an earlier case report by our group,⁹ support the finding of the current study that IgG antibodies are formed early in the course of primary CMV infections and precede the peak DNA concentration in plasma in immunocompetent subjects. In immunocompromised patients, CMV antibodies have rarely been detected before onset of CMV disease.¹²⁻¹⁶ It remains unclear, however, to what extent these results have been influenced by, for example, the use of far less sensitive methods for antibody detection¹²⁻¹⁴ or by antiviral prophylaxis after transplantation.¹⁶

Direct confirmation of viable CMV in plasma by viral cultures would have further strengthened our assumption of an increased risk of TT-CMV after transfusion of blood from donors in the early period after seroconversion. In most healthy persons, however, the concentration of CMV DNA in plasma is below the limit of detection of common viral cultures. Cultures of WBCs from newly CMV-infected immunocompetent subjects are also seldom positive (e.g., 1 of 54 samples in the study of Zanghellini and coworkers¹⁷ or 5 of 56 samples in the study of Revello and colleagues¹⁸). Therefore, the failure of our group and others¹⁷ to detect viable CMV in plasma cannot exclude the presence of low amounts of infectious virions. Even low amounts, however, might be infectious for immunocompromised patients. In the current study, we were able to detect some donors with high DNA concentrations. But as this was a retrospective study with only small aliquots of plasma samples available, no CMV cultures could be studied.

A limitation of this study is that no whole blood or WBC samples could be examined because we relied on plasma samples routinely stored for lookback studies. As low numbers of residual WBCs are contained in all blood products despite leukoreduction, it would be interesting if further studies could add information about the CMV DNA concentration and/or infectivity of these compartments.

Another limitation is the long storage time of some samples (median, approx. 4 years; range, 5 months-10 years). Prolonged storage at -20°C could have led to degradation of CMV DNA with false-negative PCR results. On the other hand, due to the short seroconversion interval this would have affected seronegative and seropositive samples equally without altering the key findings of the study.

A surprising result of this study is that, using the original samples, seroconversion was confirmed in only 11 of 93 donors (12%) tested positive for CMV antibodies within at most 35 days after negative screening results. This phenomenon was independent of the applied screening test. In only one additional donor, CMV seroconversion was confirmed in a follow-up sample. In this

donor, negative results of both confirmatory assays for the first sample tested positive in the original CMV-IgG-ELA screening assay could be explained by low antibody titers during the early humoral immune response. In 61 of 62 donors for whom later samples were available, however, even samples drawn after a mean of 368 days after the first seropositive sample tested still negative for CMV antibodies. Therefore, false-positive results of the screening test seem to be the likely explanation for the majority of nonreproducible positive results of the screening tests.

Due to the lack of availability of some original screening assays and the low sample volumes available, neither retesting of all samples with the original assay nor determination of seroconversion by concordance of a variety of screening assays could be conducted in the current study. Whether the high proportion of false-positive results could be generalized to other donor populations or if this phenomenon occurs mainly in donors with extremely short interdonation intervals must be clarified by further studies. Until results of such studies are available, the effect of false-positive results on reported CMV seroconversion rates remains unclear.

For other viruses relevant in blood donor testing, like human immunodeficiency virus or hepatitis viruses, positive results of a serologic screening assay lead to repeated testing in the same assay in duplicate. Donors with reactive results in the initial testing followed by negative results of both repeated tests are considered as tested negative. If at least one of the repeated tests shows a positive result, supplementary testing with for example Western blot or PCR is initiated. If a viral infection cannot be confirmed by supplementary testing, only the current donation is discarded, but the donor is allowed to return to the pool of healthy donors if the screening assay shows negative results on later occasions. Expanding this rule to CMV antibody testing could lower the rate of false-positive results and increase the pool of seronegative donors.

Conclusively, most primarily positive results for CMV antibodies in donors with short interdonation intervals were due to false-positive reactions. In donors with confirmed seroconversion, CMV DNA in plasma was detected more frequently and at higher concentrations in the first seropositive sample than in the last seronegative sample. As there were no window period donations with prominent viremia detectable in the current study, the suspected risk of an increased proportion of window period donations due to selection of blood products from seronegative donors seems to be rather moderate. On the other hand, avoidance of blood products from primarily seropositive donors might be especially helpful to reduce the rate of TT-CMV in risk patients, if blood products from donors with short interdonation intervals (e.g., apheresis donors) are concerned.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

No grants have been received, and none of the authors declares a conflict of interest.

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キット (ロシユ) で、Light Cycler 2.0 (ロシユ) を用いて増幅した生成物の増幅及び検出のために使われた。ミニプール・スクリーニング検査で陽性結果を示したサンプルは、個々に再検査された。個別検査で陽性反応を示したサンプルは、さらに3回検査された。 2. 抗 B19V 抗体免疫測定法 我々は、recomWell パネルボウイルス B19 ELISA IgM、IgG キット (Mikrogen, Neurid, ドイツ) でベーリング DNA プロセス III (Dade ベーリング、マールブルク、ドイツ) を使って抗 B19V 抗体を検査するために、10,032 人のドナーからの 928 のサンプルをランダムに検査した。RecomLine パネルボウイルス B19 LIA IgG、IgM キット (Mikrogen) は、B19V DNA と抗 B19V 抗体の両方の陽性結果を示したドナーでの VP-2、VPN、VP-1S、VP-2r、VP-C 及び NS-1 エピトープに対する抗体を分析するのに使われた。全ての検査は、製造者の研究所で行われた。結果は製造業者からの添付文書中の印刷物で概説された基準を用いて説明された (陽性は、$>24\text{U/mL}$; 陰性は、$>20\text{U/mL}$; 境界線は、$24 \geq \text{U/mL}$)。 3. 遡及及び追跡検査 陽性の B19V DNA 検査結果を示したドナーにおける B19V 感染の後の B19V DNA と抗 B19V 抗体力価の変化を観察するために、保管サンプルを用いた 6 人の繰り返しドナーでの遡及調査及び最初ドナーを含む 5 人のドナーでの追跡調査を行った。追跡サンプルは、2-18 週間の間隔で指標献血時後の 12-24 週間にその後の血液採取を含んだ。 4. B19V 遺伝子タイピング	1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。 妊婦、産婦、授乳婦等への投与 妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害 (流産、胎児水腫、胎児死亡) が起こる可能性がある。〕
結果 1. B19V DNA の陽性率 10,032 人の血漿交換ドナーの内の 10 人 (0.1%) が B19V DNA 検査で陽性結果を示した。10 人のドナーの内の 9 人で B19V DNA の力価は低かった (範囲、$27-3.2 \times 10^4 \text{IU/mL}$)、そして 1 人のドナーは高いウイルス力価を示した ($1.33 \times 10^8 \text{IU/mL}$) (表 2)。 2. 抗 B19V 抗体の陽性率 B19V DNA 検査で陽性結果を示した 10 人のドナーの内の 9 人は抗 B19V IgG の高い力価を持っていた、そしてまた彼らの内の 7 人は抗 B19V IgM を示した。ドナーの間の抗 B19V 抗体 (IgG のみ、或いは IgG と IgM) 陽性識別率は、60.1% (558/928) であった。高齢ドナーでの抗 B19V 抗体量は、若いドナーのそれより高かった (表 3)。8 人のドナーサンプルのプロット分析 (線-免疫分析、LIA) での B19V 抗原エピトープに対する反応性のパターンは、B19V 感染における可変的な状態を明らかにした (表 4)。ドナー-8 (D8) とドナー-9 (D9) は、B19V IgM の高い力価を持つ VP2r と VPc の抗原エピトープに対して強い反応性を示した。それによって B19V 感染の初期段階を示唆した。対照的に、ドナー-1 (D1)、ドナー-3 (D3) とドナー-4 (D4) は抗 B19V IgG の高い力価で NS-1 抗原エピトープに反応性を示した、それによって休止状態、或いは過去の B19 感染を示唆した。 3. 遡及調査と追跡調査 遡及調査と追跡調査の結果を表 5 に示した。B19V DNA 力価は、大部分のドナーで次のドネーションで減少した。D1、D2 と D3 の高い抗 B19V IgG 力価の検査は、抗体価と低いウイルス量の安定がみられた。ドナー-10 (D10) は B19V DNA の最も高い力価 ($1.33 \times 10^8 \text{IU/mL}$) を示したが、指標時に如何なる B19V 抗体も持っていないかった。しかしながら、追跡調査時に、指標時の 13 週後に、ウイルス力価は突然に $2.7 \times 10^2 \text{IU/mL}$ に減少し、抗 B19V IgG の存在が明らかにになった。(指標時に最近の感染期を示唆した) D8 と D9 は、保管サンプルで B19V 感染の根拠を示さなかった。D2、D5 と D8 は、B19V DNA と抗 B19V IgM の同時に減少する力価を示した。遡及及び追跡調査の結果は、B19V DNA の存在にもかかわらず、抗 B19V IgG 中和抗体が長期にわたり存在することを明らかにした (表 4)。	(略)

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4. B19V 塩基配列分析

6人のドナーから採取されたB19V DNAの遺伝子型は、G1遺伝子型として特定された。

討 論

B19Vに関する血漿分画製剤の安全性は、現在まで主要な関心事であった。B19Vの高力価汚染は、乳児及び免疫不全や妊婦のような感染リスクの増加した患者において深刻な問題を引き起こす可能性がある。汚染した血漿製剤の使用を介して輸血感染したB19V感染の幾つかの報告があった。我々の調査において、健康な韓国血漿交換ドナーにおけるB19V DNAの陽性率は0.1%であった。それは米国(0.83-0.88%)やドイツやオーストリア(0.27%)の様な他国のそれと比較して高くなかった。その上、1人のドナーを除いて、ドナーのウイルス量(範囲: 10^2 - 10^3 IU/mL)は低かった。これらの結果は、以前の報告の結果と一致している。近年では、抗B19V IgGの中和効果の重要性は、B19V感染で考慮されている。献血、或いはレシピエントにおける抗B19V IgGはウイルスを中和し、B19V感染を予防することができる。幾つかの報告は、抗B19V抗体の1 IU/mLがB19V DNAの4.3ログを中和することができ

ることを示す。Kleinmanらは、B19Vの最小感染量が中和抗体を持たないレシピエントで(凝固因子で) 2×10^4 IU/mL、或いは(他の血漿製品で) 10^7 IU/mLであると報告した。彼らも、 10^4 IU/mL以下のウイルス力価を含む血液製剤は感染性がないと主張した。

最近の調査は、抗B19V抗体の全体的な抗体陽性率は成人で30-60%であると報告した。B19V DNA陽性ドナーの大部分とレシピエントの60%は、抗B19V IgGを示した。輸血関連感染の予測調査(TRIPS)及びレトロウイルスの疫学に関するドナーとレシピエント(RADAR)調査は、B19V DNAの陽性率は米国において献血者で0.8%、レシピエントで1.7%であることを示した。これらの調査において、 10^3 IU/mL未満の低いウイルス量を示している全てのB19V DNA陽性ドナーは、抗B19V抗体を中和する抗体も持っていた。彼らはまた、中和抗体を持っていない、そしてB19V DNAに汚染された血液成分を輸血された21人のレシピエントは、B19V感染の如何なる根拠も示さないと報告した。ドナー血液中に含まれる中和抗B19V抗体は、B19Vを中和する可能性があった。

全ての献血者でミニ・プールB19V NATスクリーニングをしているドイツ赤十字輸血センター(GRCBC)からのデータは、特別なリスクをもつ患者を除いて、ウイルス力価がプールしたサンプルで 10^5 IU/mL以下のものを病院に提供することを明らかにした。B19V DNA陽性の血液成分が提供された理由は、血液製剤中の中和抗体(VP1とVP2に)の同時存在によるものであった。GRCBCはB19V DNA陽性ドナーの75%が、B19V感染を防御できるであろう中和抗体を持っていたと報告した。

我々の調査において、B19V DNA陽性の血漿交換ドナーの90%及び928人のランダムに選択した血漿交換ドナーの60%は、抗B19V IgGを持っていた。しかし、B19Vの高い力価(1.33×10^8 IU/mL)を含んでいる血液成分は、危険因子を持つ患者で感染に至らしめることができたであろう。B19V感染後の臨床的進展は、患者の免疫状態及び中和抗B19V IgGの存在の様な因子によって変化する。日本のグループは、輸血感染B19V感染患者の大部分は発熱や発疹の様な軽い症状だけを示したと報告した。

近年では、ナノ濾過(20-35nm孔径)の様な新しい病原体除去技術が導入され、日本赤十字は分画工程でこの方法を使用している。結論として、韓国の血漿交換ドナーにおけるB19V DNAの抗体陽性率は高くなかった。そして血漿交換ドナーの60%以上は中和抗B19V抗体を持っていた。それによって韓国での輸血によるB19V感染の可能性が深刻でないことを示唆した。従って、全ての献血者のB19V DNAスクリーニングの実施が、この時点で必須とは思われない。

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報告企業の意見	今後の対応
ヒトパルボウイルス B19 (Human parvovirus B19 : B19) は、パルボウイルス科 (Parvoviridae) パルボウイルス亜科 (Parvovirinae) エリスロウイルス属 (Erythrovirus) に属する脂質エンベロープを持たない極めて小さな (約 20～26nm) DNA ウィルスで、輸血や血漿分画製剤による伝播が報告されている。他のウィルスに比べて、血漿分画製剤の製造工程での不活化・除去が困難であり、本ウィルスの伝播リスクを完全に否定することはできないため、1996 年 11 月より、使用上の注意に B19 についての記載を行い注意喚起を図ってきた。また、原料血漿への B19 混入量低減のため、日本赤十字社では CLEIA 法を用いたドナースクリーニングが行われている。万一、原料血漿に B19 が混入したとしても、Porcine parvovirus (PPV) または Canine parvovirus (CPV) をモデルウィルスとしたウィルススクリアランス試験成績及び B19 を用いた不活化・除去試験の結果から、本剤の製造工程において不活化・除去されると考えられている。	本報告は本剤の安全性に影響を与えないと考えられるので、特段の措置はとらない。

Investigation of the Prevalence of Human Parvovirus B19 DNA in Korean Plasmapheresis Donors

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Background : To ensure the safety of plasma derivatives, some countries have been screening for the human parvovirus B19 (B19V) antigen or DNA in blood donors. We investigated the prevalence of B19V DNA and anti-B19V antibodies in Korean plasmapheresis donors to evaluate the necessity of B19V DNA screening test.

Methods : Plasma samples were collected between March and July 2008 from 10,032 plasmapheresis donors. The B19V DNA test was performed using the LightCycler 2.0 (Roche, Germany) with quantification kits. Anti-B19V IgM and IgG were tested in 928 randomly selected samples from the 10,032 donors using recomWell Parvovirus B19 ELISA IgM, IgG assay (Mikrogen, Germany). RecombLine Parvovirus B19 LIA IgG, IgM assay (Mikrogen, Germany) was used to analyze the epitopes of antibodies in donors showing positive results for B19V DNA and anti-B19V antibodies. DNA sequencing was performed to identify the genotypes.

Results : The prevalence of B19V DNA was 0.1% (10/10,032). Virus titers in B19V DNA positive donors were less than 10^5 IU/mL (range: 2.7×10^4 – 3.2×10^4 IU/mL) except for 1 donor (1.33×10^6 IU/mL). All the isolated B19V DNAs from 6 donors were identified as genotype I. Nine out of 10 B19V DNA positive donors also possessed anti-B19V IgG only or IgG and IgM. The prevalence of anti-B19V IgG was 60.1% (558/928).

Conclusions : The prevalence of B19V DNA in Korean blood donors was not high and most donors also possessed neutralizing anti-B19V antibodies. Thus, the implementation of a B19V screening test for Korean blood donors does not appear to be imperative. (*Korean J Lab Med* 2010;30:58-64)

Key Words : Human parvovirus B19, Plasmapheresis donors, Prevalence

INTRODUCTION

The safety of plasma derivatives has been reinforced with more stringent donor questionnaires, implementation of nucleic acid testing (NAT), and treatment of pathogen inactivation and elimination (PI/PE) by plasma fractionation. However, human parvovirus B19 (B19V) trans-

fusion-transmitted infection cases associated with plasma derivatives have been occasionally reported [1-3], particularly in patients with increased risks of infection, such as infants and immunocompromised or pregnant patients. The prevalence of B19V DNA among blood donors has been reported as 0.003-0.9% [4] and the anti-B19V antibody frequency as 30-75% globally [5-8]. The US Food and Drug administration (FDA) guidelines and the European regulatory requirements recommend that the viral load of B19V in the manufacturing of pooled plasma should not exceed 10^4 IU/mL [5]. The Japanese Red Cross has been screening B19V antigen since 1985 [6] and the German Red Cross implemented B19V mini pool NAT (96 pool) for blood donor screening in 2000 [7]. Additionally, many other

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countries have been implementing B19V large pool NAT for pooled plasma samples and products that are manufactured [8].

In Korea, there is no regulation to ensure the safety of plasma derivatives for B19V. The Korean Food and Drug Administration has been concerned with establishing new guidelines to limit B19V contamination in pooled plasma samples and manufacturing products. Some Korean authors have reported the occurrence of B19V antigen in plasmapheresis donors [9] and anti-B19V antibody in Korean patients [10]. We investigated the prevalence of B19V DNA in Korean plasmapheresis donors to evaluate the necessity of B19V screening.

MATERIALS AND METHODS

1. B19V DNA test

Plasma samples were collected between March and July 2008 from 10,032 plasmapheresis donors from 11 Korean Red Cross blood centers. The study was approved by the Institutional Review Board (IRB) and written informed consent was obtained from all the participants. Pooled plasma aliquots of 300 μ L from each of the 24 donors were made by automated pooling using the Tecan Genesis RSP 150 (Tecan, Mannedorf, Switzerland). Two hundred microliters of each sample was used for DNA extraction and synthetic nucleic acid was added to the sample as an internal control. MagNa Pure LC (Roche, Mannheim, Germany) and MPLC total nucleic acid isolation kit (Roche) were used for nucleic acid extraction. Real-time PCR was used for the amplification and detection of the amplified products using the Light Cycler 2.0 (Roche) with the Parvovirus B19 quantification kit (Roche). The samples that showed a positive result in the mini-pool screening test were retested individually. The samples that tested positive in the individual tests were tested an additional 3 times.

2. Anti-B19V antibody immunoassay

We randomly tested 928 samples from 10,032 donors for

the anti-B19V antibody using Behring ELISA Processor III (Dade Behring, Marburg, Germany) with recomWell Parvovirus B19 ELISA IgM, IgG kits (Mikrogen, Neurid, Germany). RecombLine Parvovirus B19 LIA IgG, IgM kits (Mikrogen) were used to analyze antibodies against VP-2, VP-N, VP-IS, VP-2r, VP-C, and NS-1 epitopes in donors showing positive results for both B19V DNA and anti-B19V antibodies. All the tests were performed according to the manufacturers' instruction. The results were interpreted using the criteria outlined in the package insert from the manufacturer (U/mL sample >24 , positive; U/mL sample <20 , negative; $20 \leq$ U/mL sample ≤ 24 , borderline).

3. Retrospective and follow-up tests

To observe the changes of B19V DNA and anti-B19V antibody titers following B19V infection in donors that showed positive B19V DNA test results, a retrospective study in the 6 repeat donors using repository samples and follow up study in 5 donors, including first time donor were undertaken. Follow-up samples involved subsequent blood collection at 12–24 weeks after the index donation time with 2–18 week intervals.

4. B19V genotyping

B19V DNA sequencing of the samples obtained from the 6 donors was performed because the volume of the sample was insufficient. B19V viral DNA was prepared by nested-PCR amplification of a 1,100 bp region spanning the NS1-VP1u junction with the primers shown in Table 1 [11, 12]. The primary PCR round was performed in a 50 μ L reaction mixture containing 5 μ L of DNA, 25 pmol of each primer (e1855f and e2960r), and 3 U of AmplitaqGold polymerase (Applied Biosystems, Foster City, CA, USA). The reaction was performed with 1 cycle at 94°C for 6 min followed by 30 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min, and a final extension at 72°C for 7 min. The primary PCR product (5 μ L) was then amplified with primers e1863f and e2953r under the same conditions with

50 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 50 sec. The PCR products were electrophoresed in agarose gel and purified using the QIAquick gel extraction kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

The purified PCR products were sequenced using a Big Dye Terminator cycle sequencing v3.1 kit (Applied Biosystems) and ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions.

B19V sequences were aligned using the BLAST program (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov/blast/blast.cgi>). Prototype B19 sequences from GenBank were used as reference sequences (GenBank accession numbers were as follows: genotype 1, M13178; genotype 2, AY044266; genotype 3, AY-

083234) [11, 12].

RESULTS

1. The prevalence of B19V DNA

Ten out of 10,032 plasmapheresis donors showed positive results in B19V DNA test (0.1%). The titers of B19V DNA in 9 of 10 donors were low (range, $27\text{--}3.2 \times 10^4$ IU/mL), and 1 donor showed high virus titer (1.33×10^8 IU/mL) (Table 2).

2. The prevalence of anti-B19V antibodies

Nine of the 10 donors who showed positive results in B19V DNA test possessed high titers of anti-B19V IgG, and 7 of them also showed anti-B19V IgM. The rate of positive identification of anti-B19V antibody (IgG only or IgG and IgM) among the donors was 60.1% (558 of 928 donors). The anti-B19V antibody levels in elderly donors were higher than those of the young donors (Table 3). The pattern of reactivity to B19V antigen epitopes in the blot assay (line-immuno assay, LIA) of the 8 donor samples revealed a variable status in B19V infection (Table 4). Donors 8 (D8) and 9 (D9) presented strong reactivity to the VP2r and VPc antigen epitopes with high titer of B19V IgM, thereby suggesting an early stage of B19V infection.

Table 1. Primers used in PCRs and sequencing of human parvovirus B19

Gene	Primer name	Nucleotide sequence (5'-3')	Location* (nucleotides)
NS1/VP1u	e1855f	CACTATGAAACTGGGCAA	1855-1873
	e2960r	ACAATTCCTCATCTGCTAC	2960-2978
	e1863f	AAACTGGGCAATAAACTACAC	1863-1883
	e2953r	CTTCATCTGCTACCGTCCAA	2953-2972
	n-P5f	CGTGAACTGTTAGTTGGGGTTG	2355-2377
	n-P5r	AATGCTGATACACAGCTTTAG	2690-2669

*The location refers to the Pvbaua sequence (accession no. M13178) except for primers n-P5f and n-P5r, which refer to the HV sequence (accession no. AF162273).

Abbreviations: f, forward primer; r, reverse primer.

Table 2. Quantitative PCR results of 10 confirmed human parvovirus B19 positive cases

Concentration of DNA (IU/mL)	Donor ID	Gender	Age	B19V DNA (IU/mL)		Anti-B19V (U/mL*)	
				Mean	95% CI	IgM	IgG
$10^4\text{--}10^5$	Donor1	M	29	2.72×10^4	$0.96 \times 10^4\text{--}5.23 \times 10^4$	Negative	147.34
$10^3\text{--}10^4$	Donor2	F	28	1.39×10^3	$0.51 \times 10^3\text{--}2.78 \times 10^3$	38.86	392.83
	Donor3	M	25	4.25×10^3	$1.15 \times 10^3\text{--}7.36 \times 10^3$	Negative	151.54
	Donor4	F	25	4.60×10^3	$4.06 \times 10^3\text{--}5.15 \times 10^3$	27.01	163.72
	Donor5	M	24	8.01×10^3	$6.55 \times 10^3\text{--}9.47 \times 10^3$	83.61	162.81
	Donor6	F	27	8.15×10^3	$2.15 \times 10^3\text{--}1.61 \times 10^4$	96.04	153.13
	Donor7	M	42	1.85×10^4	$1.53 \times 10^3\text{--}2.17 \times 10^4$	123.44	156.00
$10^4\text{--}10^5$	Donor8	M	31	2.33×10^4	$1.35 \times 10^3\text{--}3.31 \times 10^4$	124.98	140.14
	Donor9	F	24	3.20×10^4	$2.70 \times 10^4\text{--}3.69 \times 10^4$	128.31	77.36
$\geq 10^5$	Donor10	F	24	1.33×10^8	$1.05 \times 10^8\text{--}1.62 \times 10^8$	Negative	Negative

*U/mL sample >24 , positive; U/mL sample <20 , negative; $20 \leq$ U/mL sample ≤ 24 , borderline.

Abbreviations: B19V, human parvovirus B19; CI, confidence interval.

In contrast, Donors 1 (D1), 3 (D3), and 4 (D4) showed reactivity to the NS-1 antigen epitopes with high titer of anti-B19V IgG, thereby suggesting latent or past stage B19V infection.

3. Retrospective and follow-up study

The results of the retrospective and follow-up test are shown in Table 5. B19V DNA titers reduced at the following donation in most donors. The examination of high anti-B19V IgG titers in D1, D2, and D3 revealed steadiness of the antibody titer and low viral loads. Donor 10 (D10) showed the highest titer of B19V DNA (1.33×10^8 IU/

mL) but did not possess any B19V antibodies at the index time. However, at the time of follow-up, 13 weeks after the index time, the virus titer abruptly decreased to 2.7×10^2 IU/mL and revealed the presence of anti-B19V IgG. D8 and D9 did not show evidence of B19V infection in archived samples, which suggested recent infection stage at the index time. D2, D5, and D8 showed concurrent decreasing titers of B19V DNA and anti-B19V IgM. The results of the retrospective and follow-up studies revealed the presence of long-lasting neutralizing anti-B19V IgG in spite of the presence of B19V DNA (Table 4).

4. B19V sequence analysis

The genotypes of B19V DNA taken from the 6 donors were identified as the G1 genotype.

Table 3. Prevalence of human parvovirus B19 antibodies in plasmapheresis donors

Age	N of donors	Only IgG positive		IgG and IgM positive	
		Number	%*	Number	%*
16-19	30	15	50.00	0	0.00
20-29	630	356	56.90	5	0.78
30-39	164	106	64.85	1	0.61
40-49	58	37	64.41	1	1.69
50-59	35	27	77.14	0	0.00
≥60	11	10	90.91	0	0.00
Total	928	551	59.38	7	0.75

*proportion (%) of antibody positively identified within the same age group.

DISCUSSION

The safety of plasma derivatives with regard to B19V has been a major concern to date. Contamination with a high titer of B19V can cause serious problems in patients with an increased risk of infection such as infants and those who are immunocompromised or pregnant. There were some reports of transfusion-transmitted B19V infec-

Table 4. The results of the confirmatory test for the positive donors in both anti-B19V and B19V NAT

Donor ID	Ab	Antigen band reading						Status
		VP-2p	VP-N	VP-1S	VP-2r	VP-C	NS-1	
Donor1	IgG	2+	3+	3+	2+		1+	Past infection month to year
Donor2	IgM	2+	3+	3+	2+			Status after infection (weeks to months)
	IgG	2+	3+	3+	3+	1+		
Donor3	IgG	2+	3+	3+	1±		1+	Long past infection
Donor4	IgM	±	3+	2+				Status after infection (weeks to months)
	IgG	2+	3+	3+	3+	1+	1+	
Donor5	IgM	2+	3+	3+	2+			Status after infection (weeks to months)
	IgG	3+	3+	3+	3+	2+		
Donor6	IgM	2+	3+	1+	2+			Status after infection (weeks to months)
	IgG	1+	3+	3+	2+	1±		
Donor8	IgM	2+	3+	3+	3+	2+		Acute infection (infection within the last 12 weeks)
	IgG	3+	3+	2+	3+	2+		
Donor9	IgM	2+	3+	2+	3+	1+		Status after infection (weeks to months)
	IgG	2+	3+	3+	3+	1+		

Abbreviations: B19V, human parvovirus B19; NAT, Nucleic Acid Testing.

Table 5. B19V DNA and anti-B19V antibodies in retrospective and follow-up specimens

Donor ID	Donation date	B19V DNA con (IU/mL)	Anti-B19V ^a	
			IgM (U/mL)	IgG (U/mL)
Donor1	2008-05-04*	2.72 × 10 ¹	Negative	147.34
	2008-08-02		Negative	358.36
	2008-10-23	7.19 × 10 ¹	Negative	145.26
Donor2	2007-04-17	Negative	ND	ND
	2007-05-26	Negative	ND	ND
	2007-10-23	Negative	ND	ND
	2007-11-09	Negative	ND	ND
	2007-12-06	Negative	ND	ND
	2007-12-20	Negative	ND	ND
	2008-01-11	7.29 × 10 ¹	160.20	152.45
	2008-03-08	6.36 × 10 ¹	88.72	434.46
	2008-04-02	5.34 × 10 ¹	52.88	396.47
	2008-04-16*	1.39 × 10 ²	38.86	392.83
	2008-05-05	2.96 × 10 ¹	Negative	350.31
	2008-07-14	1.39 × 10 ²	Negative	329.31
	2008-09-29	Negative	Negative	162.28
Donor3	2007-12-23	1.13 × 10 ²	Negative	159.42
	2008-01-06	1.28 × 10 ²	Negative	160.24
	2008-01-27	8.87 × 10 ¹	Negative	156.70
	2008-02-17	1.24 × 10 ²	Negative	155.36
	2008-03-16*	4.25 × 10 ²	Negative	151.54
	2008-03-30	9.44 × 10 ¹	Negative	149.97
	2008-08-17	2.24 × 10 ²	Negative	135.26
Donor5	2007-05-18	Negative	ND	ND
	2007-06-01	Negative	ND	ND
	2007-06-15	Negative	ND	ND
	2008-03-06	5.78 × 10 ¹	104.40	161.61
	2008-03-22*	8.01 × 10 ²	83.61	162.81
Donor8	2008-01-15	Negative	ND	ND
	2008-03-15	Negative	ND	ND
	2008-05-19*	2.33 × 10 ³	124.98	140.14
	2008-08-06	7.14 × 10 ²	71.84	153.08
	2008-10-29	3.03 × 10 ²	Negative	144.47
Donor9	2007-08-12	Negative	ND	ND
	2007-10-06	Negative	ND	ND
	2007-11-10	Negative	ND	ND
	2008-02-02	Negative	ND	ND
	2008-02-24	Negative	ND	ND
	2008-04-10	Negative	ND	ND
	2008-04-27	Negative	ND	ND
	2008-07-15*	3.20 × 10 ¹	128.31	77.36
Donor10	2007-11-23	Negative	ND	ND
	2008-02-19	Negative	ND	ND
	2008-03-27*	1.33 × 10 ³	Negative	Negative
	2008-07-08	2.68 × 10 ²	Negative	266.54
	2008-09-24	2.85 × 10 ²	Negative	158.66

*index donation; U/mL sample >24, positive; U/mL sample <20, negative; ≥20 U/mL sample ≤24, borderline.

Abbreviations: B19V, human parvovirus B19; ND, not done.

tion through the use of tainted plasma products [13, 14]. In our study, the prevalence of B19V DNA in healthy Kore-

an plasmapheresis donors was 0.1%, which was not high compared with that of other countries such as United States (0.83–0.88%), and Germany and Austria (0.27%) [7, 15]. Additionally, the donors had low viral loads (range, 10²–10³ IU/mL), except for one donor. These results are consistent with those of previous reports [15].

Recently, the significance of the neutralizing effect of anti-B19V IgG has been considered in B19V infection. The anti-B19V IgG in donated blood or recipients can neutralize the virus and prevent B19V infection. Some reports indicate that 11 U/mL of anti-B19V antibody can neutralize 4.3 log of B19V DNA [16].

Kleinman et al. [17] reported that minimum infectious doses of B19V were 2 × 10⁴ IU/mL (in the coagulation factor) or 10⁷ IU/mL (in the other plasma products) for recipients who did not possess neutralizing antibodies. They also insisted that the blood products containing virus titers below 10⁴ IU/mL were not infectious [17].

A recent survey reported that the global prevalence of anti-B19V antibody was 30–60% in adults. Most of the B19V DNA positive donors and 60% of the recipients showed anti-B19V IgG [15]. The Transfusion Related Infection Prospective Study (TRIPS) and Retrovirus Epidemiology Donor and Recipient (RADAR) study showed that the prevalence of B19V DNA was 0.8% in blood donors and 1.7% in recipients in the United States. In these studies, all the B19V DNA positive donors showing a low viral load of less than 10² IU/mL also possessed neutralizing anti-B19V antibodies. They also reported that 21 recipients who did not possess neutralizing antibodies and transfused B19V DNA-tainted blood components did not show any evidence of B19V infection [15]. The neutralizing anti-B19V antibodies contained in the donor blood may have neutralized B19V.

Data from German Red Cross blood centers (GRCBC) that have been screening mini-pool B19V NAT in all blood donors revealed that virus titers below 10⁵ IU/mL in pooled samples released to hospitals, except in patients with special risks [5]. The reason B19V DNA positive blood components were released was because of the simultaneous presence of neutralizing antibodies (to VP1 and

VP2) in the blood products. GRCBC reported that 75% of the B19V DNA negative donors possessed neutralizing antibodies, which could protect against B19V infection.

In our study, 90% of the B19V DNA positive plasmapheresis donors and 60% of the 928 randomly selected plasmapheresis donors possessed anti-B19V IgG. However, the blood components containing high titers of B19V (1.33×10^8 IU/mL) could lead to infection in patients with risk factors. The clinical progression following B19V infection would vary according to factors such as the patient's immune status and the presence of neutralizing anti-B19V IgG. Japanese groups [18, 19] have reported that most of the transfusion transmitted B19V infected patients show only mild symptoms such as a fever or rash.

Recently, new pathogen elimination techniques such as nanofiltration (20–35 nm pore size) have been introduced and the Japanese Red Cross has been using this method during fractionation.

In conclusion, the prevalence of the B19V DNA in Korean plasmapheresis donors was not high and more than 60% of the plasmapheresis donors possessed neutralizing anti-B19V antibodies, thereby suggesting that the possibility of transfusion transmitted B19V infection in Korea is not serious. Therefore, the implementation of B19V DNA screening for all blood donors does not seem to be warranted at this time.

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医薬品
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研究報告 調査報告書

識別番号・報告回数	報告日	第一報入手日 2013年08月28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	①②抗 HBs 人免疫グロブリン ③④乾燥抗 HBs 人免疫グロブリン ⑤ ポリエチレングリコール処理抗 HBs 人免疫グロブリン	公表国 イタリア		
販売名 (企業名)	①抗 HBs 人免疫グロブリン筋注 200 単位/1mL「日赤」 (日本血液製剤機構) ②抗 HBs 人免疫グロブリン筋注 1000 単位/5mL「日赤」 (日本血液製剤機構) ③へブスブリン筋注用 200 単位 (日本血液製剤機構) ④へブスブリン筋注用 1000 単位 (日本血液製剤機構) ⑤へブスブリン IH 静注 1000 単位 (日本血液製剤機構)	研究報告の 公表状況 Transfusion 2013; 53(7): 1421-1428		
研究報告の概要	いくつもの新興感染症 (EID) が輸血感染していることが繰り返し確認されていることから、血液の安全性に対して潜在的リスクを引き起こす EID はこれまでに知られていないよりも多いかもしれないという認識が高まっている。大半の国では、既知の疾患 (ヒト免疫不全ウイルス、B 型肝炎ウイルス、C 型肝炎ウイルスなど) から血液の安全性を確保するために多種多様な安全対策が講じられている。このため、供血者の選択手順、スクリーニング検査、および製造工程における病原体の除去/不活化により、輸血感染リスクを非常に低いレベルまで下げることができている。こうした対策にもかかわらず、多数の EID の輸血感染が記録されていることから、新たなアウトブレイクが起きた場合には追加的措置をとる必要があるかもしれないことが示唆される。しかし、アウトブレイク中に特定の EID の伝播予防措置を実行するのはコストがかかり、血液供給の不足を招くおそれがあるため、そのような措置を支持するエビデンスが得られない場合には妥当ではないかもしれない。そのため、タイムリーなリスクアセスメントのほかに、EID がアウトブレイクした後、またはアウトブレイクの後半に行われている。アウトブレイクの初期段階、またはそれが実際に起きる前に血液の安全性に対するその影響を予想できるリスク評価モデルが構築されれば、アウトブレイクの初期段階で安全対策を実行できると考えられる。血液の安全性に対する輸血感染リスクを EID アウトブレイク中にタイムリーに評価できるようなモデルの開発が欧州疾病予防管理センターにより開始され、European Up-Front Risk Assessment Tool が作成されるに至った (EURFRAT; http://euferratool.ecdc.europa.eu/)。本稿では、EURFRAT のモデル構造と仮説、ならびに最近のアウトブレイクのデータにこれを応用した結果について記載する。 公表文献から入手した、特定の病原体による感染症の臨床的および疫学的特性をサーベイランスデータと組み合わせ、供血者集団が感染しているリスクから受血者が感染するリスクまでの輸血感染リスクを算出するモデルの設計に用いた。イタリアで最近起きたチタングニアのアウトブレイクを用いて、本モデルの応用方法を示す。数種の介入シナリオを解析した。なかでも、献血血液の検査期間を延長した場合の、受血者の感染獲得リスクに対する影響を解析した。さらに、アウトブレイク地域を訪問した供血者が自国に戻って感染血液を献血する際のリスクも評価した。 材料および方法 まず、本モデルの仮説と構造を示し、次に特定のデータセットに対するこの応用方法を説明する。 モデルの説明 EID の特性ならびに輸血業務内での処理の詳細を反映した汎用モデルを構築した。輸血チェーン内の 5 つの連続するステップを特定した。各ステップにおける感染リスクは以下のように説明される。1) 供血者が感染しているリスク、2) 感染血液が献血されるリスク、3) 感染した血液成分がリリースされるリスク、4) 最終血液製剤にウイルスが存在するリスク、5) 受血者に感染が伝播するリスクである (図 1)。			
使用上の注意記載状況・ その他参考事項等	代表としてへブスブリン IH 静注 1000 単位の記載を示す。 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 抗体陰性であることを確認している。更に、プールの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高力価の抗 HBs 抗体を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗 HBs 人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60°C、10 時間の液状加熱処理及びウイルス除去膜による過処理を施しているが、投与に際しては、次の点に十分注意すること。			

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具体的には、供血者には一般集団の一部として、その曝露の程度に応じてアウトブレイク中に感染する一定の確率がある。献血前には供血者に対し供血者健康調査票 (DHQ) への記入が求められるが、これには曝露の履歴、リスク因子、または既知の感染症の症状に関する一連の質問が含まれている。この調査は、潜在的な感染供血者を一定の有効性で検出するもので、また、感染の状態にかかわらず供血者のコンプライアンスに準じて利用される。供血者は、全血輸血、血漿輸血などその献血タイプに応じて分類される (それぞれに特定の献血頻度がある)。各献血血液は検査を受けてからその成分が後の処理段階 (病原体の除去/不活化など) に進められ、最終血液製剤が製造される。最終的にこれらの製剤が受血者に輸血されることになる。これら5つの各ステップからのアウトプットは1) 供血者集団における感染有病率の変化、2) 感染血液が献血される回数、3) リリースされる血液成分による感染、4) 最終製剤による感染、5) 受血者の感染数である。以下には、本モデルに基づいている仮説を示す。計算全般に用いている詳細な式は、付録 S1 に記載している (本誌オンライン版で補足情報として閲覧可)。

献血時点で供血者が感染している確率 (ステップ1) は、供血者集団における感染の有病率に比例すると仮定している。これは、発生率 (アウトブレイクの評価中に曝露集団から報告された新規症例数) から、感染期間に関する知識を用いて計算する。本モデルにおける感染期間は、急性感染患者の一部が慢性感染に進行するような EID に応用できるような、急性相と慢性相の感染期間の和として考えている (図2)。この有病率をさらに、供血者の相対リスク (供血者の感染リスクを一般集団の感染リスクと比較したもの) により補正する。さらに、未発症例の割合に関する補正係数も加える。すなわち、無症状態で医療機関を受診しなかったため、または誤診により、発症例として報告されなかった感染患者の割合である。大半の EID の場合、特にアウトブレイクの初期では、供血者の相対リスクおよび未発症例の割合はよく分かっていない。これらの変数について情報が得られない場合は、供血者のリスクは一般集団のリスクと同じであると仮定し、また未発症例の割合は、無症候性の症例の割合以上であると仮定する。有病率も、供血者のデータを直接用いて定量化でき (献血血液の検査の有無を問わず)、アウトブレイク地域を一時的に訪問した供血者が自国で感染血液を供血するリスクも推定される。

ステップ2では、感染供血者により行われる可能性がある献血回数を、ステップ1で推定した有病率、献血行動、実施した DHQ 調査により感染供血者が特定される確率を用いて推定する。DHQ により、感染症状を示す感染供血者がスクリーニングされる。したがって、症候性感染者の感染期間はここで、発症前の感染期間 (critical infectivity [感染力が強い] 期間と呼ばれる) と、発症後の感染期間に分けられる。この critical infectivity 期間は、感染供血者から感染した献血血液が生じる頻度を決定する。すなわち、critical infectivity 期間が献血間隔 (2回の献血の間の日数) より長い場合、1回目の献血が感染期間の初期であったと想定すると、感染供血者は感染した献血血液を複数回提供すると予想される。そのため感染血液の合計献血回数は、有病率、供血者集団の大きさ、および感染者の予想献血頻度の積として推定される。さらにこの数値を、DHQ のスクリーニングで除外されたであろう症候性感染供血者により行われる献血の割合について補正する。

感染献血血液の数、診断検査、血液の処理、病原体の除去/不活化、その他の安全対策によりさらに減少する。診断検査はその検査項目と有効性に応じて、リリースされる感染成分の数を減少させるが、血液の処理と病原体の除去/不活化は、感染した最終製剤の数を減少させる。感染供血者からの献血血液は、輸血の受血者に対して、一定の感染率で感染性をもつと推定される。この確率は、既知の多くの病原体について 100%未満であるが、本モデルではワーストケースのシナリオとするため、初期値として 100%と設定している。受血者が感染するリスクは、その感染症に対する個々の免疫性によっても異なる。

免疫性をもたない者は、特定の感染徴候を示す。大半の EID では、特定の免疫性をもつ受血者の割合、および感染血液製剤の輸血後に特定の徴候が発現する割合の推定値は入手されていない。そのような場合、すべての受血者が十分に感染しやすく、したがって疾患の重症度は一般集団のそれと同じであると推定する。受血者の感染リスクは、感染した最終製剤が投与された非免疫受血者における感染者数として計算される。この数値はさらに、急性重症感染者、慢性感染者、および死者数に分けられる。

記述される全推定値には不確実性が伴う。この不確実性は、データに固有のものか (サンプリング誤差)、または感染力の遷移率 (transition rate) および感染性にとどまっている時間 (duration of the infectivity) の不確実性によるものと考えられる。このモデルでは、モデルのインプットと変数に関する不確実性の範囲が特定できる。曝露集団あたりの発症例の割合はベータ分布を呈し、感染力の遷移率および感染性にとどまっている時間は三角分布を示す (その最頻値と範囲は文献のデータに示されている) と推定される。モデルのインプットおよび変数はさらにその分布からサンプリングされ、各数値セットについてモデル推定値が算出される。このシミュレーションの結果から、それぞれのモデルのアウトプットについて確率分布が得られ、そこから平均値、中央値、および信頼区間 (CI) が得られる。

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アウトブレイクデータへの応用

モデルを検証するため、イタリアで起きたチクングニアのアウトブレイクの最近発表されたデータを用いた。データは、表1の最初の4列に示しているように、このオランダの研究から抽出した。15週間のアウトブレイク期間中、住民3,977,508人の地域で合計247例の感染例が確定された。発表されている研究の結果と比較できるよう、症候性感染症の割合(85%)と感染性にとどまっている期間については、同様の数値を用いた。感染性を有する期間は、症候性の感染者と無症候性の感染者で同様であると想定し(8日間)、感染から発症まで2日間と想定した。計算に用いたcritical infectivity期間は、症候性の感染者で2日間、無症候性の感染者で8日間とした。平均で年2回全血献血している献血者は、140,216人いた。症候性および無症候性の感染症について、critical infectivity期間がそれぞれ2日間と8日間である確率が最も高いとき、変数値の分布を用いたシミュレーションでは、広めの範囲として1~12日間を用いた。既報では、イタリアの輸血業務に関する詳細はあまり報告されていないことから、我々は計算上、以下のことを想定した：安全対策が実行されているにもかかわらず、献血血液は献血者に対して感染性をもっていない。全血献血から3種類の血液成分が製造される。最終製剤の製造には、血液成分をプールしない。献血者は感染に対して免疫をもっていない。感染した献血者の0.1%が急性重症感染を発症する。

このアウトブレイク中に輸血感染症例は認められなかったものの、タイムリーなリスクアセスメントを行うことにより、このEIDによりもたらされる血液の安全性に対するリスクが推定できるものと考えた。本モデルを用いて感染の有病率を算出し、それにより求められる感染供血者/ウイルス血症の献血者を、毎週(Method 1)および累積して(Method 2)通知される症例数を用いて算出した。有病率は、固定インプットデータと可変的な分布変数を用いて算出した。アウトブレイクのピーク時(10週目)における輸血感染リスクを、献血者の有病率から始め、献血者における潜在的な感染者数に至るまで算出した。

当時、イタリアで血液を管轄している省庁(Italian blood service)により、関係地域におけるアウトブレイクの影響を最小限に抑えるための追加措置がいくつかとられていた。1つの措置は、検査期間を2日間または5日間とするものであった。我々のモデルから、そのような措置をとった場合ととらなかった場合で収集される、ウイルス血症の献血血液数が推定された。またこのモデルにより、来訪者が自国でウイルス血症血液を献血する輸血感染リスクを、DHQを実施した場合と非実施の場合とで解析した。後者については、献血者がアウトブレイクのピーク時に1週間訪問し、帰国前に感染し、その翌日から年2回の割合で献血したものと想定した。回答者が完全に遵守するDHQについて、有効性は85%と想定した。

結果

アウトブレイクピーク時の評価により、毎週報告された症例を用いた場合と累積症例を用いた場合の献血者10万人あたりの有病率はそれぞれ1.07人(95%CI: 0.38-2.03)と0.39人(95%CI: 0.15-0.71)、感染血の献血回数それぞれ0.04回(95%CI: 0.01~0.10)と0.02回(95%CI: 0.002~0.06)になると推定された。この推定値により、感染した血液成分数(ステップ3のアウトカム)は、毎週報告された症例を用いた場合と累積症例を用いた場合とでそれぞれ0.13回分と0.05回分、感染した最終製剤数(ステップ4)は0.13回分と0.05回分、献血者の重度感染者数は0.0001人と0.00005人になる。

図3に、2007年のイタリアにおけるチクングニアのアウトブレイク中でのウイルス血症患者の推定数と、ウイルス血症献血者の推定数を要約する。この図から、固定モデル変数を用いた感染の有病率推定値は、ランダム分布を用いた推定値より低いことが分かる。これは、一定の歪分布(skewed variable distribution)から求められた平均値は固定変数値よりも高いためである。感染期間の分布を2.9日間(1~5日間)と定めていたなら、ランダム分布モデルの結果は固定モデルの結果に一致していたと思われる。アウトブレイクのピーク時には、累積推定値(Method 2)が週推定値(Method 1)に比べきわめて低いが、これは、Method 2の場合、比較的長い流行時間単位で発生率を平均化する傾向があるためである。

図4は、アウトブレイク期間にウイルス血症が献血される週単位の推定数を検査日数別に示している。検査期間が2日間と5日間の場合、回収した5378回分の献血血液からそれぞれ0.027回分と0.0025回分の血液がアウトブレイクのピーク時にウイルス血症性であったと推定される。この数値はそれぞれ、197,333回分の献血血液中1回分と、2,170,665回分の献血血液中1回分がウイルス血症であることに相当する。イタリアでは、38万回分あたり1回分のウイルス血症輸血血液が関与していると推定されていることから、後者については許容できると考えられる。ウイルス血症の献血血液の数は、検査日数を7日間以上としたとき0になると推定される。

我々のモデルによって得られた推定値は、図5に示す通り、Liimbrunoらの発表した推定値によく一致している。我々のモデルではウイルス

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ス血症の全症例数を推定しているのに対し、既報の研究は無症候性症例のみを表していることに注意するべきである。既報の研究により既に結論が得られていた通り、このアウトブレイク期間の症候性症例は、ウイルス血症の献血血液にほとんど影響しなかったと考えられる。さらに本モデルから、アウトブレイク地域を訪問した供血者が自国にて感染血液を献血する確率は 10 万人あたり 0.30 人 (95%CI: 0.01 ~ 0.65) であることが推定された。DHQ が実施されたと想定すると、この者が感染血液を献血するリスクは、10 万人あたり 0.458 人である。そのような DHQ が実施されなかった場合、このリスクは 10 万人あたり 0.515 人に増加する。

考察

アウトブレイク情報を用いた輸血感染リスクの定量化に本モデルをどのように利用できるかを示した。これは、供血者集団における感染獲得の確率から始まり、ウイルス血症の献血血液の推定数、さらには受血者集団における輸血感染リスクに至るまでのものである。さらに、チンクニアの例を用いて、ユーザがどのようにして、様々なアウトカムシナリオの解析に本モデルを応用できるかを示した。週単位のウイルス血症献血血液の推定数は、政治家たちがどの時点で追加の安全対策を行うべきかを判断する際に役立つと考えられる。また、このモデルは、その後の輸血感染リスクから受血者の重症感染発現リスクに至るまで、またアウトブレイク地域を訪問した供血者からのリスクについても推定できた。その結果の比較により、我々のモデルで得られる推定値は、オリジナルの研究の数値と類似していることが示されている。さらに本モデルからは、様々な検疫期間について、検疫が輸血感染リスクの封じ込めにどれほど有効かが示された。輸血感染リスクの包括的な推定値を示すこのようなモデルは、アウトブレイクが生じた際のリスクアセスメントに有用であるかもしれない。この EURAT モデルは、すべての EID に、また輸血チェーンにおける様々な安全確保の介入に適用することができる。この他にも、輸血に対する感染症のリスクを推定するモデルが発表されている。1 つのモデルは、アウトブレイク地域における献血血液感染のリスクを直接的に計算し (<http://ewrtest.ecdc.europa.eu/blood/>)、その他のモデルは、特異的 EID の輸血感染リスクを定量化している。これらには、様々な介入のコストや効果を考慮したものも、考慮していないものもある。Kleinman らが開発した広範囲に及ぶモデルでは、新興病原体のリスクを定量化することで同様の目的を目指している。このモデルはリスク管理を目的に開発されたものだが、受血者の医療費の損失に対する新興病原体の破滅的影響を評価するために用いられている。しかし、EURAT モデルの主な目的は、継続中の EID アウトブレイクによる特定の輸血感染リスクを解析することである。本モデルは汎用的であるという特徴から、短期/中期/長期のリスク期間をもつ EID のアウトブレイク期間中に入手される限られた情報を用い、供血者における感染リスクから受血者の感染リスクに至るまでの大まかな輸血感染リスクを求めるために利用することができる。これは、本モデルの要素が EID の最も基本的な特性、ならびに輸血制度に考えられる安全対策を把握するよう、慎重に選択されたためである。EURAT モデルは、保健当局や輸血専門機関がモデリングの知識背景の有無にかかわらず利用できるよう、簡単に使用しやすいユーザ向けインターフェースとなっている。費用対効果などさらなる解析が必要と考えられる場合は、我々のモデルの結果を利用することもできる。

このモデルは実務的な利便性を持っているものの、いくつかの限界がある。その 1 つは、リスクアセスメントのための有病率の推定値は規定される時間単位により影響される、ということである。累積的に推定された有病率は週単位で推定された有病率に比べ、流行のピークに達するまでは低く推定され、単位時間あたりの症例数が減った場合には高く推定される。これは、アウトブレイクの時間単位全体を通して、発生率を平均化したことに基づいている。つまり、短い時間間隔でリスクを評価すれば精度は改善すると考えられるが、症例数の変動による不確実性に大きく影響される可能性もある。ただしこのモデルは、アウトブレイクデータが入手できれば、適正なリスクアセスメントを行う時間単位を特定するために用いることができる。もう 1 つの限界は、慢性感染症に進行している感染患者の集団によるリスクがその急性相のリスクと重複してしまいう点である。これにより、慢性感染者の割合が顕著に高い EID の場合、真のリスクを過大評価してしまう可能性がある。

リスクの許容性についても議論が続けられている。今日まで、一般集団および EID の特定集団において許容可能な輸血感染リスクとはどのようなものかについて、コンセンサスは確立されていない。すべての国の血液供給機関が最大限の血液の安全性を追求している。しかし、輸血感染リスクをもつ EID の数が増えつつあるとき、すべての国とは言わずとも一部の国にとっては、実行可能なすべての措置を行うことはできない。EURAT モデルは、リスク軽減によって得られる安全対策の有効性を評価し、残るリスクの推定値を求めることに役立つ。本モデルの目的は、アウトブレイクの完全なリスクアセスメントを行うことではない。この第一の目的は、(追加的な) 安全確保の介入に関わる意思決定を支えるべく、輸血感染リスクの規模を推定することである。

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汎用性をもつ本 EUPRAT モデルは、限られた量の病原体情報とアウトブレイクデータを用い、チクングニアやその他の EID の輸血感染リスクを定量化するために応用できる。モデルのこのような柔軟性により、様々な安全対策に関する輸血感染リスクの評価、およびアウトブレイク地域を訪問した供血者のリスクの評価も可能である。本モデルの結果は、輸血機関および保健当局による標的を絞った予防措置を支持するために用いることができる。	
報告企業の意見	
本モデルの検証に用いられたチクングニアウイルス (Chikungunya virus: CHIKV) は、トガウイルス科 (Togaviridae) アルファウイルス属 (Alphavirus) に分類される直径 70nm のエンベロープを有する球状の RNA ウィルスで、蚊 (ヤブカ属のネッタイシマカやヒトスジシマカ) によって媒介される。万一、原料血漿に CHIKV が混入したとしても、Bovine viral diarrhoea virus (BVDV) をモデルウイルスとしたウィルススクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	今後の対応 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。

TRANSFUSION COMPLICATIONS

Modeling the transmission risk of emerging infectious diseases through blood transfusion

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BACKGROUND: A timely risk assessment is desired to guide decisions on preventive transfusion safety measures during emerging infectious disease (EID) outbreaks. The European Up-Front Risk Assessment Tool (EUFRAT) model was developed to provide quantitative transmission risk estimates of EIDs through blood transfusion.

STUDY DESIGN AND METHODS: The generic model comprises five sequential steps to estimate the infection risks in the blood transfusion chain: 1) the prevalence of infection in the donor population, 2) the risk of obtaining infected donations, 3) infected components, 4) infected blood products, and 5) the risk of transmitting the infection to recipients. The model uses inputs from epidemiologic characteristics of an EID and transfusion practice. The model was applied to data from a recent chikungunya outbreak in Italy.

RESULTS: Based on data from the outbreak peak, an estimated prevalence of 1.07 (95% confidence interval [CI], 0.38-2.03) per 100,000 donors would lead to 0.04 infected donations (95% CI, 0.01-0.10), 0.13 infected blood components, 0.13 infected end products, and 0.0001 severe infections in recipients. This estimated risk can be reduced by increasing the duration of quarantine of the donated blood and becomes zero after 7 or more days of quarantine. The model also estimated the probability of a donor returning from the outbreak area and subsequently donating infected blood in his home country to be 0.30 (95% CI, 0.01-0.65) per 100,000.

CONCLUSION: The model can be used to quantify EID outbreak risks to blood transfusion recipients and the effect of targeted safety interventions and as such support public health decision-making.

Repeated identification of transfusion transmission (TT) of some emerging infectious diseases (EIDs) raised the awareness that there may be more EIDs than previously known posing a potential risk for blood safety.¹⁻⁴ In most countries a whole range of safety measures are in place to ascertain blood safety from known diseases (such as human immunodeficiency virus, hepatitis B virus, and hepatitis C virus). This resulted in reduction of TT risks to very low levels by means of donor selection procedures, screening tests, and pathogen removal and/or inactivation in the production process.^{5,6} Despite these measures the TT of a number of EIDs was documented, suggesting that additional measures may be required if a new outbreak occurs. However, implementing measures to prevent transmission of specific EIDs during outbreaks is costly, may lead to shortages in blood supply, and may be unjustified when supporting evidence for such measures is lacking. Timely risk assessment is therefore desirable. To date, risk assessments are mostly performed after or at a later phase of an EID outbreak.⁷⁻⁹ A model that can assess risks in the initial

ABBREVIATIONS: DHQ = donor health questionnaire; EID(s) = emerging infectious disease(s); EUFRAT = European Up-Front Risk Assessment Tool; TT = transfusion transmission.

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phase of the outbreak or even before it actually occurs to anticipate its impact on blood safety has the advantage that safety measures can be implemented at an early outbreak stage. The development of such a model, allowing a timely assessment of the TT risk to blood safety during an EID outbreak, was initiated by the European Centre for Disease Prevention and Control and resulted in the European Up-Front Risk Assessment Tool (EUFRAAT; <http://eufraatool.ecdc.europa.eu/>). Here we describe the model structure and assumptions of EUFRAAT and its application to data from a recent outbreak.

Surveillance data combined with clinical and epidemiologic characteristics of infections with specific pathogens from published literature were used to design a model for calculating the TT risk, starting from the risk of contracting the infection in a donor population up to the risk of recipients getting infected. The recent outbreak of chikungunya in Italy is used to illustrate the application of the model.⁹⁻¹¹ Several intervention scenarios were analyzed, among others the effects of increasing the quarantine duration of blood donations on the recipient risk of acquiring infection. Also, the risk from a donor who visited the outbreak area donating infected blood in his or her home country was assessed.

MATERIALS AND METHODS

We first describe the model assumptions and structure and then how we applied it to a specific data set.

Description of the model

A generic model that reflects the EID characteristics and the processing details within the blood transfusion practice was developed. Five sequential steps in the blood transfusion chain were identified and the risks of infection in each of the steps were accounted for: 1) the risk of a donor being infectious, 2) the risk of yielding infected donations, 3) infected released components, 4) infected end products, and 5) the risk of recipients contracting the infection (Fig. 1). Specifically, donors as part of a general population have a certain probability of being infectious during an outbreak depending on their exposure. Before donating, donors are asked to fill out a donor health questionnaire (DHQ), containing a set of questions that are related to history of exposure, risk factors, or symptoms of known infection. The questionnaire detects the potentially infectious donors with certain effectiveness and is applied according to the donors' compliance that is independent of infection status. Donors are categorized

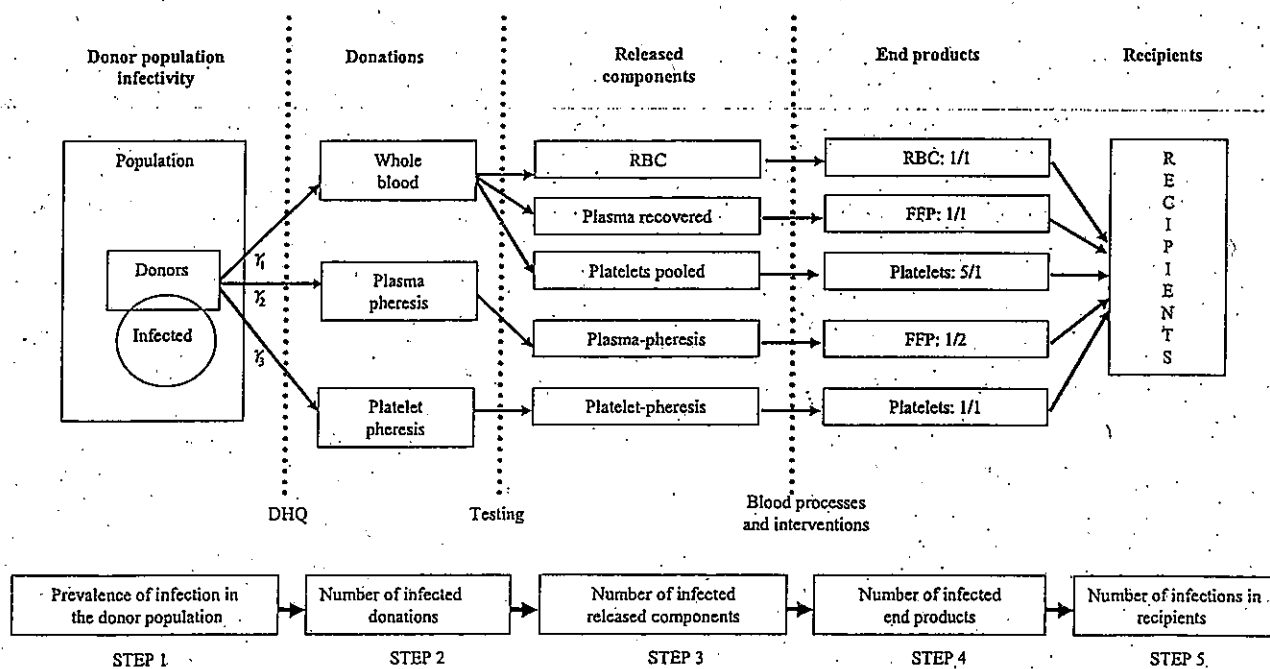


Fig. 1. Risk model flow scheme. The risks of infection in the transfusion chain are estimated in five steps; starting from the risk of obtaining infection in the donor population up to the risk of contracting the infection in recipients, accounting for various safety measures in transfusion practice, among others DHQ, diagnostic tests on the donated blood, processing steps, pathogen removal and/or inactivation, or other processes. The outcomes of each step are: 1) prevalence of infection in the donor population, 2) number of infected donations, 3) number of infected components, 4) number of infected end products, and 5) number of infections in recipients.

according to their donation type, for example, whole blood or plasma, with a particular donation frequency. Each donation is tested before its components are released to further processing steps including pathogen removal and/or inactivation to produce final blood products. Finally, these products will be transfused to recipients. The outputs from each of the five steps are 1) the changing prevalence of infection in the donor population, 2) the number of infected donations, 3) infected released components, 4) infected end products, and 5) the number of infections in recipients. In the following we describe the assumptions on which the model is based; detailed formulas for all calculations can be found in Appendix S1 (available as supporting information in the online version of this paper).

The probability of a donor being infectious at the moment of donation (Step 1) is assumed to be proportional to the prevalence of infection in the donor population. This is computed from the incidence rate, that is, number of new cases reported from an exposed population during the outbreak assessment, using knowledge about the infectious period. The infectious period in the model is taken as a sum of the infectious period of acute and chronic phases to allow for application to EID where a proportion of acutely infected individuals progress to chronic infection (Fig. 2). The prevalence is then corrected by donor relative risk, that is, the risk of a donor contracting the infection compared with that of the general population. Furthermore, there is a correction factor for the

proportion of undetected cases, that is, the proportion of infected individuals who are not reported as cases due to lack of symptoms, not seeking health care, or due to misdiagnosis. For most EIDs, a donor's relative risk and the proportion of undetected cases are not well known, especially at the beginning of an outbreak. If no information about these variables is available, we assume that a donor's risk equals that of the general population and that the proportion of undetected cases is larger or equal to the fraction of asymptomatic cases. The prevalence can also be quantified using donor data directly, with or without testing the donations, and to estimate the risk of a donor who has temporarily visited the outbreak area donating infected blood in his home country.

In Step 2, the number of donations potentially made by infectious donors is estimated using the prevalence estimated in the previous step, the donation behavior, and the probability that an infectious donor is identified by the DHQ implemented. The DHQ screens out those infectious donors who show symptoms of infection. Consequently, the infectious period of the symptomatically infected individuals used here is subdivided into the infectious period before symptom onset, referred to as critical infectivity, and after. This critical infectivity determines the frequency of yielding infected donations from infectious donors; if the critical infectivity is longer than the interdonation interval, that is, number of days between two donations, it is expected that an infectious donor will give an infected donation more than once assuming that first donation is

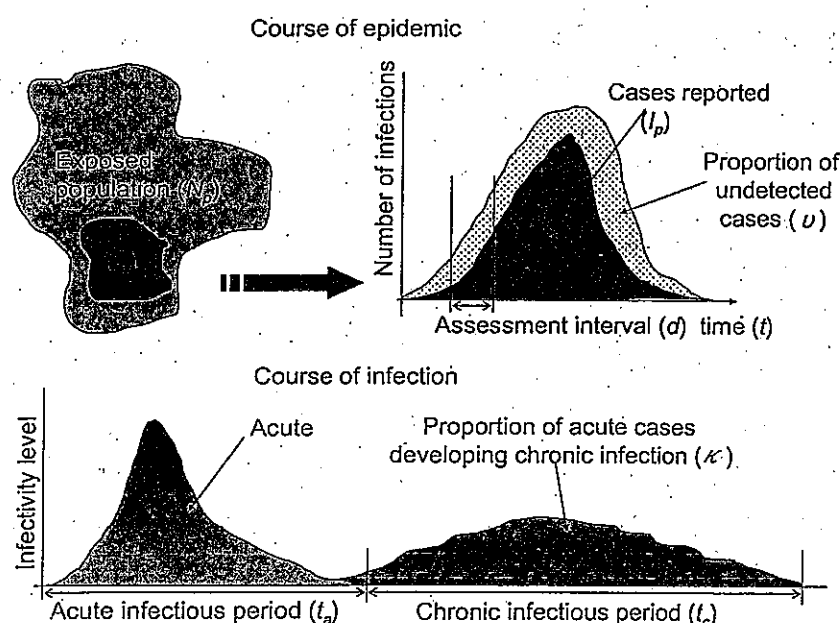


Fig. 2. Estimating the prevalence of infection in donor population. The prevalence of infection p is estimated from the number of cases reported I_p in the exposed population N_p during the assessment interval d , accounting for the proportion of undetected cases v and the infectious period for acute t_a and a proportion κ of acute individuals that progress to chronic infection t_c . Donors are assumed to have similar risk of acquiring the infection as the general population ($R_d = 1$).

at beginning of infectious period. The total number of infected donations is therefore estimated as the product of the prevalence, size of the donor population, and the expected frequency of infected donations. This number is then corrected for the proportion of donations made by the symptomatically infectious donors that would be screened out by the DHQ.

The number of infected donations will be reduced further by diagnostic testing, blood processing, pathogen removal and/or inactivation, or other safety measures. A diagnostic test, depending on its coverage and effectiveness, reduces the number of infected released components, whereas the latter two measures reduce the number of infected end products. It is assumed that the blood donated by infectious donors is infectious for transfusion recipients with a certain transmission probability. This probability is less than 100% for many known agents¹² but in the model its default value is set to 100% to capture a worst-case scenario. The recipients' risk of getting infected also depends on their specific immunity against the infection.

Individuals without immunity will have a particular infection manifestation. Estimates of the proportion of recipients with specific immunity and the proportion of developing specific manifestations after receiving infected blood products are not available for most EIDs. In that case, it is assumed that all recipients are fully susceptible to infection and that disease severity is the same as in the general population. The recipients' risk of contracting the infection is calculated as the number of infections in nonimmune recipients who received the infected end products. This number is then further subdivided into a number of acute severe infections, chronic infections, and the number of deaths.

All estimates described are subject to uncertainty. This uncertainty may be inherent in the data (sampling error) or it might be due to uncertainty in transition rate and duration of the infectivity. The model allows specifying uncertainty ranges for model input and variables. We assume that the proportion of cases per exposed population is beta distributed¹³ and that transition rates and durations have a triangular distribution where modes and ranges are specified from literature data. Model input and variable values are then sampled from their distributions and for each set of values model estimates are computed. The simulation outcome therefore results in a probability distribution for each of the model outputs from which the mean, median, and confidence intervals (CIs) are then obtained.

Application to outbreak data

Recently published data from an outbreak of chikungunya in Italy were used to test the model.⁹ Data were abstracted from the original study as shown in the first four columns of Table 1. A total number of 247 cases were identified in an area with a population of 3,977,508 inhabitants within an outbreak period of 15 weeks. To allow a comparison with results of that published study we used similar values for the proportion of symptomatic infections (85%) and the duration of infectivity. The infectious period is assumed to be of equal duration for symptomatically and asymptotically infected individuals (8 days) and symptom onset is 2 days after becoming infectious; the critical infectivities used in the calculations were 2 and 8 days for symptomatic and asymptomatic donors, respectively. There are 140,216 donors that donate whole blood on average twice a year. In the simulation using distributions of variable values a

TABLE 1. Number of notified cases (weekly and cumulatively) during 15 weeks of the chikungunya outbreak in Italy in 2007^a and the number of viremic donations (in per 100,000) estimated from weekly cases as a function of the quarantine period

Epidemic week	Begin date*	Weekly cases	Cumulative cases	Estimated number of viremic donations (per 100,000) per quarantine period (days)		
				0	2	5
1	17/06/2007	1	1	92	57	5
2	24/06/2007	0	1	0	0	0
3	01/07/2007	1	2	92	57	5
4	08/07/2007	1	3	92	57	5
5	15/07/2007	8	11	730	454	41
6	22/07/2007	10	21	912	568	52
7	29/07/2007	26	47	2371	1476	134
8	05/08/2007	42	89	3831	2385	217
9	12/08/2007	38	127	3466	2158	196
10	19/08/2007	48	175	4378	2725	248
11	26/08/2007	26	201	2371	1476	134
12	02/09/2007	25	226	2280	1419	129
13	09/09/2007	8	234	730	454	41
14	16/09/2007	9	243	821	511	46
15	23/09/2007	4	247	365	227	21

* Day/month/year.

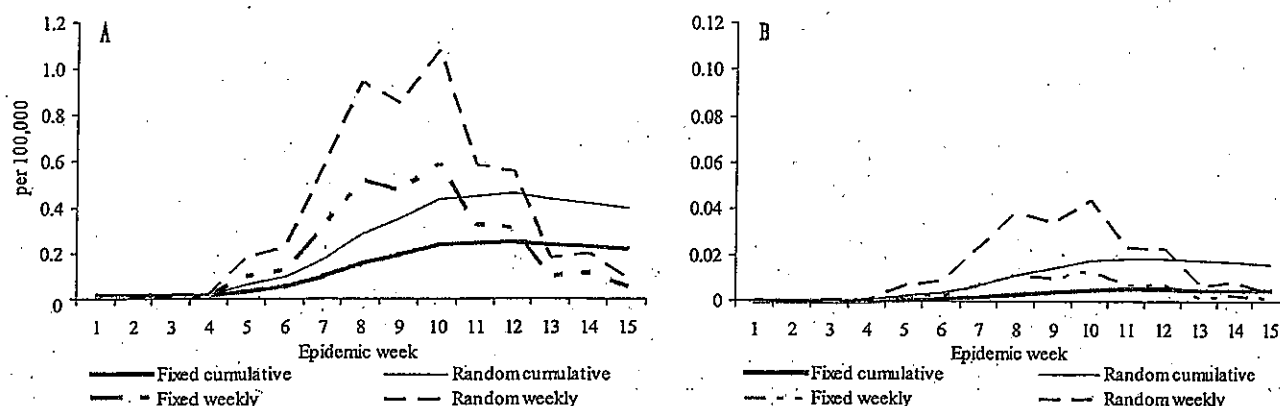


Fig. 3. Estimated number of viremic cases per 100,000 donor population (A) and the resulting number of viremic donations (B) during the chikungunya outbreak in Italy, 2007.⁸ The numbers of viremic cases were estimated using fixed model variables or random distribution variable values based on weekly notified cases (dashed lines) and cumulative number of cases (solid lines).

wider range of 1 to 12 days is used where 2 and 8 days are the most likely values for symptomatic and asymptomatic infections, respectively.¹⁴ Few details on Italian transfusion practice are given in the published study, and thus for calculation purposes we assumed that the donated blood remains infectious to recipients despite the implemented measures; from a whole blood donation three components are produced; components are not pooled to form end products; recipients have no immunity against the infection; and 0.1% of the infected recipients develop acute severe disease manifestations.

Despite the absence of transfusion-transmitted cases documented during that outbreak, a timely risk assessment would have allowed estimating the risk to blood safety posed by this EID. We used the model to calculate the prevalence of infection and the resulting number of infected or viremic donations using the number of cases notified weekly (Method 1) and cumulatively (Method 2). The prevalence was calculated using fixed input data and using variable distributions values. An illustration of TT risks at the outbreak peak (Week 10), starting from the donor prevalence up to the potential number of infections in recipients, was calculated.

Several additional measures were implemented by the Italian blood service at that time to minimize the impact of the outbreak in the affected region. One measure was to implement a quarantine period of either 2 or 5 days. Our model estimated the number of viremic donations that would have been collected with and without such measure. The model also analyzed the TT risk of a visiting donor donating viremic blood in his home country, with or without the implementation of a DHQ. The latter was calculated by assuming that the donor visited the area during the outbreak peak for a week, getting infected at the moment before returning to his or her home country, and subsequently commenced his or

her twice-yearly blood donation 1 day after. The DHQ to which he fully complied is assumed to be 85% effective.

RESULTS

An assessment at the outbreak peak would have resulted in an estimated prevalence of 1.07 (95% CI, 0.38-2.03) and a prevalence of 0.39 (95% CI, 0.15-0.71) per 100,000 among donors and 0.04 (95% CI, 0.01-0.10) and 0.02 (95% CI, 0.002-0.06) infected donations using weekly and cumulative cases, respectively. These estimates lead to 0.13 and 0.05 infected blood components (outcome of Step 3), 0.13 and 0.05 infected end products (Step 4), and 0.0001 and 0.00005 severe infections in recipients (Step 5), again using weekly and cumulative cases, respectively.

Figure 3 summarizes the estimated number of viremic individuals and the estimated number of viremic donations during a chikungunya outbreak in Italy in 2007. The figure shows that the prevalence of infection estimates using fixed model variables give lower estimates than the ones estimated using random distributions. This is because the mean value generated from the defined skewed variable distribution is higher than the fixed variable value; results of random distribution model would have been consistent with results of the fixed model if the distribution of infectious period was defined as 2.9 (range, 1-5) days. During the outbreak peak, the cumulative estimates (Method 2) give a substantially lower estimate than weekly estimates (Method 1) because Method 2 tends to average out the incidence at a longer epidemic time unit.

Figure 4 shows the weekly estimated number of viremic donations during the outbreak as a function of the number of quarantine days. For 2 and 5 days of quarantine 0.027 and 0.0025, respectively, from the 5378 donations collected are expected to have been viremic at the outbreak peak. These numbers correspond to 1 in 197,333

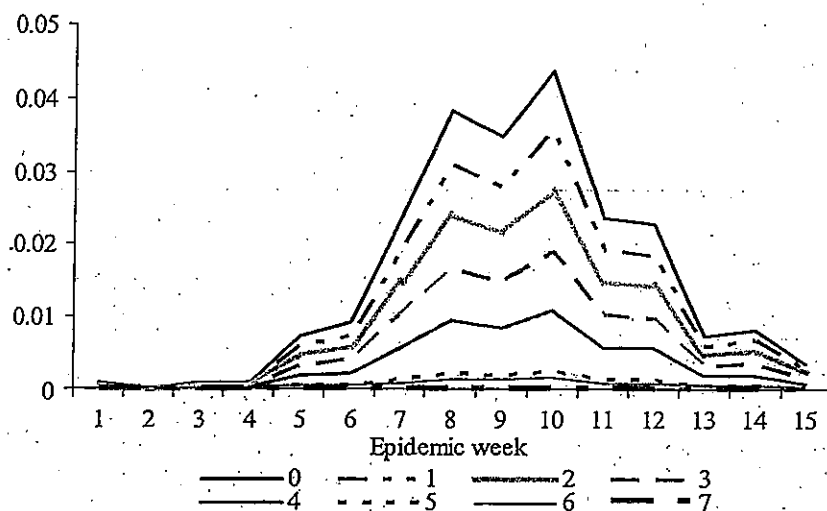


Fig. 4. Estimated number of viremic donations that would have been collected during the chikungunya outbreak in Italy in 2007 for various options for the number of days of quarantine.

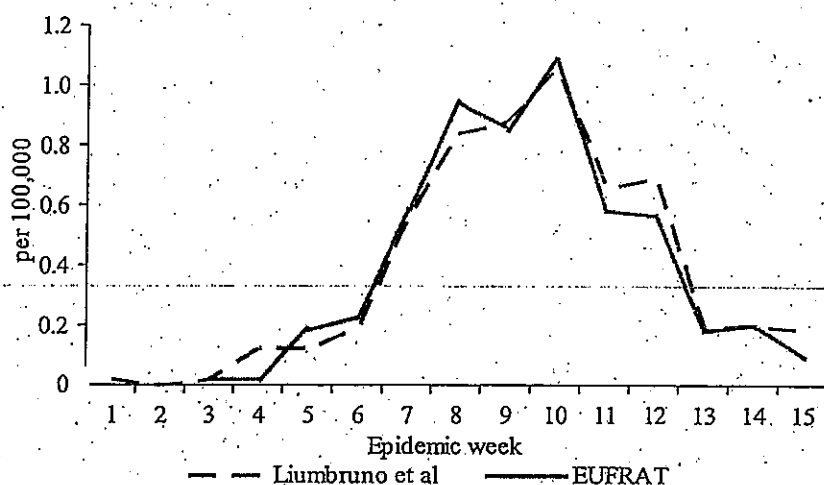


Fig. 5. Comparison of number of viremic cases (per 100,000) estimated by EUFRAT model and by Liunbruno and colleagues⁹ during chikungunya outbreak in Italy, 2007.

donations and 1 in 2,170,665 donations being viremic, respectively. The latter would be considered acceptable in Italy as it is below the one viremic blood donation per 380,000 thresholds.⁹ The number of viremic donations is expected to become zero after at least 7 days of quarantine.

The estimate obtained with our model, as shown in Fig. 5, agrees well with the published estimate by Liunbruno and colleagues.⁹ It should be noted that our model estimates the number of all viremic cases whereas the published study represents only asymptomatic cases. It appears that the symptomatic cases during that outbreak had a negligible contribution to the possible viremic donations, as already concluded by the published study.

Our model estimated the probability of the donor who visited the outbreak area donating infectious blood in his home country to be 0.30 (95% CI, 0.01-0.65) per 100,000. Assuming that a DHQ is implemented, he is at risk of giving infected donations of 0.458 per 100,000. If there were no such DHQ implemented, this risk would have increased to 0.515 per 100,000.

DISCUSSION

It was demonstrated how the model can be used to quantify the TT risks using outbreak information, starting from the probability of acquiring infection in a donor population, the expected number of viremic donations, up to the

TT risk in the recipient population. The chikungunya example was used to illustrate how users may apply the model in analyzing various outcome scenarios. The estimated number of viremic donations per week would help the policy makers decide at which time point additional safety measures need to be taken. The model also estimated the subsequent TT risks up to the risk of developing severe infection among the recipients, and the risk from a donor visiting the outbreak area. A comparison of results shows that our model estimates are similar to that of the original study. Furthermore, the model illustrated what the effect of quarantine would be on containing the TT risks for varying duration of quarantine. Such a model that gives comprehensive estimates of TT risks may prove useful for risk assessment in an outbreak situation.

The EUFRAT model can be applied for any emerging infection and various safety interventions in the transfusion chain. Other models for estimating the risk of infectious diseases to blood transfusion have been published. One model straightforwardly calculates the risk of infected blood donations in an outbreak area (<http://ewrtest.ecdc.europa.eu/blood/>) while others quantify the TT risk of a specific EID, with or without taking into account the costs and effects of various interventions.^{7,15,16} An extensive model developed by Kleinman and colleagues¹⁷ shares a similar objective by quantifying the risk of emerging pathogens. This model, which was developed for risk management purposes, was used to assess the potential catastrophic impact of a newly emerging pathogen in terms of loss of health and medical cost of recipients incurred. The primary aim of the EUFRAT model, however, is to allow an analysis of the particular TT risk from an ongoing EID outbreak. The generic feature of the model can be employed to provide rough TT risk estimates, starting from the risk of infection among donors up to the recipients', using limited information available during outbreaks of EIDs with a short, intermediate, or long risk period. This is because the elements of the model were carefully selected to capture the most basic characteristics of EIDs and the possible safety measures in the transfusion system. The EUFRAT model is presented in a simple and easy-to-use user interface intended for public health officers or transfusion regulators with or without modeling background. Results of our model can be further used for cost-effectiveness analyses if such additional analysis is deemed necessary.

Despite the practical usability of the model there are some limitations. One of these is the prevalence estimate that is sensitive to the time unit defined for the risk assessment. The prevalence estimated cumulatively compared with estimated weekly tends to provide lower estimates before reaching the epidemic peak and overestimates it when the numbers of cases per unit time drop. This is because it is based on averaging the incidence throughout the time unit of the outbreak. This implies that assessing

the risk in a smaller time interval may improve the accuracy but it is more affected by uncertainties due to fluctuations in the numbers of cases. However, the model can be used to specify the time unit that provides an optimal risk assessment given outbreak data is available. Another limitation is that the risk contributed by a proportion of infected individuals progressing to chronic infection overlaps with the risk of their acute phase; this may overestimate the true risk of EIDs with a significant proportion of chronic infection.

There is an ongoing debate on risk acceptability. To date, there is no established consensus on what can be considered an acceptable TT risk in general and in particular for EIDs. The blood supply organizations in all countries try to pursue maximum blood safety. However, one can imagine that implementation of all measures feasible is unattainable for some, if not for all countries when considering the increasing number of EIDs that pose TT risks. The EUFRAT model assists in evaluating the effectiveness of available safety measures by means of risk reduction obtained and to provide estimates for the residual risks. The purpose of the model is not to perform a complete outbreak risk assessment. Instead, its primary goal is to estimate the extent of the TT risks to support decision-making concerning (additional) safety interventions.

The generic EUFRAT model can be applied to quantify the TT risks of chikungunya and other EIDs using a limited amount of information concerning the pathogen and outbreak data. The flexibility of the model allows evaluating TT risks for various safety measures and to assess the risks of a visiting donor. The outcomes of the model can be useful to support targeted preventive measures for transfusion regulators and public health officers.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Formulas used for calculating model outcomes.

[Correction added after online publication 31-Oct-2012: The appendix has been updated.]

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日		第一報入手日		新医薬品等の区分		総合機構処理欄	
一般的名称		研究報告の公表状況		http://www.ansm.sante.fr/S-informer/Informations-de-securite-Retraits-de-lots-et-de-produits/Medicaments-Derives-du-Sang-LFB-Biomedicaments-Rappel-de-lots4		公表国 仏国		使用上の注意記載状況・その他参考事項等 重要な基本的注意 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病 (vCJD) 等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。	
販売名(企業名)		—		—		—			
研究報告の概要		LFB BIOMEDICAMENTS は、仏 ANSM(医薬品・保健製品安全庁)の要請で、散発性クロイツフェルト・ヤコブ病の可能性がある供血者の血漿を用いた血液由来製剤 (MDS) の特定ロットを回収する。この回収は、輸血の安全性政策上の予防措置として行われる。血液製剤による散発的クロイツフェルト・ヤコブ病の伝播症例は報告されていない。また、血液製剤の製造工程には、プリオンに對し有効と考えられるステッピングが含まれる。 la circulaire DGS/SQ4 n° 98/231 du 9 avril 1998 に基づき、この回収では公衆衛生コード L 1111-2 により要求されている、通知の影響を受けた製品を投与された個人の情報は必要ではない。 実際には血液製剤による散発的クロイツフェルト・ヤコブ病の伝播は、科学的知見における理論上のリスクで、新たな証明されたリスクではない。 • Aclotine 100UI/mL-1000UI/10mL (antithrombin III (human)) • Clairryg 50mg/mL-10g/200mL (immunoglobulin) • Factane 100UI/mL-1000UI/10mL (factor VIII)							
報告企業の意見		今後の対応 今後ともプリオン病に関する安全性情報等に留意していく。							
仏国における散発性 CJD に起因する製剤の回収に関する情報である。理論上のリスクであって、新たに証明され特定されたリスクではないとのことである。なお、当社血漿分画製剤はフランス由来の原料血漿を用いていない。									

Accueil > S'informer > Informations de... > Médicaments Dérivés du Sang - LFB Biomédicaments - Rappel de lots

Médicaments Dérivés du Sang - LFB Biomédicaments - Rappel de lots

09/08/2013

MED 13/B029



Le laboratoire LFB BIOMÉDICAMENTS procède, à la demande de l'ANSM, au rappel des lots des médicaments dérivés du sang (MDS) mentionnés ci-après issus du plasma d'un donneur de sang ayant développé un cas probable de maladie de Creutzfeldt-Jakob sous sa forme sporadique.

Ce rappel est effectué par mesure de précaution dans le cadre de la politique de sécurité transfusionnelle.

Aucun cas de transmission de la forme sporadique de la maladie de Creutzfeldt-Jakob par les MDS n'a été rapporté. En outre, les procédés de fabrication des MDS comportent des étapes considérées comme efficaces pour l'élimination des prions.

Conformément à la circulaire DGS/SQ4 n°98/231 du 9 avril 1998, un tel rappel n'appelle pas une information nominative, telle que prévue par l'article L 1111-2 du code de la santé publique, des patients ayant reçu les produits des lots concernés par le rappel. En effet, la transmission par les médicaments dérivés du sang de la forme sporadique de la maladie de Creutzfeldt-Jakob constitue en l'état actuel des connaissances scientifiques un risque théorique et non pas un risque nouveau avéré et identifié.

Les médicaments des lots mentionnés dans cette liste et qui auraient fait l'objet d'une rétrocession sont également concernés par ce rappel. Dans ce cas cependant, une information spécifique sur le motif du rappel pourra être apportée aux patients actuellement détenteurs de ces produits.

Liste des lots concernés par le rappel LFB BIOMÉDICAMENTS du 08 aout 2013

- Aclotine 100 UI/ml - 1000 UI/10 ml - Code CIP 34009 561 938 82
Lot : 12L02600 (Exp : 12/2013)
- Clairyg 50 mg/ml - 10g/200 ml - Code CIP 34009 576 190 46
Lot : 12L02514 (Exp : 01/2014)
- Factane 100 UI/ml - 1000 UI/10 ml - Code CIP 34009 562 117 84
Lots : 12L07205 (Exp : 02/2014) - 12L07208 (Exp : 02/2014) - 12L07209 (Exp : 02/2014)

LFB BIOMEDICAMENTS の血液由来製剤のロット回収

LFB BIOMEDICAMENTS は、仏 ANSM(医薬品・保健製品安全庁)の要請で、散発性クロイツフェルト・ヤコブ病の発症の可能性がある供血者の血漿を用いた下記の血液由来製剤 (MDS) のロットを回収する。

この回収は、輸血の安全性政策上の予防措置として行われる。

血液製剤による散発的クロイツフェルト・ヤコブ病の伝播症例は報告されていない。加えて、血液製剤の製造工程には、プリオンに対し有効と考えられるステップが含まれる。

周知された DGS/SQ4 No.98/231 April 9 1998 に基づき、この回収では公衆衛生コード L 1111-2 において要求されているとおり、回収の影響を受けた製品が投与された個人の情報は必要とされない。実際、血液製剤による散発的クロイツフェルト・ヤコブ病の伝播は、現状の科学的知見における理論上のリスクであり、新しい証明された特定されたリスクではない。

このリストに記載されている医薬品の返却品も回収の対象である。しかし、この場合、回収の理由についての具体的な情報は、これらの製品を現在保持する患者に与えることができる。

2013 年 8 月 8 日の LFB BIOMEDICAMENTS による回収の影響を受けるロット

•Aclotine 100 UI/ml - 1000 UI/10 ml - Code CIP 34009 561 938 82

Lot : 12L02600 (Exp : 12/2013)

•Clairyg 50 mg/ml - 10g/200 ml - Code CIP 34009 576 190 46

Lot : 12L02514 (Exp : 01/2014)

•Factane 100 UI/ml - 1000 UI/10 ml - Code CIP 34009 562 117 84

Lots : 12L07205 (Exp : 02/2014) - 12L07208 (Exp : 02/2014) - 12L07209 (Exp : 02/2014)

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2013. 5. 31	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿			公表国	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)	研究報告の公表状況	ProMED 20130531.1746730	OIE	
研究報告の概要	○BSE最新情報：国際獣疫事務局(OIE)公式疾病ステータス OIEは2013年の総会において、イスラエル、イタリア、日本、オランダ、スロベニア及び米国は「無視できるウシ海綿状脳症(BSE)リスク」であると認定した。ブルガリア及びコスタリカについては、「管理されたBSEリスク」であると認定した。既に公式のステータスが認められている国についての変更はない。				
報告企業の意見	今後の対応 今後も引き続き情報の収集に努める。				
OIEは2013年の総会で、イスラエル、イタリア、日本、オランダ、スロベニア及び米国は「無視できるBSEリスク」であると認定したとの報告である。					
使用上の注意記載状況・ その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク					



Published Date: 2013-05-31 02:39:12
 Subject: PRO/AH> BSE update: OIE official disease status
 Archive Number: 20130531.1746730

BSE UPDATE: OIE OFFICIAL DISEASE STATUS

A ProMED-mail post
<http://www.promedmail.org>
 ProMED-mail is a program of the
 International Society for Infectious Diseases
<http://www.isid.org>

Date: Wed 29 May 2013
 Source: OIE 81st General Session of the World Assembly of Delegates of the OIE, press release [summ., edited]
<http://tinyurl.com/ohxqbg2>

Bovine spongiform encephalopathy (BSE) or "mad cow disease"

During this year's [2013] General Session, the World Assembly of national Delegates recognised Israel, Italy, Japan, The Netherlands, Slovenia, and the United States of America as having a "negligible bovine spongiform encephalopathy (BSE) risk".

Bulgaria and Costa Rica were recognised as having "controlled risk" status with regard to BSE.

The official status of all the countries that already had an officially recognised status remains unchanged.

communicated by:
 ProMED-mail
[<promed@promedmail.org>](mailto:promed@promedmail.org)

[In May 2012, following the recommendation of the Scientific Commission based upon received questionnaires, the following 19 OIE member countries were officially recognised by the General Session as having a "negligible BSE risk" in accordance with chapter 11.5 of the Terrestrial Code: (total = total number of BSE cases reported; "first" and "last" = the year of the 1st and of the last reported BSE cases in this country):

Country / total / first / last
Argentina / 0
Australia / 0
Austria / 8 / 2000 / 2010
Belgium / 125 / 2000 / 2006
Brazil / 0
Chile / 0
Colombia / 0
Denmark / 15 / 2000 / 2009
Finland / 1 / 2001 / 2001
Iceland / 0
India / 0
New Zealand / 0
Norway / 0
Panama / 0
Paraguay / 0
Peru / 0

Singapore / 0
Sweden / 0
Uruguay / 0

During the May 2013 General Session, the following countries have been added to the "negligible BSE risk" group, in line with the recommendation of the Scientific Commission:

Country/ total / first / last / comment

Israel / 1 / 2002 / 2002 / single 2002 case in a 10 year old cow. MBM [meat bone meal] import ban July 1990, total MBM feed ban August 1996
Italy / 142 / 2001 / 2009 / -
Japan / 36 / 2001 / 2009 / -
Netherlands / 62 / 2000 / 2011 / the 2011 case was "atypical" BSE (L-type)
Slovenia / 8 / 2001 / 2007 / -
USA / 3 / 2005/ 2006 / the 2011 case one "atypical" BSE

The requirements for a member to be recognised as having a "negligible BSE" risk are laid out in article 11.5.3 of the Terrestrial Code; it can be accessed at http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.11.5.htm:

Countries enjoying the status of "negligible BSE risk", which is the highest possible status according to the terrestrial code, are liable to relatively lenient BSE surveillance and control requirements. They must submit an annual reconfirmation form by the end of November every year.

As the official BSE status of a country or zone is determined on the basis of an overall assessment of risk, the occurrence of a new BSE case implies a re-assessment of the official risk status only in the event of a change in the epidemiological situation indicating failure of the BSE risk mitigating measures in place.

When the Scientific Commission determines that the conditions to demonstrate compliance with the relevant requirements of the Terrestrial Code are no longer met, a disease status may be suspended. The Scientific Commission may decide to reinstate the suspended status when a member country has submitted an application which fulfills all the requirements requested for the recovery of official disease status laid out in the relevant chapters of the Terrestrial Code. The suspensions and recoveries of disease status are announced by the director general of the OIE in consultation with the Scientific Commission and the list of these is kept up to date until adoption of a new Resolution by the World Assembly in the following May.

Following the addition of Bulgaria and Costa Rica to the list of countries recognised as having "controlled risk" status with regard to BSE, the updates list includes the following 27 countries:

Bulgaria, Canada, Chinese Taipei, Costa Rica, Croatia, Cyprus, Czech Republic, Estonia, France, Germany, Greece, Hungary, Ireland, Korea (Rep. of), Latvia, Lichtenstein, Lithuania, Luxembourg, Malta, Mexico, Nicaragua, Poland, Portugal, Slovak Republic, Spain, Switzerland, and United Kingdom.

For background on BSE, including diagnostic procedures, please refer to http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.06_BSE.pdf.

The impact of this zoonotic disease, manifested in humans as vCJD, is reflected in the large (774) number of BSE postings in the ProMED-mail archives since November 1994. - Mod.AS]

See Also

2011

BSE - Netherlands: (FR), OIE [20110122.027](#)

2004

BSE - France: epidemic reviewed [20040704.1796](#)

2002

BSE guidelines: WHO [20030130.0264](#)

BSE, source, calf milk replacers: suspected [20020621.4563](#)

1998

BSE, USDA risk assessment - USA [19980427.0803](#)

BSE: official inquiry - UK [19980131.0196](#)

1997

BSE: European Parliament Inquiry draft report [19970118.0097](#)

1996

Spongiform encephalopathy (24): WHO report on BSE [19960405.0647](#)

Spongiform encephalopathy (25): Review of BSE/CJD [19960405.0650](#)

1995

BSE/FSE/TSE [19950330.0170](#)

.....arn/mj/sh

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医薬品 研究報告 調査報告書

識別番号・報告回数	報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
		2013. 8. 12	該当なし	
一般的名称	新鮮凍結人血漿	O'Brien SF, Scalia V, Goldman M, Fan W, Yi QL, Dines IR, Huang M, Ndao M, Fearon MA. Transfusion. 2013 Aug;53(8):1706-13. doi: 10.1111/j.1537-2995.2012.03950.x. Epub 2012 Nov 12.	公表国 カナダ	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)	研究報告の公表状況		
研究報告の概要	○ <i>Trypanosoma cruzi</i> (T. cruzi) 選択的検査: カナダ血液サービズでの実施1年の結果 背景: 多様な検査戦略は、シャーマーガス病非流行国や低流行国における感染リスクを減少させる可能性がある。カナダ血液サービズにおけるリスク献血者の選択的検査実施後1年の結果が報告された。 研究デザインと方法: 2009年2月から全ての献血者に中南米滞在歴や自身の出身地及び母親・母方の祖母の出身地について問診を実施し、リスク献血者からは血小板製剤の製造を中止している。2010年5月以降、リスク献血者に <i>T. cruzi</i> 抗体検査を行い、陽性の献血者にはリスク因子について面談し、遡及調査を実施した。 結果: 献血者421,979人中、リスク献血者7,255人(1.72%)をスクリーニングした。リスク因子の内訳は、中南米出身(50.6%)、母または母方の祖母が中南米出身(28%)、6カ月以上の中南米滞在歴(19%)であった。16人のリスク献血者は <i>T. cruzi</i> 抗体検査で繰り返し反応し、そのうち13人の陽性が確定した。13人中11人は中南米出身(パラグアイ9人、アルゼンチン2人)で、他の2人は、カナダ出身であるが両者共に短期の中南米渡航歴があり、母親が中南米出身者であった。遡及調査により以前の献血148件(176製剤が輸血された)が評価され、そのうち28%の受血者が検査を受けたが、陽性者はいなかった。 結論: 選択的検査を用いることで、偽陽性結果による受血者の減少を抑え、血液供給に対するリスクを軽減させた。大部分の陽性献血者はリスクのある国の出身であり、パラグアイからの移民に集中していた。居住または渡航のみについては、明確なリスク因子ではなかった。			
	使用上の注意記載状況・その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、細菌、原虫等の感染、vCJD等の伝播のリスク			
報告企業の意見	今後の対応 日本赤十字社は、輸血感染症対策として献血時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーマーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「血液製剤の安全性確保と安定供給のための新興・再興感染症の研究」班と共同して検討している。また、中南米出身者(母親が出身を含む)、通算4週間以上の中南米滞在歴を有する献血者からの血液は、血漿分画製剤の原料にのみ使用する対策を実施している。今後引き続き情報の収集に努める。			
カナダ血液サービズにおける <i>T. cruzi</i> 選択的検査の開始から1年が経過し、大部分の陽性献血者はリスクのある国の出身であったとの報告である。				

DONOR INFECTIOUS DISEASE TESTING

Selective testing for *Trypanosoma cruzi*: the first year after implementation at Canadian Blood Services

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BACKGROUND: Various testing strategies may reduce the risk of Chagas disease transmission in nonendemic, low-prevalence countries. Results of the first year of selective testing of at-risk donors at Canadian Blood Services are reported.

STUDY DESIGN AND METHODS: Since February 2009, platelets were not produced from at-risk donors. Since May 2010, at-risk donors were tested for *Trypanosoma cruzi* antibodies. Donors testing positive were interviewed about risk factors, and lookback studies were initiated.

RESULTS: There were 7255 at-risk donors of 421,979 donors screened (1.72%). Risk factors were born in Latin America (50.6%), mother or maternal grandmother born in Latin America (28%), and 6 months or more travel history or residence in Latin America (19%).

Sixteen (16) at-risk donors had *T. cruzi* repeat-reactive test results of whom 13 confirmed positive. Eleven of 13 were born in Latin America (nine in Paraguay and two in Argentina), and the other two were born in Canada but had short-term travel history and mothers who had been born in Latin America. Ten of the donors spoke German as their first language (all of those born in Paraguay and one born in Canada). There were 148 previous donations (176 components transfused) evaluated by lookback, of which 28% of recipients could be tested. None were positive.

CONCLUSION: Selective testing has mitigated a small risk to the blood supply with very few false-positive results. Most positive donors were born in a risk country, with a concentration of German-speaking immigrants from Paraguay. Residency or travel alone were not clear risk factors.

The protozoan parasite *Trypanosoma cruzi* is endemic in parts of Mexico and Central and South America where it is carried by a triatomine insect vector (reduviid bug). It is generally encountered in rural areas, particularly in homes with dirt floors, adobe or stone walls, and palm-thatched roofs where the insect can live in the cracks and crevices.¹ Many people are unaware of their infection, but infected individuals sometimes develop a short-term, localized swelling at the site of the bite (Chagoma) and/or flu-like symptoms. After initial infection, there is often life-long chronic persistence of the parasite, with detectable antibody and intermittent parasitemia. Up to 30% of infected individuals will develop chronic Chagas disease affecting the smooth muscle of the heart or gastrointestinal tract after 20 to 30 years. Infection usually occurs after being bitten by the insect, but individuals can also be infected congenitally and by blood transfusion. In the United States and Canada, 10 transfusion-transmitted cases have been reported (six definite, two probable, and two possible); largely associated with platelet (PLT) transfusions.² Autochthonous infections

ABBREVIATIONS: CBS = Canadian Blood Services; DHQ = donor history questionnaire; IB = immunoblot; S/CO = sample-to-cutoff ratio.

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occasionally occur in the United States^{3,4} with risk in donors mainly associated with birth in Latin America and possibly travel history.⁵ Cases of oral transmission of Chagas disease via contaminated food have also been reported.⁶

With the implementation of insecticide spraying programs to control the insect vector, as well as screening of the blood supply, the prevalence of *T. cruzi* infection in Latin America has been decreasing from an estimated 16 to 18 million people in the mid-1980s to 7.6 million in 2006.⁷ In nonendemic countries such as Canada, *T. cruzi* infection is associated with immigration from Latin America, and the potential for transfusion transmission is expected to continue for many years to come, with an estimated 1218 infected immigrants currently residing in Canada.⁷

In January 2007 US blood operators began testing all donations for *T. cruzi* antibody, and after approximately 2 years of testing 19.3 million donations had been screened, with 2775 (0.014%) repeat-reactive and 667 donations confirmed positive.⁸ Most US blood operators have now switched to testing all donors once as a more cost-effective approach⁹ and as incident infections have not been identified,¹⁰ questioning about recent travel is not considered necessary.

In Canada, there are no known insect vectors, but two transfusion-transmitted cases have been reported from donors who had lived in South America.^{11,12} However, the risk was believed to be low based on immigration patterns and a seroprevalence study of at-risk donors in one Canadian city, which identified no *T. cruzi*-positive donors.¹³ Héma-Québec, the blood operator responsible for blood collection in the province of Québec, began selective testing of at-risk donors based on risk questioning using the Ortho *T. cruzi* enzyme-linked immunosorbent assay (ELISA) test systems assay in March 2009 identifying four confirmed positive donations to date of approximately 8300 donations tested (G. Delage, Héma-Québec, personal communication, 2012). At Canadian Blood Services (CBS), in anticipation of Canadian licensure of the Abbott PRISM Chagas assay, risk questioning was introduced in February 2009 and donations from at-risk donors were not used to produce PLTs or transfusable plasma. In May 2010 CBS began selective testing of all at-risk donors for antibody to *T. cruzi* and carried out additional risk factor interviews of donors who were confirmed positive. We present results of the first year of selective testing and describe donor risk factors and the outcome of lookback investigations on donations from *T. cruzi* antibody-positive donors.

MATERIALS AND METHODS

Implementation of risk factor screening and selective testing

Beginning in February 2009 all donors were asked the following questions on the self-administered part of the donor history questionnaire (DHQ):

Have you spent a total of 6 months or more in a continuous period in Mexico, Central America, or South America?

Were you born in Mexico, Central America, or South America?

Was your mother or grandmother born in Mexico, Central America, or South America?

The last question was designed to capture the risk of vertical transmission¹⁴⁻¹⁶ and donors answering "yes" were further questioned to determine if the maternal grandmother had been born in a risk country. All donors assessed as having risk were flagged in the computer database so that even if the donor failed to acknowledge risk on a subsequent donation, she or he would still be considered as having risk. Information about Chagas disease and testing was included in the mandatory predonation reading materials. From February 2009 to May 2010, donors with risk continued to donate but their donation was not used to prepare PLTs or transfusable plasma. As of May 17, 2010, all donations from donors identified as having risk were tested, and the study period was May 17, 2010, to May 17, 2011.

Laboratory methods

Testing was carried out on each donation from at-risk donors using the Abbott PRISM Chagas assay (Abbott Laboratories, Wiesbaden, Germany). Confirmatory or supplemental testing on repeat-reactive donors was carried out by both the National Reference Centre for Parasitology (McGill University Centre for Tropical Disease, Montreal, Québec, Canada) using a combination of an immunoblot (IB) assay and a polymerase chain reaction (PCR) assay^{17,18} and by Quest Diagnostics (Fairfax, VA) using a radioimmunoassay (RIPA).¹⁹ For details of primers used in the PCR assay, please see Ndao and coworkers.¹⁸ In addition, the repeat-reactive samples were tested on the Ortho *T. cruzi* ELISA test system (Ortho Clinical Diagnostics, Raritan, NJ). Donors were considered confirmed positive if both the IB assay and the RIPA was positive. All donors with repeat-reactive screening results were informed of their results and deferred from future donation, regardless of results of confirmatory testing.

Donor interviews

All donors with confirmed positive tests were invited to participate in a scripted telephone interview with a nurse. Donors were reasked the screening questions from the DHQ plus additional questions including demographic questions, their country of birth, the type of housing they lived in, and travel history.

Lookback

Lookback was initiated for all donations that were confirmed positive for antibody to *T. cruzi*. A computer search

of donor records was carried out to identify all previous donations, all components produced from these donations, and the hospitals to which these were released. Records were identified as far back as computerized records were available (back to 1987). Hospital blood banks were asked to arrange testing of the recipients of all these components and to report the results to CBS.

National Epidemiology Donor Database

Statistical analysis was based on data from the National Epidemiology Donor Database, which is maintained with computer software (SAS, SAS Institute, Inc., Cary, NC). This database contains donation and deferral data, the donor's answers to risk questions on each donation, as well as a code for having ever been assessed as having risk (based on the DHQ screening questions and subsequent questioning) on all Canadian blood donors except those in the province of Québec. All allogeneic blood donations (whole blood, plasma, and PLT donations) produced between May 17, 2010, and May 17, 2011, were included.

Statistical analysis

To estimate the number of positive donors expected to be identified by selective testing once implemented, the annual number of donors making a donation was multiplied by the percentage of donors with risk travel from a 2006 survey,¹³ and this number was multiplied by the point estimate of positive donors identified in seroprevalence studies.^{13,20} A confidence interval (CI) was estimated to include both the uncertainty around the percentage of donors with travel history and the percentage of positive donors with travel history using Monte Carlo simulation. The donor surveys had assessed risk based on birth in an endemic country and cumulative travel of 6 months or more; thus the same question for the strongest predictor, birth in an endemic country, but different from the criteria that were ultimately implemented for travel risk. Additionally, the donor surveys did not include vertical transmission risk.

RESULTS

Laboratory results

Of the 7255 at-risk donors tested (14,587 donations) during the first year of selective testing, there were 16 repeat-reactive donations (sample-to-cutoff ratio [S/CO] of greater than or equal to 1.0) on the Abbott PRISM Chagas assay (see Table 1). Of these, 13 were confirmed antibody-positive based on a positive result on both the IB assay and

the RIPA. Two of these donors also had a positive result on PCR testing; one was a 25-year-old donor born in Canada, and the other was a 63-year-old donor born in Paraguay. As shown in Table 1 in sequential order, the mean S/CO was not always predictive of confirmatory results. Although most samples with positive confirmatory test results had S/CO of greater than 4, there were two that were just above the cutoff. Of the three false-positive repeat-reactive donors (negative on both the IB assay and the RIPA), two had a S/CO just above the cutoff; however, one of the donors had a high S/CO (9.4). All confirmed positive samples tested on the Ortho *T. cruzi* ELISA test system were reactive (S/CO ratios > 1), and of the three samples considered to be false-reactive Donation 11 tested negative (S/CO = 0.3) and the other two were not tested.

Predicted number of cases

Based on an estimated 1,000,000 donations from 400,000 donors with 2% having risk background, and using a prevalence estimate from the 1998 Toronto study (0 positive donors; 95% CI, 0%-0.28%)¹³ it was expected that the number of cases would be between 0 and 20 if at risk donations were tested. Since the Toronto study did not identify any positive donations, the analysis was also carried out using the rate of *T. cruzi* antibody positives among donors with risk factors from a larger study in Los Angeles²⁰ (147 cases, 0.2% of at-risk donors; 95% CI, 0.16%-0.22%), which predicted that there would be between 7 and 15 positive donations. The 13 positive donations identified in the first year of selective testing were within the expected CIs for both estimates, although the criteria for identifying at-risk donors varied somewhat.

TABLE 1. Test results of donations reactive on the Abbott PRISM screening assay*

Donation	Abbott PRISM (S/CO)	Confirmatory testing		
		IB assay	RIPA	PCR
1	10.3	Positive	Positive	Negative
2	11.6	Positive	Positive	Positive
3	6.4	Positive	Positive	Not tested
4	1.3	Positive	Positive	Negative
5	7.0	Positive	Positive	Negative
6†	9.4	Negative	Negative	Negative
7	11.4	Positive	Positive	Negative
8	10.5	Positive	Positive	Positive
9	4.9	Positive	Positive	Negative
10	8.5	Positive	Positive	Negative
11†	1.4	Negative	Negative	Negative
12	4.4	Positive	Positive	Negative
13	9.3	Positive	Positive	Negative
14	1.2	Positive	Positive	Negative
15	16.0	Positive	Positive	Negative
16†	1.0	Negative	Negative	Negative

* All donations are listed in the order identified.

† Donations considered false reactive (based on IB assay and RIPA).

Donor risk factors

As shown in Table 2, there were 7255 (1.72%) who reported risk factors of 421,979 donors who completed the DHQ. Approximately half said they were born in Mexico, Central America, or South America, mostly with other risk factors as well. There were 28% whose only risk was that their mother or grandmother was born in a risk country and 19% who had only spent 6 months or more continuously in a risk country. In total there were 16 repeat-reactive donors identified by a risk question, of whom 13 (0.18% of donors with risk factors) were confirmed positive. Two donors were from British Columbia, five were from Ontario, and six were from Manitoba.

All 13 confirmed positive donors agreed to participate in the follow-up interview. As shown in Table 3, all donors had risk factors. Eleven of the 13 donors had been born in an endemic country (nine in Paraguay and two in Argentina); and four donors had mothers or grandmothers born in Russia. In addition most of these donors had lived in a house with a dirt floor and/or mud walls. The two donors that had been born in Canada had not lived in Mexico or Central or South America but both had mothers born in South America. They also each had several travel events to South America of 2 to 3 months' duration. Also, 10 of the 13 donors spoke German as their first language (all nine donors who were born in Paraguay and one Canadian-born donor) and the other three spoke Spanish (the two donors born in Argentina and one Canadian-born donor).

Lookback results

For the 13 positive donors, 148 previous donations were identified from which 397 blood components were produced (see Fig. 1). In total, 176 of these components were transfused (116 red blood cells [RBCs], 37 PLTs, and 23 other products) of which 66 were traced to a live recipient, six recipients were not identified, and 104 recipients deceased. In total, there were 50 components (28% of components transfused) for which there were recipient test results and of these 47 reported negative results (either antibody or PCR assay, 27 RBCs, six PLTs, and 14 other) and three recipients of RBCs (from two *T. cruzi* antibody-positive, but PCR-negative donors) were PCR positive although two tested antibody negative (the other was not tested). All were asymptomatic and testing occurred at least 6 months after the implicated transfusion.

DISCUSSION

We implemented selective testing for *T. cruzi* based on risk screening questions; in the first year of testing, 1.72% of donors were identified as being potentially at risk, and 13 confirmed positive donors were identified (1.8/1000 high-risk donors), showing that there was a small risk to

TABLE 2. Percentage of donors answering affirmatively to risk questions

Risk question	Number of donors		Percentage (95% CI)
	Only risk	More than one risk	
Were you born in Mexico, Central America, or South America?	81	3594	0.87 (0.84-0.90)
Have you spent a total of 6 months or more in a continuous period in Mexico, Central America, or South America?	1359	3547	1.16 (1.13-1.20)
Was your mother or grandmother born in Mexico, Central America, or South America?	2062	3354	1.28 (1.25-1.32)
Total: yes to any of the above.		7255	1.72 (1.68-1.76)

* Only mother's or maternal grandmother's country of birth was considered in risk assessment.

TABLE 3. Risk factors from postdonation interviews with 13 donors positive for antibody to *T. cruzi*

Donor*	Country of birth	Mother/maternal grandmother	At risk housing	First language	Note
1	Argentina (Mendoza)	Argentina/France	Yes	Spanish	Visited Paraguay twice for 2 months each time
2	Canada	Bolivia/Paraguay	No	German	
3	Paraguay (Chaco)	Paraguay/Canada	No	German	Traveled to Ecuador three times: once for 3 months and twice for 2 months
4	Paraguay (Neuland)	Russia/Russia	Yes	German	
5	Paraguay (Chaco)	Not Latin America/Russia	Yes	German	
7	Paraguay (Philadelphia)	Paraguay/Russia	Yes	German	
8	Paraguay (Chaco)	Russia/Russia	Yes	German	
9	Argentina (San Juan)	Italy/Not Latin America	Yes	Spanish	
10	Canada	Ecuador/Ecuador	No	Spanish	
12	Paraguay	Canada/Not Latin America	Yes	German	
13	Paraguay (Chaco)	Not Latin America/Not Latin America	Yes	German	
14	Paraguay (Chaco)	Not Latin America/Not Latin America	Yes	German	
15	Paraguay	Unknown/Unknown	No (but in rural area)	German	

* Donors 6, 11, and 16 had false-positive results and were not interviewed.

the blood supply. Before implementation of selective testing we estimated that the expected number of cases in the first year of testing would be low and found the actual result to be within the expected CI. This was in spite of having imperfect estimates of seroprevalence (that is, no national estimate) and variation between the risk factor criteria used in the seroprevalence studies and those used in implementation, although the main risk factor, birth in a risk country, was present in the seroprevalence studies. This underscores the value of studies and data modeling as the basis for decision making.

There were 3 of 16 repeat-reactive donations considered to be false reactive because they were negative on both the IB assay and RIPA. Of the 13 true-positive donors, two (15%) were positive on PCR testing, which is similar to the 13% reported by the American Red Cross³ and marginally higher than 4.5% reported by Blood Systems.⁵ PCR-positive donations may be more likely to transmit infection but donors could be intermittently PCR positive and results may depend on the parasite strain, parasite load, and primers used.²¹⁻²³ Our data also indicate that a S/CO of greater than or equal to 1 usually indicates a true-positive result; therefore, in a selective testing population, the testing kits demonstrated excellent specificity. However, over the threshold of 1, the strength of the signal is not a good predictor of positivity, since some samples just above the S/CO were true positives and one sample that was higher than several of the true positives did not confirm. In the United States, of 2775 repeat-reactive donations 2718 had confirmatory RIPA results reported, and 677 (25%) had positive results.⁸ However, these results are not directly comparable because different assays were used, and a different donor population (all donors rather than high risk donors) were tested.

Although risk factor interviews cannot identify the actual timing and source of infection, they show that birth in a risk country was by far the most common risk factor among *T. cruzi* antibody-positive donors. All documented transfusion-transmitted infections in the United States, Canada, and Spain involved donors born in an endemic area.² In the United States, preliminary data from the American Red Cross showed that of *T. cruzi*-positive donors 28% were born in Mexico, 26% in the United States, 16% in El Salvador, and 11% in Bolivia.³ This is quite different from our data in which 70% were born in Paraguay, 15% in Argentina, and 15% in Canada. In Canada the most common countries of origin for Latin American immigrants are (in order) Guyana, Mexico, El Salvador, Colombia, and Chile: only approximately 5% are from Argentina and less than 2% are from Paraguay;²⁴ thus *T. cruzi*-positive donors were disproportionately from countries from which Canada has relatively few immigrants. As Canada's immigration policies tend to encourage immigration from educated city dwellers who would have little exposure to the insect vector in their country of origin,

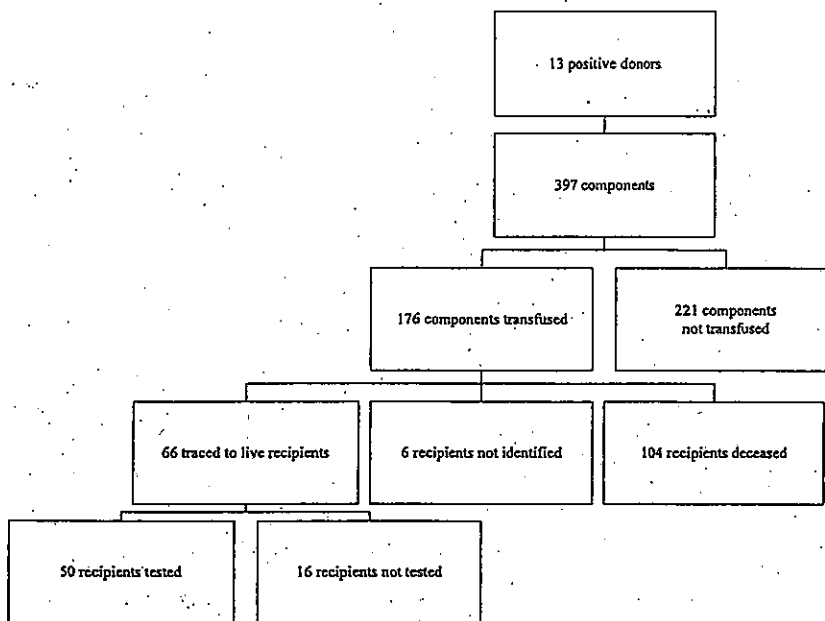


Fig. 1. Disposition of units prepared from donors who had tested positive for *T. cruzi* (the lookback investigation).

and as 8 of the 11 donors born in Latin America recall living in the type of housing where contact with the insect vector is possible (mud walls and thatched roofs), it appears that most of the risk is confined to a small atypical subset of Latin American immigrants. Furthermore, 10 of the *T. cruzi* antibody-positive donors spoke German as their first language, which is also at odds with the typical Latin American immigrant who is more likely to speak Spanish or Portuguese. These are similar to the two documented cases of transfusion transmission in Canada, which involved donors from a German-speaking community who had lived in South America, both donating at the same collection site in Winnipeg.^{11,12} Most of the German-speaking donors were from Paraguay where German communities are established with both economic immigrants and German Mennonites that had been living in Russia (the so-called Russian Mennonites).²⁵

Many countries in Latin America have implemented steps to reduce the incidence of *T. cruzi* infection such as insect spraying, provision of higher quality housing, and educational programs for children, successfully reducing new infections especially in younger people.²⁶ The risk in Canada is mainly from immigrants infected many years ago, which will continue for many years to come. Indeed, more than 4 years of tracking at the American Red Cross failed to identify any incident cases.¹⁰ As a screening question, birth in Latin America identifies most donors with risk but screening for vertical transmission risk is important^{14,16} as two donors born in Canada may have been infected by their mothers through vertical transmission. A

stay of 6 months or more in an endemic country was not the sole risk factor for any *T. cruzi*-positive donors suggesting that travel alone is rarely a risk factor consistent with experience in other countries,^{3,27,28} although travel history was reported to be a better predictor than country of birth alone in one US study.⁵

To date, the lookback investigations have identified three asymptomatic but PCR-positive recipients of RBCs. The physicians were then requested to perform serologic testing and the two that complied obtained negative antibody results. Given that implicated transfusions occurred at least 6 months before testing and seroconversion would be expected, that the implicated donors were PCR negative, and that the recipients received RBCs whereas reported cases have involved PLT transfusions, it was felt that these were unlikely to be true positive cases. Transfusion transmission has been reported

in less than half of recipients of infected blood in Latin America (14%-18% in Argentina, Brazil, and Chile; up to 49% in Bolivia).²⁹ However, since implementation of testing in the United States, two transfusion-transmitted cases have been identified³⁰ of 224 transfusions (0.83%), perhaps suggesting lower transfusion transmission rates in the United States. Our results are consistent with low risk, but as only about one-quarter of transfused components could be traced to a live recipient and only six PLT transfusions were involved, it is difficult to draw any firm conclusions.

In Canada selective testing of at-risk donors represents a paradigm shift in approach from universal testing (every donor, every donation). Selective testing policies to reduce the low risk from imported Chagas infections are a cost-effective approach³¹ and have been implemented in other countries such as Spain, France, and the United Kingdom^{27,28,32,33} with similar low-risk mainly related to birth in Latin America. The US situation is somewhat different in that there is a small proportion of donors with infections acquired in the United States,⁴ which can be addressed by testing of all donors at least once. Endemic cases of Chagas disease have never been described in Canada. Our experience shows that there is very little loss of donors due to false-positive results using a selective testing approach. As with any selective testing approach there is a chance that positive donations may not be selected for testing and the sensitivity of the questions was not addressed in this study. However, our analysis showed that birth in a risk country is the most common risk factor

and this should be relatively easy for donors to answer correctly in screening. We are currently performing a study in donors who have not declared any risk factors on the DHQ to better understand the potential risk in these donors.

CONFLICT OF INTEREST

No conflicts of interest to disclose.

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医薬品 研究報告 調査報告書

識別番号・報告回数	報告日		第一報入手日	新医薬品等の区分	総合機構処理欄
			2013. 6. 24	該当なし	
一般的名称	新鮮凍結人血漿	研究報告の公表状況	Emerging Infectious Disease Journal, Vol.19 No.7; Available from: http://wwwnc.cdc.gov/eid/article/19/7/12-1576.htm		公表国 ベネズエラ
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」J120(日本赤十字社) 新鮮凍結血漿-LR「日赤」J240(日本赤十字社) 新鮮凍結血漿-LR「日赤」J480(日本赤十字社)				
研究報告の概要	○ベネズエラにおけるシャギーガス病経口感染源の分子疫学的追跡 中南米では、経口感染によるシャギーガス病集団発生の報告が増加している。ベネズエラの都市周辺部2カ所における集団発生事例1は2009年4月初旬、首都カラカスから北西約50kmに位置するバルガス州Chichirivicheの小学校で、生徒71人、成人14人がシャギーガス病を発病し、33人に原虫血症が確認された。事例2は2010年5月、カラカスの南西部Antimanoの同じ食堂で食事をしていた35人に、<i>Trypanosoma cruzi</i> (<i>T. cruzi</i>) 感染が疑われ、検査したところ、15人が<i>T. cruzi</i>抗体陽性であり、14人に原虫血症が確認された。 感染源を特定するため、各地域で同時期に採取された<i>T. cruzi</i>非ヒト由来サンプルと、ベネズエラ全域のヒト由来及び非ヒト由来サンプルから246件の<i>T. cruzi</i>株及びクローンを分類し、解析を行ったところ、2件の経口感染のヒト分離株は、ベネズエラ全域から採取したヒトの非経口感染株とは明確に異なっていた。媒介動物による非経口感染株は、広範な地理的分布にもかかわらず全て近縁種であった。比較して、50km離れているだけの2件の経口感染株は非常に多様であるが発生地域における非ヒト株内にクラスターを形成しており、局所的な発生を示唆していた。さらに、遺伝的分化の指標である<i>Fst</i>値を算出した結果、経口感染株と地域の環境株間の関連性が支持された。 調査の結果、Antimanoの集団発生で原因となった食品は、同地域で汚染されていたことが明らかとなり、この地域で見られる齧歯類とサンガメによる非ヒト感染サイクルが感染源と考えられた。原虫感染の解明には、経口及び非経口感染によるヒト症例の分離株間の遺伝的差異の解析が重要である。				
報告企業の意見	ベネズエラでシャギーガス病の経口感染集団発生が増加しており、2件の感染事例について高解像度マイクロサテライト・タイピングを用いた解析を行ったところ、1件について感染原因となった食品は、当該地域で汚染されていたことが判明したとの報告である。				
今後の対応	日本赤十字社は、輸血感染症対策として献血時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャギーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「血液製剤の安全性確保と安定供給のための新興・再興感染症の研究」班と共同して検討している。また、中南米出身者(母親が出身を含む)、通算4週間以上の中南米滞在歴を有する献血者からの血液は、血漿分画製剤の原料のみ使用する対策を実施している。今後も引き続き情報の収集に努める。				
使用上の注意記載状況・その他参考事項等	新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」J120 新鮮凍結血漿-LR「日赤」J240 新鮮凍結血漿-LR「日赤」J480 血液を介するウイルス、細菌、原虫等の感染、vCJD等の伝播のリスク				

Molecular Epidemiologic Source Tracking of Orally Transmitted Chagas Disease, Venezuela

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Oral outbreaks of Chagas disease are increasingly reported in Latin America. The transitory presence of *Trypanosoma cruzi* parasites within contaminated foods, and the rapid consumption of those foods, precludes precise identification of outbreak origin. We report source attribution for 2 peri-urban oral outbreaks of Chagas disease in Venezuela via high resolution microsatellite typing.

Rapid urbanization presents new challenges for Chagas disease control in Latin America. Foci of disease are now reported in slums surrounding several Andean cities (1–3). Oral transmission is believed responsible for recent outbreaks of Chagas disease, most of which were characterized by atypically severe symptoms (4,5). Many cases have occurred in urban settings (5,6), amplifying the size and effect of the outbreaks.

Sources of orally transmitted disease outbreaks vary, but contaminated food and juices are often blamed. However, after a contaminated food is eaten, it may take weeks for the onset of clinical signs and symptoms, and direct molecular and cytological incrimination of a par-

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ticular batch of food/beverage has not been possible (5). Thus, evidence pointing to particular foodstuffs is often circumstantial.

Molecular epidemiologic analyses of human and environmental isolates are routinely used to track the source of outbreaks caused by foodborne pathogens. High-resolution molecular markers have been developed and validated for *Trypanosoma cruzi*, the parasite that causes Chagas disease (7,8). These markers, used in conjunction with careful sampling, can identify the source of foodborne outbreaks.

The Study

We studied 2 outbreaks of orally transmitted Chagas disease (120 cases, 5 deaths). The first occurred in Chichiriviche, Vargas State, a coastal community (population ≈800 persons) ≈50 km northwest of Caracas, Venezuela. The outbreak occurred at a primary school where food was prepared on site. In early April 2009, a total of 71 children (6–13 years of age) who attended the morning school shift and 14 adults became ill. Exposure of these persons to *T. cruzi* was established by use of IgM and IgG ELISA. Parasitemia was observed in 33 of the patients with serologic results positive for *T. cruzi* infection (9,10).

The second outbreak occurred in Antimano, a peri-urban slum southwest of central Caracas (online Technical Appendix Figure 1, wwwnc.cdc.gov/EID/article/19/7/12-1576-Techapp1.pdf). In May 2010, 35 patients with suspected *T. cruzi* infection were examined at Hospital Miguel Perez Carreno in Caracas. Patients reported that they regularly ate at the same local communal canteen. Among the patients tested, 15 were positive for *T. cruzi* IgM and IgG (9). Parasitemia in 14 patients was confirmed indirectly by hemoculture. Of the 35 patients, 21 (2 adults, 19 children) were hospitalized.

To enable outbreak source attribution, we undertook intensive additional sampling of contemporary, nonhuman sources local to each outbreak and of human and nonhuman sources from more distant localities throughout Venezuela. In total, 246 *T. cruzi* strains and clones were typed for 23 microsatellite markers (online Technical Appendix 1 Table) (8). A list of the samples and their sites of origin is in online Technical Appendix 2 (wwwnc.cdc.gov/EID/article/19/7/12-1576-Techapp1.xlsx).

Individual level sample clustering was defined first by constructing a neighbor joining tree based on pairwise distances between multilocus genotypes (Figure 1). A second analysis used *K*-means clustering and discriminant analysis of principal components (Figure 2) (11). To assess connectivity between human and nonhuman outbreak cases, pairwise genetic differentiation (F_{ST}) was calculated (Table 1). Population-level genetic diversity was assessed first by calculating allelic richness then private allele frequency

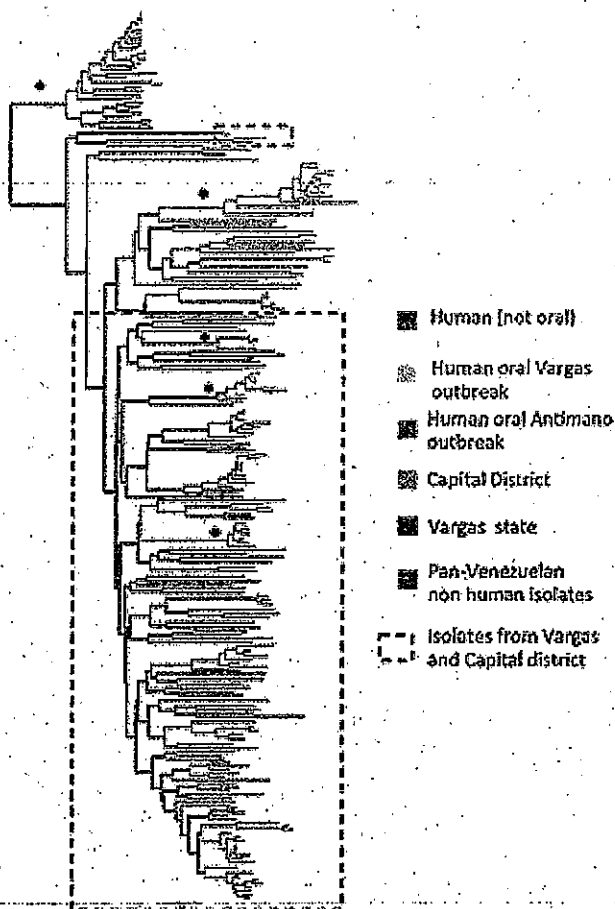


Figure 1. Unrooted neighbor joining tree showing genetic clustering among *Trypanosoma cruzi* isolates from 2 outbreaks of oral disease in northern Venezuela. Based on pairwise genetic distances, (1 - proportion of shared alleles) between multilocus microsatellite profiles (23 loci) generated from 246 isolates and clones. Black circles indicate nodes with >60% bootstrap support. Branch color key is shown. Dashed boxes indicate isolates associated with the outbreaks.

over loci between each human–nonhuman population pair (Table 2). Geographic sampling distribution is shown in online Technical Appendix 1 Figure 2.

Clustering results determined by discriminant analysis of principal components and neighbor joining were broadly congruent. In the former, 19 clusters were defined; sample

allocations are included in online Technical Appendix 2. Substantial overlap existed between some clusters, especially those from Capital and Vargas States (e.g., those labeled “x” in Figure 2), while others were highly distinct (e.g., cluster 8 in Figure 2). Human isolates from both oral outbreaks are extremely distinct from non-orally transmitted isolates collected from humans throughout Venezuela. Almost all these presumably vector-transmitted strains are closely related to one another, despite their geographic dispersal (cluster 8 in Figure 2). By comparison, oral outbreak strains that were isolated a mere 50 km apart (clusters 2, 5, 7, 9, 15–17) are far more globally diverse. Unlike most human isolates in Venezuela, which are distinct from nonhuman strains, oral outbreak isolates are interspersed among nonhuman strains from Venezuela. Furthermore, samples from both outbreaks clustered among nonhuman strains local to that outbreak, clearly indicating a local origin. Oral samples from each outbreak are polyphyletic with respect to strains from their immediate environment, a finding consistent with multiple contamination events or multiclonal infection sources.

F_{ST} values further support connectivity between outbreak and local environmental samples in both Antimano and Chichiriviche (Table 1). A lack of private alleles between human and nonhuman isolates also supports a local source for the Chichiriviche outbreak (Table 2). F_{ST} values in the 4-way comparison between outbreak and local environmental strains are, however, somewhat equivocal with respect to the entire dataset (Table 1). Cluster analysis showed that the human and nonhuman strains from Chichiriviche interspersed with strains from other states in Venezuela (Figure 2). Thus, we cannot confirm a uniquely local origin for the Chichiriviche outbreak, despite a low value for F_{ST} , and it is possible that some contaminating strains originated elsewhere.

Conclusions

This study demonstrates the value of rigorous molecular epidemiologic analysis of orally transmitted *T. cruzi* outbreaks, including the importance of appropriate sampling to identify the origin of the infecting strains. The foodstuff that propagated the peri-urban outbreak in Antimano was certainly contaminated locally. An active nonhu-

Table 1. F_{ST} values in a 4-way comparison for differentiation between *Trypanosoma cruzi* isolates derived from humans and the local environment during an outbreak of orally transmitted Chagás disease in 2 areas of Venezuela*

Isolate, location	Human isolates from		Nonhuman isolates from	
	Antimano	Chichiriviche	Antimano	Chichiriviche
Human				
Antimano		0.000	0.000	0.000
Chichiriviche	0.201		0.000	0.004
Nonhuman				
Antimano	0.093	0.170		0.000
Chichiriviche	0.088	0.053	0.079	

*Lower left shows linearized F_{ST} (genetic differentiation) values; upper right shows associated p values.

Table 2. Sample size corrected diversity between *Trypanosoma cruzi* isolates derived from humans and the local environment during an outbreak of orally transmitted Chagas disease in 2 areas of Venezuela*

Isolate, location	No. isolates/no. genotypes	Sample size corrected allelic richness $\pm$ SE	Mean no. private alleles/locus $\pm$ SE
Human			
Antimano	30/26	2.735 $\pm$ 0.291	0.32 $\pm$ 0.113
Chichiriviche	12/9	3.459 $\pm$ 0.412	0
Nonhuman			
Antimano	107/91	2.946 $\pm$ 0.320	0.86 $\pm$ 0.203
Chichiriviche	13/13	3.443 $\pm$ 0.409	0

man transmission cycle in the slums of Caracas, maintained by *Rattus rattus* rodents and *Panstrongylus geniculatus* triatomines, is the likely source. The Chichiriviche outbreak, however, has potential sources both in and outside the immediate area. As found in Chagas disease outbreaks linked to açai palm fruit in Brazil (12), the *T. cruzi* parasite can survive for several days in some foodstuffs (13). Also, triatomines can survive for months in harvested crops; thus, multiple hygiene interventions are potentially necessary along the food production line (14). Nonetheless, if the foodstuff implicated was prepared locally, local contamination represents the most likely source of the outbreak. Study of additional nonhuman strains from Chichiriviche is necessary to support this assertion.

Crucial to understanding parasite transmission in general, we believe, are genetic differences between strains

from orally and non-orally transmitted human cases. All TcI strains appear to be infective to humans and adapted to long-term carriage (8). However, the presence of a common, reduced-diversity TcI genotype cluster (TcI_{DOM}) among a high proportion of human Chagas disease cases in South America is also well established (7,8). We originally hypothesized that TcI_{DOM} was maintained, despite the presence of sympatric and infective sylvatic strains, because of low parasite transmission efficiency by invasive sylvatic vectors (8). Oral transmission is likely to be much more efficient. Thus, unlike TcI_{DOM} strains, those from orally transmitted *T. cruzi* cases demonstrated high genetic diversity and clearly originated from local nonhuman *T. cruzi* populations. However, it is also true that all TcI_{DOM} strains we isolated originated from patients with chronic infection, and all orally transmitted cases were in the acute phase. We cannot, therefore, rule

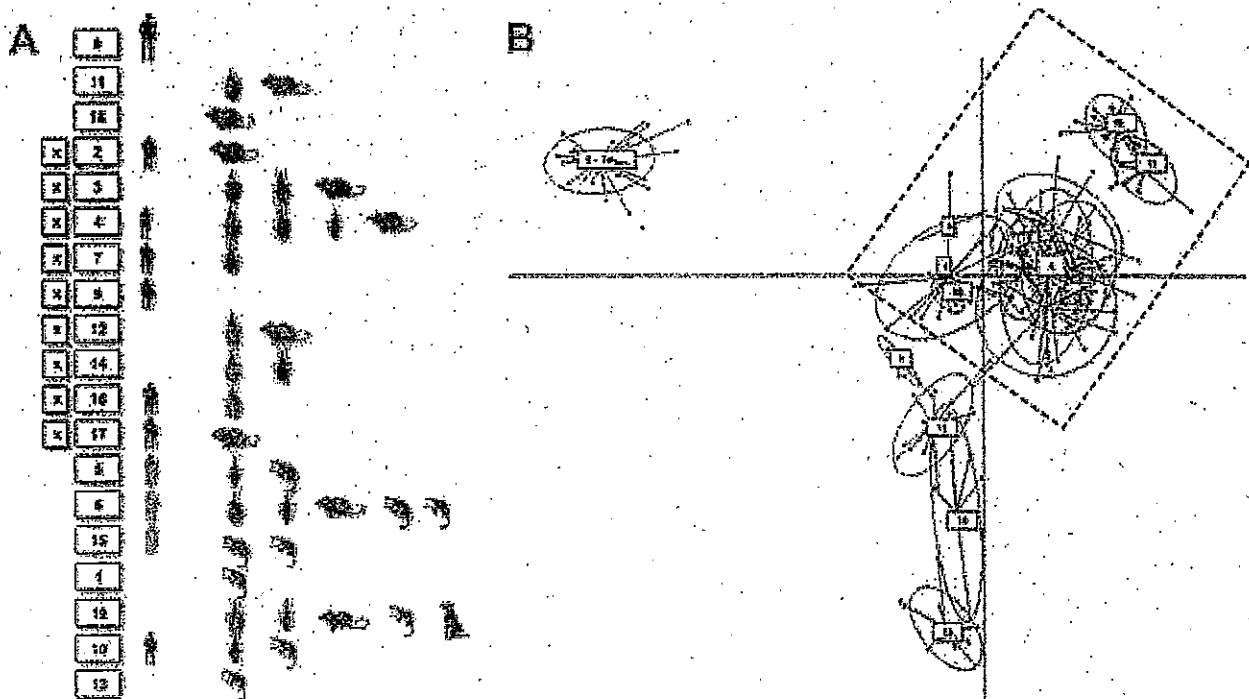


Figure 2. Discriminant analysis of principal components showing genetic clustering among *Trypanosoma cruzi* isolates from 2 outbreaks of oral disease in northern Venezuela. Six principal components were retained, explaining 80% of the diversity. Ellipses correspond to the optimal (as defined by the Bayesian information criterion minimum) number of population clusters among the genotypes analyzed. Images indicate sample host origin (human, rodent, marsupial, or triatomine), while colors correspond to the key in Figure 1: A full list of samples and population assignments (numbered boxes) is included in online Technical Appendix 2 (wwwnc.cdc.gov/EID/article/19/7/12-1576-Techapp1.xlsx). Dashed box indicates the isolates associated with the outbreaks.

out a role for immune selection in driving the frequency of TcL_{DOM} infections among humans; such selection represents an intriguing topic for future enquiry.

Molecular tools and reference datasets are now available to determine the source of acute Chagas disease outbreaks within days of their occurrence. The plummeting cost of such analyses means it is time to apply population genetic techniques and markers developed for trypanosomes as genuine epidemiologic tools.

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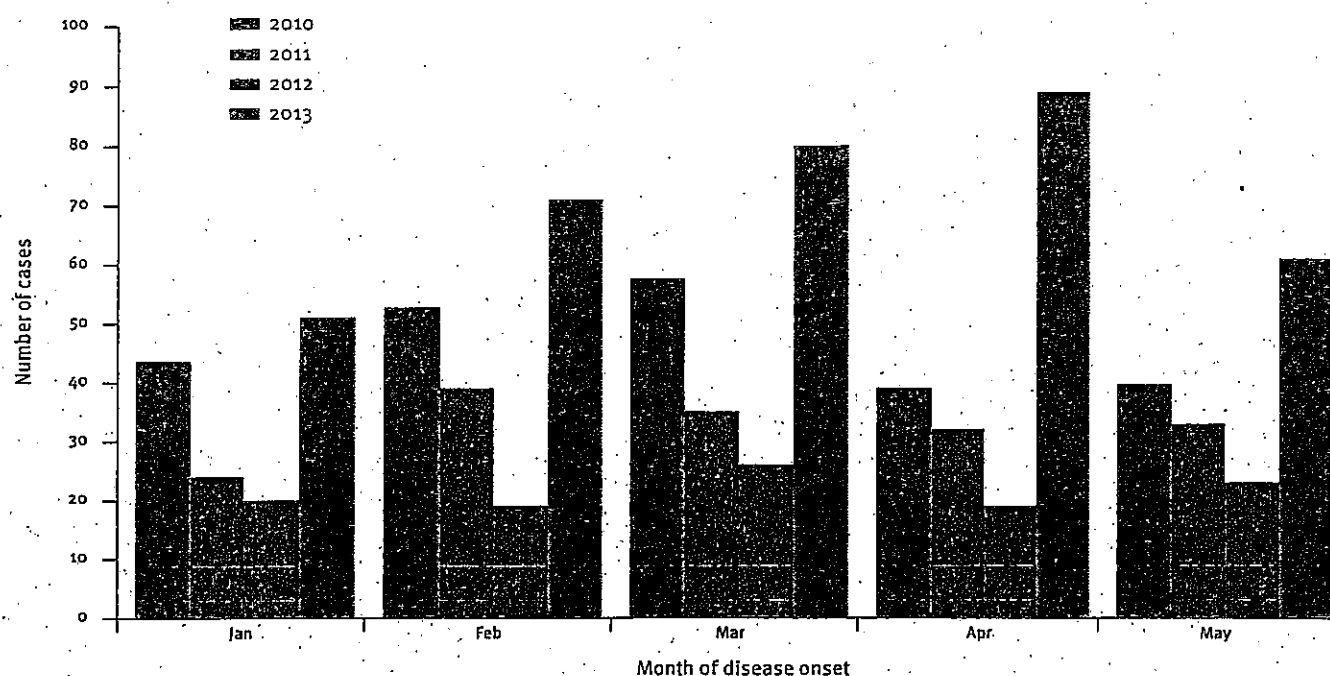
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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2013. 7. 8	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿	研究報告の公表状況	Rizzo C, Alfonsi V, Bruni R, et al.; Central Task Force on Hepatitis A. Euro Surveill. 2013 Jul 4;18(27). pii: 20518.	公表国 イタリア	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」J120(日本赤十字社) 新鮮凍結血漿-LR「日赤」J240(日本赤十字社) 新鮮凍結血漿-LR「日赤」J480(日本赤十字社)				
研究報告の概要	○イタリアにおけるA型肝炎の集団発生: 2013年5月31日付速報 イタリアのA型肝炎感染水準は低または中程度とされ、集団発生事例は1996～1997年及び2004年に南イタリアで、生の魚介類摂取に関連して報告された。しかし2013年1月以降、北イタリアにおいてA型肝炎の患者数が増加している。また5月には、ドイツ、オランダ、ポーランドで、北イタリア(トレントとボルツァーノ)のスキー旅行に関連したA型肝炎患者が15人発生したと報告された。トレントで発生した5人のイタリア人患者のHAV遺伝子解析結果は、ドイツとオランダの患者の解析結果と一致した。トレント及びボルツァーノで後方視的疫学調査を行ったところ、A型肝炎患者において、唯一共通して摂取されていた食品は冷凍ミックスベリーのあり、この期間中に消費されたとみられるミックスベリーからHAVが検出された。 汚染食品の分布とこれらへの曝露リスクについて明確にするため、ウイルスを保有する可能性のある他の食品と共に、冷凍ミックスベリーのサーベイランスが強化されている。保健当局は感染源の調査と感染拡大を防ぐための対策を行っている。				
報告企業の意見	2013年1月以降、北イタリアでA型肝炎が増加しており、患者の多くが共通して摂取していた食品である冷凍ミックスベリーからHAVが検出されたとの報告である。				
今後の対応	日本赤十字社は、輸血感染症対策として、ALT高値を排除するとともに、A型肝炎については治癒後6カ月間、家族に発症した人がある場合は1カ月間献血不適としている。また、厚生労働科学研究「経口感染によるウイルス性肝炎(A型及びE型)の感染防止、病態解明、遺伝的多様性及び治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。今後も情報の収集に努める。				
使用上の注意記載状況・その他参考事項等	新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」J120 新鮮凍結血漿-LR「日赤」J240 新鮮凍結血漿-LR「日赤」J480 血液を介するウイルス、細菌、原虫等の感染、vCJD等の伝播のリスク				

FIGURE 1

Distribution of hepatitis A cases in Italy, January–May 2010 to January–May 2013



Source: Sentinel surveillance system for acute viral hepatitis (Sistema Epidemiologico Integrato Epatiti Virali Acute - SEIEVA)

population [2] to date, a decreasing trend in the incidence of HAV has been observed, to 1.1 cases, 0.7 and 0.8 per 100,000 population in 2010, 2011, and 2012, respectively [7].

The 2013 hepatitis A outbreak in Italy

From 1 January 2013 to 31 May 2013 a total number of 352 cases of hepatitis A were reported to SEIEVA surveillance system, corresponding to a 70%, 54% and 34% increase in HAV notifications compared to the same period in 2012, 2011 and 2010, respectively (Figure 1).

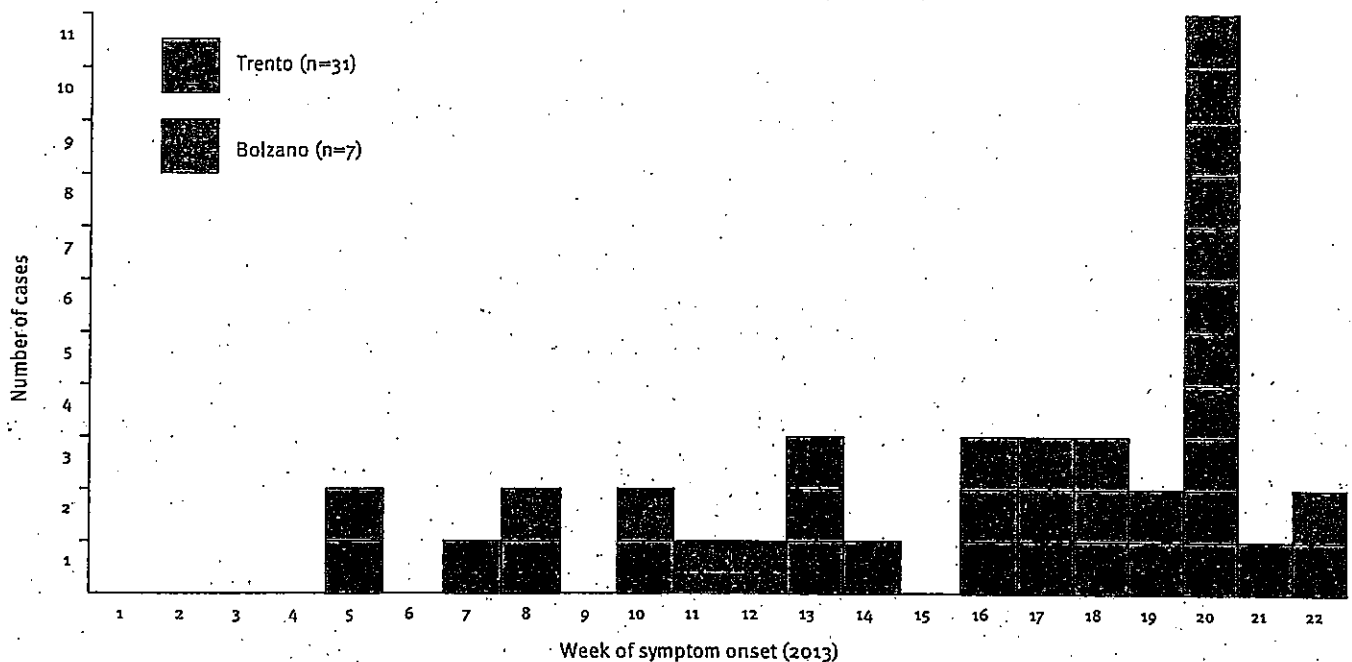
The highest increase in the number of cases was observed in seven northern Italian regions (Trento and Bolzano, Emilia-Romagna, Lombardy, Friuli Venezia Giulia, Piedmont, and Veneto) that accounted for 193/352 (55%) of the total cases recorded in 2013. In these seven regions, the cumulative incidence was 2.66 per 100,000 population in the five-month reference period. Another region that showed an increase in the number of cases in 2013 is Apulia, in southern Italy, which recorded a 22% increase in the number of cases in 2013; 77 of the 352 cases were reported from this region.

The distribution of cases by age group and year is reported in Figure 2. The mean age of cases was 35 years (range: 2–63 years) and the median was 39 years; 23 cases (12%) were recorded in children under 14 years. The cases were equally distributed among men and women: 55% of the cases were men and 45% were women. A total of 159 persons were hospitalised, with the majority of hospitalised cases in the age group of 35–54 years. As of 31 May 2013, no acute liver failures and deaths occurred. Four cases had been vaccinated against hepatitis A, with one dose within the three weeks before the onset of symptoms, so these were not considered vaccine failures.

With regard to the risk factors, among those who answered the questionnaire (193 cases), 3% (7/193) reported to have travelled to Egypt, 17% (33/193) reported to have eaten raw seafood and 20% (37/193) mixed berries in the six weeks before the symptom onset. When considering risk factors distribution after the end of April (date of introduction of the question on the consumption of frozen mixed berries), the majority of cases (37 of 46) reported having consumed frozen mixed berries.

FIGURE 3

Hepatitis A cases by week of symptom onset, provinces of Trento and Bolzano, Italy, January–May 2013 (n=38)



Source: Sentinel surveillance system for acute viral hepatitis (Sistema Epidemiologico Integrato Epatiti Virali Acute - SEIEVA)

from the province of Trento; however, this case had been vaccinated with one dose within the three weeks before the onset of symptoms, so this was not considered a vaccine failure. Preliminary epidemiological investigation for the identification of risk factors and common exposures focused on consumption of contaminated food as no epidemiological link between the cases could be confirmed. The only common food consumed by all cases was mixed berries or food containing mixed berries (cakes).

Serum samples were collected during the acute phase of the disease from five of the 38 cases, all from the Trento province. The sequence of the VP1/2A region of the HAV 1A virus obtained from all of them (with GenBank accession number KF182323) showed a 100% nucleotides homology with sequences of the isolates from the German and Dutch cases.

Investigation of food items implicated

The preliminary epidemiological investigation in the provinces of Trento and Bolzano showed that the only common food consumed by different cases was mixed berries or food containing mixed berries (cakes). Moreover, the hypothesis was strongly supported by the results of an epidemiological investigation conducted in a family cluster in Veneto region. Part of the mixed berries (redcurrant, blackberries, raspberries,

blueberries) that the cases indicated to have eaten within the period of time compatible with the onset of clinical symptoms were still available and were sampled. The analysis for HAV detection in the sample of mixed berries provided positive results. As a consequence, on 17 May, the Italian Ministry of Health (which is the food safety authority at national level) communicated these findings through the European Rapid Alert System for Food and Feed (RASFF). Following these preliminary positive results, the surveillance of these food items was intensified. More samples of berries were collected throughout the country once they were identified as potential risk factors, and two sampled berries in Trento were found positive for HAV. On 30 May, two additional RASFF notifications were issued to inform about new HAV findings in frozen mixed berries from Italy. Environmental investigations have been done on the mixed frozen berries suppliers of raw material in six different countries. Results on samples collected are pending at the time of the present rapid communication.

Control measures

On 23 May the Ministry of Health (the General Direction for Prevention together with the food safety authority) published a note for RHAs in order to enhance surveillance and awareness of HAV recommending to report within 24 hours any new HAV cases, to collect

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医薬品 研究報告 調査報告書

識別番号・報告回数	総合機構処理欄	
一般的名称	第一報入手日 2013. 5. 16	新医薬品等の区分 該当なし
販売名(企業名)	報告日	公表国 ブラジル
新鮮凍結人血漿	研究報告の公表状況	
新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」J120(日本赤十字社) 新鮮凍結血漿-LR「日赤」J240(日本赤十字社) 新鮮凍結血漿-LR「日赤」J480(日本赤十字社)	Am J Infect Control. 2013 May;41(5):471-2. doi: 10.1016/j.ajic.2012.05.016. Epub 2012 Oct 22.	
研究報告の概要	○針刺し事故後に曝露後予防措置を受けた医療従事者のHIVセロコンバージョン曝露後予防措置を講じたにもかかわらずHIVに感染したブラジルの医療従事者の症例について報告する。 2007年12月、42歳看護師が、患者の血糖値測定の際に針で母指を刺し、わずかな出血を伴う傷を負ったが、すぐに洗浄した。当該患者はAIDSを発症しており、CD4数は11/μLであったが、抗HIV治療を受けておらず、2008年2月に呼吸器合併症と敗血症により死亡した。 看護師は曝露時、ELISA法によるHIV、HBV、HCV抗体検査で陰性であった。曝露後2時間以内にジドブジン、ラミブジン、ロピナビル、リトナビルによる曝露後予防措置が開始され、28日間継続した。追跡期間中、急性HIV感染の徴候や症状はなく、2008年1月、3月、5月、5月はHIV 抗体陰性であったが、6月に陽性となり、7月にはPCR法で陽性となりHIV-1サブタイプCと同定された。 2011年2月、抗レトロウイルス治療を開始した。定性分析によると、HIVは非核酸系逆転写酵素阻害剤(K103N突然変異遺伝子)に高い抵抗性を示し、エトラビルを除きエファビレンツ、デビルジン及びネビラピンに強い耐性を持つことが分かった。現在AIDSの徴候や症状を示していないが、抑うつ障害のため受診している。 この症例では、曝露後予防措置を行ったにもかかわらずHIVセロコンバージョンが起こったが、曝露後予防措置の重要性は否定されるべきではない。本症例で曝露後予防措置が失敗した理由として、原因患者のCD4数が低く、数週間で死亡したこと、高ウイルス量であったことが考えられる。	
報告企業の意見	今後の対応	
AIDS患者の血糖値測定を行った際に針刺し事故を起こしてしまった医療従事者が、曝露後予防措置を講じたにもかかわらず、HIVに感染した症例報告である。	今後引き続き情報の収集に努める。	
使用上の注意記載状況・その他参考事項等	新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」J120 新鮮凍結血漿-LR「日赤」J240 新鮮凍結血漿-LR「日赤」J480 血液を介するウイルス、細菌、原虫等の感染、vCJD等の伝播のリスク	

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Role of clinical diagnosis of ventilator-associated pneumonia

To the Editor:

Ventilator-associated pneumonia (VAP) is an important nosocomial infection associated with increased morbidity and mortality. VAP is defined as pneumonia that occurs more than 48 hours after initiation of mechanical ventilation.¹ It is difficult to diagnose VAP accurately, and a high index of suspicion is required. VAP is clinically suspected usually based on the presence of fever (temperature $>38.3^{\circ}\text{C}$), blood leukocytosis ($>10,000/\text{mm}^3$), or leukopenia ($<4,000/\text{mm}^3$), purulent tracheal secretions, and a new and/or persistent radiographic infiltrate. Pugin et al proposed the clinical pulmonary infection score (CPIS), based on 6 clinical parameters (0–2 points each), such as fever, leukocyte count, quantity and purulence of tracheal secretions, oxygenation, type of radiographic abnormality, and results of sputum culture and Gram's stain.²

We performed a prospective study over a period of 15 months to determine the value of various clinical parameters in diagnosing VAP. VAP was diagnosed in patients with a quantitative endotracheal aspirate culture showing $\geq 10^5$ colony-forming units/mL after 48 hours of mechanical ventilation. A total of 200 patients was prospectively evaluated, of whom 36 (18%) developed VAP during their intensive care unit stay. The mean CPIS of VAP group (8.9 ± 8.262) was significantly higher than that of non-VAP group (2.5 ± 2.022) ($P < .0001$). There was a significant and very strong positive correlation between CPIS >6 and VAP (Pearson correlation, 0.909; $P < .0001$). The CPIS >6 had 100% sensitivity, 96.3% specificity, 85.7% positive predictive value, and 100% negative predictive value in diagnosing VAP. Similarly, Niederman had shown that a clinical definition of VAP based on CPIS >6 was associated with a high likelihood of pneumonia with a sensitivity of 93% and a specificity of 100%

when compared with quantitative bronchoalveolar lavage culture.³ Therefore, CPIS >6 may be considered diagnostic of VAP. In the critically ill patients on mechanical ventilation, the clinical symptoms and signs of pneumonia may vary. In our study, logistic regression analysis showed that temperature $\geq 38.5^{\circ}\text{C}$ ($P = .008$), oxygenation ($\text{PaO}_2/\text{FiO}_2 \text{ mm Hg} \leq 240$; $P < .0001$), and abnormal chest x-ray ($P = .029$) were independent predictors of VAP. Therefore, presence of these parameters should raise the suspicion of VAP in patients on mechanical ventilation for >48 hours. The presence of asymmetric pulmonary infiltrates on chest radiograph consistent with VAP may sometimes be caused by various noninfectious conditions. However, certain chest radiograph findings, such as progressive rapid cavitation of the pulmonary infiltrate, an air space process abutting a fissure, and a single air bronchogram, are associated with 96% specificity for diagnosing VAP and therefore can be reliably used.¹

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Conflicts of interest: None to report.

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HIV seroconversion in a health care worker who underwent postexposure prophylaxis following needlestick injury

To the Editor:

The recommendation to use postexposure prophylaxis (PEP) after occupational exposure to HIV did not exist before 1990. An article published in 1997 reports that the use of prophylaxis with zidovudine could reduce by 81% the risk of HIV infection in

cases of accidents involving exposure to biologic material.¹ In 1998, the Centers for Disease Control and Prevention recommended an expanded regimen that includes the addition of a protease inhibitor (indinavir or nelfinavir) for HIV exposures that pose an increased risk for transmission or where resistance to one or more of the antiretroviral agents recommended for PEP is known or suspected.² Since that time, because of viral strains resistant to several classes of drugs, other antiretroviral drugs have been incorporated into the PEP recommendations following occupational exposure to HIV.³

We describe a case of HIV seroconversion in a health care worker who underwent PEP after accidental HIV exposure in Brazil. In December 2007 a nursing assistant aged 42 years working in an infectious disease unit experienced a thumb injury while performing a hemogluco-test. The health care worker wore a latex glove during the procedure yet was injured while collecting the test device, the lancet of which had not been completely enclosed and was left on the table after the procedure. The percutaneous injury on the surface of the thumb caused slight bleeding that was immediately washed. The source patient was diagnosed with AIDS, had a CD4 count of 11 cells/mm³, but was not receiving any anti-HIV treatment. The patient had a history of neglect or irregular use of antiretroviral drugs (efavirenz, zidovudine, and lamivudine) and was diagnosed with pulmonary tuberculosis. There was no information regarding viral load or antiretroviral resistance.

During February 2008, the source patient died of respiratory complications and sepsis. Due to the rapid disease progression, tests for viral load and resistance to antiretroviral drugs could not be concluded. Antibodies for hepatitis B virus and hepatitis C virus were negative.

The health care worker showed negative antibodies for HIV enzyme-linked immunosorbent assay (ELISA), hepatitis B virus, and hepatitis C virus at the time of exposure. PEP was initiated within the first 2 hours, with zidovudine, lamivudine, lopinavir, and ritonavir, and maintained for 28 days. There were no reports of complications or interruptions. The use of lopinavir and ritonavir rather than efavirenz was based on information that the health care worker has epilepsy and makes regular use of carbamazepine. During the follow-up period, she did not present signs or symptoms of acute HIV infection. Antibodies for HIV ELISA in January, March, and May 2008 were negative. However, during June 2008, ELISA was positive and immunofluorescence for HIV was undetermined. Qualitative polymerase chain reaction HIV DNA test was positive for HIV-1 subtype C in July 2008.

During February 2011, the health care worker began antiretroviral therapy. Qualitative analysis showed that the health care worker's HIV had high resistance to non-nucleoside reverse-transcriptase inhibitors (K103N mutation gene) and showed high resistance to efavirenz, delavirdine, and nevirapine, but sensitivity to efavirenz. There were no mutations related to nucleoside analog reverse-transcriptase inhibitors and resistance to nucleotide analog reverse-transcriptase inhibitors. The health care worker did not present any signs or symptoms of AIDS, but began psychiatric evaluation due to depressive disorder. All other possible risk factors for HIV were ruled out and the health care worker's sexual partner was determined to be HIV negative.

Despite the fact that our case report describes HIV seroconversion in a health care worker who underwent PEP, the importance of its use should not be diminished. A possible cause of PEP failure in this case is the severity of illness of the source patient, who died few weeks after the accident with low CD4 and a probably high viral load.

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Comparative efficacy of commercially available alcohol-based hand rubs and World Health Organization-recommended hand rubs

To the Editor:

Edmonds et al conducted a comprehensive study leading to the following conclusion: Formulation matters.¹ However, the study design suffers from limitations challenging the conclusions.

The study was conducted according to an outdated version of test method American Society for Testing and Materials International (ASTM) E 1174, version-94 ("old version"), which was introduced in 1996 and is not state of the art because it was amended and reapproved twice since then.^{2,3} The current version is ASTM 1174-06 ("current version").⁴ Although various test conditions of ASTM 1174 had been adapted since 1996, in particular 3 of these adaptations seem to be highly relevant for interpretation of test results presented by Edmonds et al:

1. The current version is also valid for waterless formulations like alcohol-based hand rubs (ABHR), whereas the old version had been exclusively developed for rinse-off products.

This is a very relevant aspect when testing waterless formulations. Consequently, the number of repeated test washes may be reduced or eliminated which is in line with World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) recommendations that hands should only be treated with ABHR when not visibly soiled.^{5,6} A repeated hand contamination and treatment with ABHR without in-between handwashings as demanded by the old version is contradictory to these

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2013年7月1日	新医薬品等の区分 該当なし。	総合機構処理欄
一般的名称	別紙のとおり。	研究報告の 公表状況	Taiwan Centers for Disease Control Press Releases (2013-06-21)	公表国 台湾	使用上の注意記載状況・ その他参考事項等 記載なし。
販売名(企業名)	別紙のとおり。				
問題点：台湾で、鳥インフルエンザ A (H6N1) ウイルスの初めての人感染例が報告された。 台湾 CDC は、軽度の肺炎を発症した台湾中央部の飲食店で働く 20 歳の女性から鳥インフルエンザ (H6N1) ウイルスが検出されたとの報告を受取った。患者は、発熱、咳等の症状を呈した後、入院し、軽度の肺炎も発症したが、オセルタミビルを処方され、完全に回復した。アデノウイルスを含む鳥インフルエンザ (H6N1) ウイルス以外の 23 種のウイルスについては調べられたが、全て陰性であった。患者に海外渡航歴は無く、家禽やその他鳥との接触も無かった。患者の居住地の周囲 1km 以内にある 2 つの家禽農場が調べられたが、鳥インフルエンザ (H6N1) ウイルスは検出されなかった。また、患者と密接な接触のあった 36 人のうち、4 人もインフルエンザ様症状を呈したが、いずれも鳥インフルエンザ (H6N1) ウイルスには感染しておらず、疫学的調査の結果からは人-人感染を示す証拠は得られなかった。 研究報告の概要					
報告企業の意見			今後の対応		
別紙のとおり。			今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。		

一般的名稱	①人血清アルブミン、②人血清アルブミン*、③人血清アルブミン、④人免疫グロブリン、⑤人免疫グロブリン*、⑥乾燥ペプシン処理人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅷ因子、⑭乾燥濃縮人血液凝固第Ⅸ因子、⑮乾燥濃縮人血液凝固第Ⅹ因子、⑯乾燥濃縮人血液凝固第Ⅺ因子、⑰乾燥濃縮人血液凝固第Ⅻ因子、⑱乾燥抗破傷風人免疫グロブリン、⑲抗HBs人免疫グロブリン、⑳抗HBs人免疫グロブリン、㉑抗HBs人免疫グロブリン、㉒抗HBs人免疫グロブリン、㉓トロンビン、㉔フィブリノゲン加第ⅤⅢ因子*、㉕フィブリノゲン加第ⅤⅢ因子、㉖乾燥濃縮人アンチトロンビンⅢ、㉗ヒスタミン加人免疫グロブリン製剤、㉘人血清アルブミン*、㉙人血清アルブミン、㉚乾燥ペプシン処理人免疫グロブリン*、㉛乾燥濃縮人アンチトロンビンⅢ
販売名（企業名）	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン25“化血研”*、④ガンマーグロブリン筋注450mg/3ml「化血研」、⑤ガンマーグロブリン筋注1500mg/10ml「化血研」、⑥献血グロブリン注射用2500mg「化血研」、⑦献血ベニコロンーⅠ静注用500mg、⑧献血ベニコロンーⅠ静注用1000mg、⑨献血ベニコロンーⅠ静注用2500mg、⑩献血ベニコロンーⅠ静注用5000mg、⑪ベニコロン*、⑫注射用アナクトC2,500単位、⑬コンファクトF注射用250、⑭コンファクトF注射用500、⑮コンファクトF注射用1000、⑯ノバクトM静注用400単位、⑰ノバクトM静注用800単位、⑱ノバクトM静注用1600単位、⑲デタノセーラ筋注用250単位、⑳ヘパトセーラ筋注200単位/ml、㉑ヘパトセーラ筋注200単位/ml、㉒ヘパトセーラ筋注1000単位/5ml、㉓トロンビン“化血研”、㉔ボルヒール*、㉕ボルヒール組織接着用、㉖アンスロビンP500注射用、㉗ヒスタグロビン皮下注用、㉘アルブミン20%化血研*、㉙アルブミン5%化血研*、㉚静注グロブリン*、㉛アンスロビンP1500注射用
報告企業の意見	インフルエンザウイルスは70～120nmの球形または多形性で、核酸は8本の分節状マイナース本鎖RNA、エンベロープを有し、エンベロープの表面に存在する赤血球凝集素(HA)とノイラミダーゼ(NA)の抗原性により16種類のHA亜型および9種類のNA亜型に分類される。今回の報告は、鳥インフルエンザA(H6N1)の初めての人感染例が報告された、というものである。 上記製剤の製造工程には、冷エタノール分画工程及びウイルス除去膜ろ過工程といった原理の異なるウイルスクリアランス工程が導入されており、各工程のウイルスクリアランス効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬第1047号、平成11年8月30日）」に基づき、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告した鳥インフルエンザA(H6N1)のモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス(BVDV)が該当すると考えられるが、上記工程のBVDVクリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤による鳥インフルエンザA(H6N1)への感染報告例は無い。

＊：現在製造を行っていない

INF2013-004

Press Releases

Laboratory-confirmed case of human infection with avian influenza A(H6N1) virus in Taiwan recovered; Taiwan CDC urges public to take precautions to stay healthy (2013-06-21)



On May 20, 2013, the Taiwan Centers for Disease Control (Taiwan CDC) received a report from a hospital concerning a case of human infection with unsubtypeable influenza A virus in a 20-year-old female presenting mild pneumonia who resides in central Taiwan. The virus isolated from the respiratory specimen from the case was then submitted to Taiwan CDC for further identification. After conducting whole genome sequencing, the National Influenza Center (NIC) at Taiwan CDC identified the virus to be a novel avian-origin influenza A (H6N1) virus. A total of 36 close contacts of the case have been traced for follow-up. 4 of them experienced influenza-like illness symptoms, but none of them has been found to be infected with influenza A (H6N1) virus. Taiwan CDC continues to closely monitor the influenza activity and advises the public to seek immediate medical attention when experiencing any influenza-like illness with respiratory distress.

According to the epidemiological investigation, the case works at a breakfast shop. She had not traveled out of the country and had not been exposed to any poultry or bird. On May 5, she developed symptoms, including fever, cough, headache and muscle ache. On May 8, when her fever persisted and she developed shortness of breath, she sought medical attention at a hospital and was hospitalized for treatment. Her chest X-ray showed mild pneumonia. After administering Oseltamivir, her symptoms improved the next day. On May 11, she was discharged from the hospital. As of now, she has fully recovered. Influenza A (H6N1) virus was isolated from the respiratory specimen collected from the case on May 7. The case was found to have an antibody titer of 1:20 in the serum specimen collected on May 24. Another serum specimen was collected from the case on June 8 and the antibody titer in this specimen was found to be 1:40. Besides testing the respiratory specimen collected from the case for influenza viruses, the NIC also tested the specimen for 23 other common respiratory viruses such as adenovirus, respiratory syncytial virus, coronavirus, enterovirus and rhinovirus and the specimen tested negative for all these viruses. The agricultural authority has collected specimens from the poultry from the two poultry farms located within the 1-km perimeter of the case's residence. No avian influenza A (H6N1) virus has been detected in any of the specimens.

Beginning 1999, besides testing specimens from patients with notifiable infectious diseases such as influenza-related complications, Taiwan CDC regularly collects specimens from patients with influenza-like illness in the community and hospitalized patients with pneumonia of unknown origin to test for influenza viruses and systematically monitors the domestic influenza virus activity and the actual clinical situation (patient visits for influenza-like illness). Each year, on average, approximately 10,000 to 20,000 specimens were tested. As of now, an accumulative total of 250,000 specimens have been tested. 86,000 influenza virus strains have been isolated and identified from the specimens. Besides this H6N1 strain and the imported H7N9 strain detected in 2013, no other non-H1/H3 subtypes of influenza A viruses have been detected in humans in Taiwan.

An expert meeting attended by the agricultural authority, related medical experts and veterinary experts was convened to discuss the case. Based on the results of the epidemiological investigation, the case presenting mild pneumonia is a sporadic case and testing of the close contacts of the cases has not yielded any evidence of human-to-human transmission of this virus in the community. Both the health and agricultural authorities will continue to reinforce avian influenza surveillance in humans, poultry and the environment.

Avian influenza A (H6N1) virus is a low-pathogenic avian influenza virus commonly circulating in the domestic bird population and the virus had not been previously detected in humans. The genome sequence of the virus isolated from the case is found to be avian-origin and closely resembles avian influenza A (H6N1) virus in domestic poultry. The virus is also sensitive to influenza antivirals, including Oseltamivir and Zanamivir according to the sequencing data.

To prevent avian-to-human transmission of avian influenza infections, poultry vendors are advised to take personal precautions and the general public to avoid unnecessary direct contact with poultry and birds or their droppings. In addition, the public is also advised to

practice good personal hygiene such as washing hands frequently and consuming only thoroughly cooked poultry and eggs to reduce the risk of infection. If influenza-like illness symptoms or conjunctivitis develop, please put on a surgical mask, seek immediate medical attention and inform your doctor of your exposure history to birds/poultry, job contents and travel history. For more information, please call the toll-free Communicable Disease-Reporting and Care Hotline, 1922, or 0800-001922 if calling from a cell phone, or visit the Taiwan CDC's website at <http://www.cdc.gov.tw>.

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	回	報告日 年 月 日	第一報入手日 2013 年 7 月 1 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称					
販売名 (企業名)		研究報告の公表状況	ProMED mail: Avian Influenza, Human: Taiwan, H6N1 http://www.promedmail.org/direct.php?id=20130621.1785829	公表国 台湾	
台湾で鳥インフルエンザ A (H6N1) の人への感染が報告された。 2013 年 5 月 20 日、台湾 Centers for Disease Control (TCDC) は、軽度の肺炎症状を呈した 20 歳の女性が分類不能の A 型インフル ルエンザに感染したことを報告した。呼吸器試料から分離されたウイルスの全遺伝子解析の結果、ウイルスは鳥由来の新型インフ ルエンザ A (H6N1) であったと発表した。患者と接触があった 36 人に対し追跡調査が実施された。4 人がインフルエンザ様症状を 呈していたが、いずれの症例からもインフルエンザ A (H6N1) は検出されなかった。 疫学的調査によると、患者は食堂で働いており、国外旅行の経験はなく、また家禽や鳥との接触もなかった。2013 年 5 月 5 日、 患者は、発熱、咳、頭痛および筋肉痛を発現し、5 月 8 日に呼吸困難が発現した。この間、発熱は継続していた。患者は病院を受 診。胸部 X 線検査で軽度の肺炎所見が得られた。Oseltamivir が投与され、翌日に症状は改善した。2013 年 5 月 11 日、患者は退 院した。現在、完全に回復している。 鳥インフルエンザ A (H6N1) は鳥に対して低病原性で、家禽間で感染が継続している。一方、このウイルスが人から検出されたとの 報告はない。患者から検出したウイルスの遺伝子配列は、家禽から得た A (H6N1) ウイルスのそれと極めて類似していた。このウイ ルスは、Oseltamivir や Zanamivir 等の抗ウイルス薬に対し感受性があった。 農業当局は、患者の住居から 1 km 以内にある 2 つの家禽農場から試料採取を行ったが、鳥インフルエンザ A (H6N1) は検出されなか った。この患者は散発発生であると考えられた。患者と接触があった人を調べた限り、人から人への感染の証拠はなかった。厚生 および農業行政当局は、引き続き、人、家禽及び野鳥における鳥インフルエンザ活性の監視を強化している。					
研究報告の概要 BYL-2013-419 ProMED mail: Avian Influenza, Human: Taiwan, H6N1 http://www.promedmail.org/direct.php?id=20130621.1785829 使用上の注意記載状況・ その他参考事項等					
報告企業の意見		今後の対応			
鳥インフルエンザ A (H6N1) による人への感染症の第 1 報で ある。現在、本ウイルスによる感染報告は本報告だけである。 散発症例であると考えられている。 コージネイト FS およびコージネイト FS バイオセツトの製造 工程における病原体除去・不活化処理は、ウイルス及び細菌 に対して有効であることが報告されている。		現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も、新規人畜共通感染症や新たなウイルス感染症に関する情報収集 に努める。			



Global Alert and Response (GAR)

Human infection with influenza A(H7N9) virus in China

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1 APRIL 2013 - On 31 March 2013, the China Health and Family Planning Commission notified the World Health Organization (WHO) of three cases of human infection with influenza A(H7N9). The cases were laboratory confirmed on 29 March by China CDC. Laboratory testing for influenza A (H3N2), A (H1N1)pdm09 and A(H5N1), as well as for novel coronavirus, has been negative.

The cases were reported from Shanghai (2 cases) and Anhui province (1 case). All three cases presented with respiratory tract infection with progression to severe pneumonia and breathing difficulties. Disease onset was between 19 February and 15 March 2013. Two of the cases died. The third case is currently in critical condition.

To date no epidemiological link between the cases has been identified. An investigation including follow-up of contacts is ongoing. So far no further cases have been identified among the 88 identified contacts under follow up.

Investigations into the source of infection and mode of transmission are ongoing.

The Chinese government is actively investigating this event and has instituted enhanced surveillance, laboratory strengthening and training of health care professionals for detection, reporting and treatment.

WHO is in contact with the national authorities and is following the event closely. It will issue updates as new information becomes available.

Related links

[More on human infection with influenza A\(H7N9\) virus](#)



Influenza at the human-animal interface

Summary and assessment as of 4 July 2013

Human infection with avian influenza A(H5N1) viruses

From 2003 through 3 July 2013, 633 laboratory-confirmed human cases with avian influenza A(H5N1) virus infection, of which 377 died, have been officially reported to WHO from 15 countries.

Since the last update on 4 June 2013, three new laboratory-confirmed human cases with influenza A(H5N1) virus infection were reported to WHO from Cambodia (two) and Indonesia (one). Two of these patients died.

Cambodia reported one recent fatal case in a 6-year-old girl from Kampot province, and one retrospective diagnosis in a 58-year old man from Phnom Penh province who had onset of disease on 4 January 2013. Indonesia reported one new case in a 2-year-old boy from West Java. This was the first case of human infection with influenza A(H5N1) reported from Indonesia in 2013. All cases are considered to be sporadic, with no evidence of community-level transmission.

As influenza A(H5N1) virus is circulating widely in poultry in both countries, additional sporadic human cases or small clusters might be expected in the future.

Table 1: Laboratory-confirmed human cases of avian influenza A(H5N1) virus infection (4 June 2013– 3 July 2013)

Country	Province	Age	Sex	Date of onset	Date of Hospitalisation	Oseltamivir treatment Start date	Date of death	Exposure to
Cambodia	Kampot	6 years	F	24/06/2013	28/06/2013	28/06/2013	28/06/2013	Sick and dead poultry in village
	Phnom Penh	58 years	M	4/01/2013	6/01/2013	NA	NA	unknown
Indonesia	West Java	2 years	M	10/06/2013	17/06/2013	NA	19/06/2013	Live bird market

NA: not applicable or not available

Overall public health risk assessment for avian influenza A(H5N1) viruses: Whenever influenza viruses are circulating in poultry, sporadic infections or small clusters of human cases are possible, especially in people exposed to infected household poultry or contaminated environments. However, currently, this influenza A(H5N1) virus does not appear to transmit easily among people and therefore the risk of community-level spread of this virus remains low. Therefore, the overall public health risk associated with this virus remains unchanged.

Figure 1: Epidemiological curve of avian influenza A(H5N1) cases in humans by country and month of onset

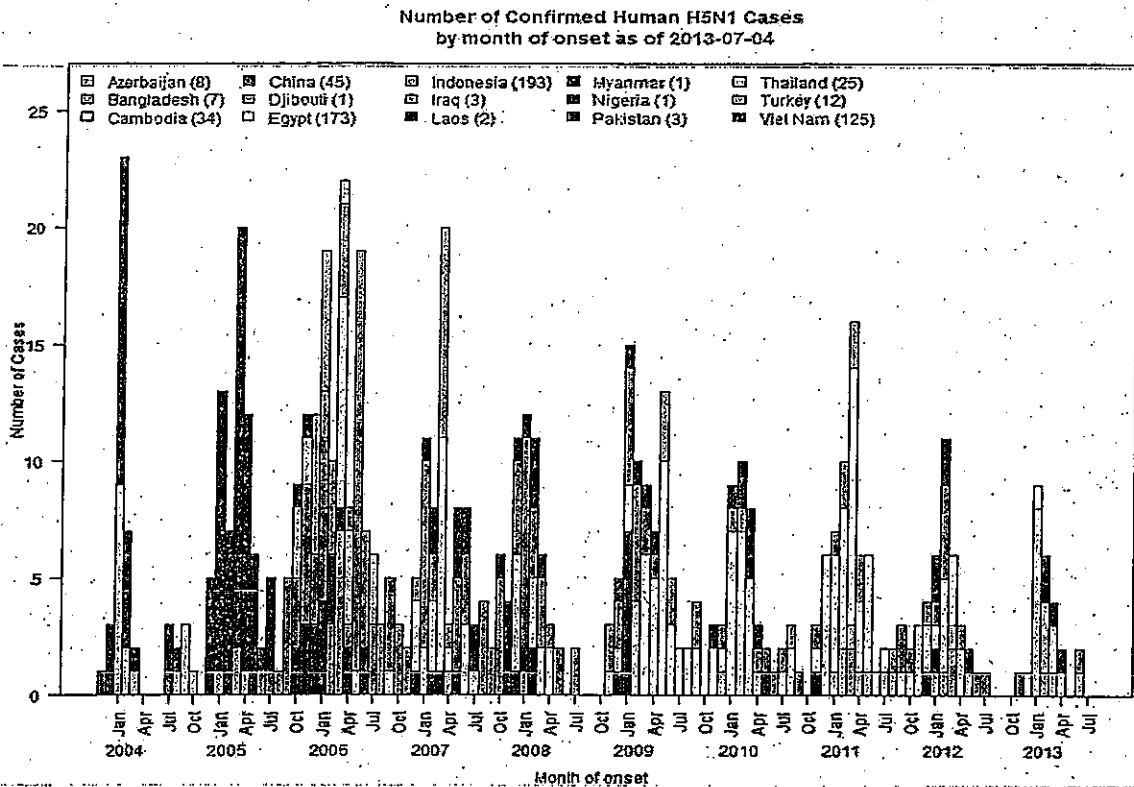
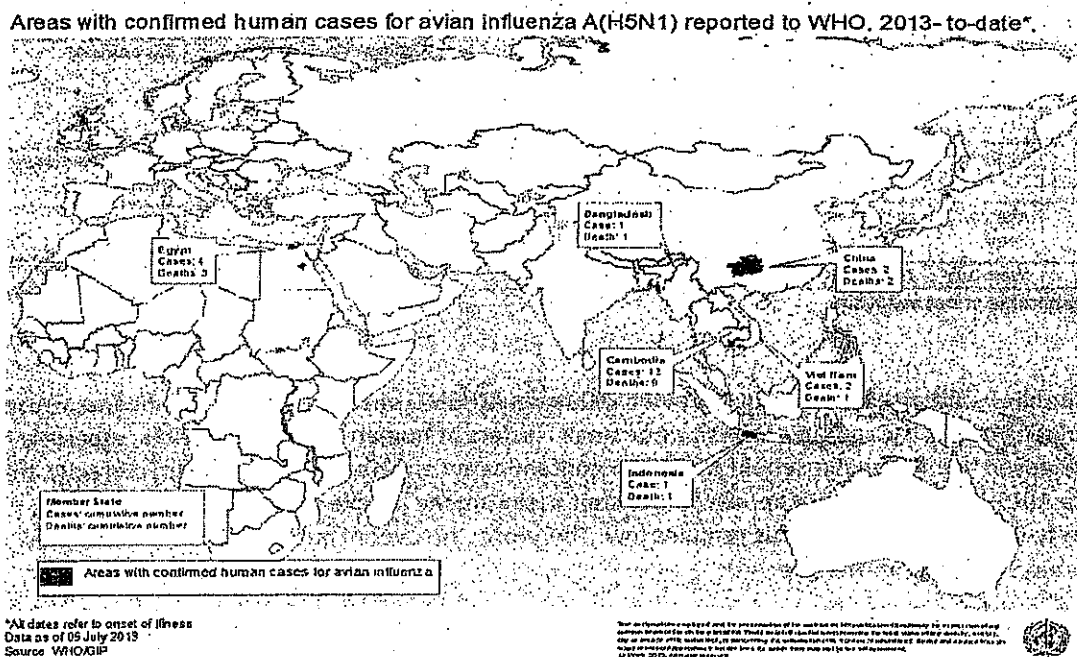


Figure 2: Map of avian influenza A(H5N1) cases in humans in 2013



Human infection with other non-seasonal influenza viruses

Avian influenza A(H7N9)

China has been reporting cases of human infection with avian influenza A(H7N9) virus since the end of March 2013. Since the last update of 4 June 2013, one retrospectively detected case has been reported from China. The patient was from Jiangsu province and had onset of illness on 25 April, was hospitalized on 26 April and discharged on 2 May. Both seasonal human influenza A(H3N2) and avian influenza A(H7N9) virus were detected in the throat swab from this patient. As of 4 July 2013, 133 human cases with influenza A(H7N9) infection were reported to WHO, including 43 deaths and 3 cases remaining in hospital. Most human cases presented with pneumonia.

Most cases with H7N9 infections have reported contact with poultry or live animal markets. Knowledge about the main virus reservoirs and the extent and distribution of the virus in animals remains limited. The incidence of human infections with avian influenza A(H7N9) seems to have decreased sharply after live animal market closure in the main affected provinces and municipalities. There are several possible reasons for this decrease, including decreased potential for human exposure after live animal market closure, but also greater public awareness, with subsequent change in behaviour. Also, the influenza A(H7N9) virus might follow a seasonal outbreak pattern similar to that of other avian influenza viruses, with more frequent transmission in poultry and to humans in winter months in temperate zones. Given the reopening of some live animal markets from the end of June, combined with the potential continued circulation of the virus in poultry, reports of additional human cases and infections in animals would not be unexpected, especially as the autumn approaches.

Although four small family clusters have been reported among previous cases, evidence does not support sustained human-to-human transmission.

Overall public health risk assessment for avian influenza A(H7N9) virus: Sporadic human cases and small clusters would not be unexpected in previously affected and possibly neighbouring areas/countries of China, and potentially in travellers from these areas returning to other countries. Continued vigilance is needed within affected and neighbouring areas to detect infections in animals and humans. WHO advises countries to continue surveillance and other preparedness actions, including ensuring appropriate laboratory capacity. All human infections with non-seasonal influenza viruses, such as avian influenza A(H7N9), are reportable to WHO under IHR (2005).

Current technical information as well as guidance related to avian influenza can be found at http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/index.html

Avian influenza A(H6N1)

Taipei Centers for Disease Control (Taipei CDC) reported one human case with infection with influenza A(H6N1) virus in a 20-year-old woman. She developed symptoms, including fever, cough, headache and muscle ache on 5 May 2013. She was hospitalized on 8 May with mild pneumonia. She received oseltamivir, recovered and was discharged from the hospital on 11 May.

Influenza A(H6N1) virus was isolated from the respiratory specimen collected on 7 May. Serum specimens collected on 24 May and 8 June showed antibody titres against H6N1 virus of 1:20 and 1:40, respectively. The case did not report exposure to poultry or other birds and has no travel history out of

the Taiwan Island. Specimens from poultry from two poultry farms located within a 1-km perimeter of the case's residence tested negative for avian influenza A(H6N1).

A total of 36 close contacts were identified and followed up. Four of these experienced influenza-like illness (ILI); throat swabs and paired serological samples were found to be negative for avian influenza A(H6N1) virus.

Avian influenza A(H6N1) virus is a low-pathogenic avian influenza virus commonly circulating in domestic bird populations¹ in the region and throughout the world. Although previous serological studies have documented antibodies to influenza A(H6) in humans,^{2,3,4} this is the first report of a symptomatic human infection with avian influenza A(H6N1) virus. Taipei CDC reports that the genome sequence of the virus isolated from the case is highly similar to avian influenza A(H6N1) viruses circulating in domestic poultry. The virus is reported to be sensitive to influenza antivirals, including oseltamivir and zanamivir, based on sequencing data.

Overall public health risk assessment for avian influenza A(H6N1) virus: Based on current epidemiological information this seems to be a sporadic human infection with avian influenza A(H6N1) virus. Both health and agricultural authorities continue to enhance influenza surveillance in humans, poultry and the environment in the region, especially in the context of identifying H7N9 virus-associated events. As with other avian influenza virus infections in humans, sporadic cases of human infection with avian influenza H6N1 virus would not be unexpected, as this virus is circulating widely in the local poultry population.

Influenza A(H3N2) variant virus infections in humans

The United States of America (USA) has reported four cases of human infection with influenza A(H3N2)v in the state of Indiana. The first case had onset of symptoms on 18 June. These are the first human cases reported in 2013. The total number of human infections with influenza A(H3N2)v virus reported in the USA was 12 in 2011 and 309 in 2012. Most cases in previous years were associated with exposure to swine at agricultural fairs.

None of the four new cases were hospitalized, no ongoing human to human transmission has been identified, and all four cases reported close contact with swine in the week before onset of illness. One virus sample has been fully characterized by CDC and is 99% similar to H3N2v viruses identified in the 309 human infections that occurred in the United States in 2012. Investigations around cases and contacts are ongoing.

Limited serological studies indicate that adults may have some pre-existing immunity to this virus

¹ Cheung CL et al. Establishment of influenza A virus (H6N1) in minor poultry species in Southern China. J Virology 81:10401-412, 2007.

² Shortridge KF. Pandemic influenza: a zoonosis? Semin Respir Infect 7:11-25, 1992.

³ Myers P et al. Infection due to 3 avian influenza subtypes in United States veterinarians. Clinical Infectious Diseases 45:4-9, 2007.

⁴ Kayali G et al. Evidence of previous avian influenza infection among US turkey workers. Zoonoses Public Health 57:265-72, 2010.

but children do not. Seasonal vaccines do not provide cross-protection to influenza A(H3N2)v in adults or children. Three candidate vaccine viruses specific for A(H3N2)v9 have been developed in the USA and could be used to produce an (H3N2)v vaccine if needed.

For more information see: <http://www.cdc.gov/flu/swineflu/h3n2v-cases.htm>.

Overall public health risk assessment for influenza A(H3N2)v viruses: Further human cases and small clusters may be expected, as this virus is circulating in the swine population in the USA and the season of agricultural fairs has begun. Close monitoring of the situation, including continued characterization of the virus to detect any changes, is warranted.

Animal outbreaks with highly pathogenic avian influenza viruses with potential public health impact

Overall, official reports of animal influenza outbreaks are at their expected seasonal levels (http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/WI). Normally, with the onset of summer in the northern hemisphere, there is a downward trend in the number of reports of influenza events in birds. Owing in part to the emergence of avian influenza A(H7N9) virus and infections of humans with this virus in China, there is enhanced surveillance for various subtypes of avian influenza in both humans and animals in China, the countries neighbouring China, and globally. It is therefore expected that more influenza A(H5) and A(H7) events in humans and animals will be detected and reported, as well as identification of infections with a variety of other subtypes and reassortants. Most infections that might be reported in humans will likely be mild and sporadic, and will not change the overall public health risk assessment. However, it is critical that these events be reported through the appropriate channels and viruses be collected and fully characterized in appropriate animal or human health influenza reference laboratories.

Because of the constantly evolving nature of influenza viruses, WHO continues to stress the importance of global monitoring of influenza viruses in animals and people and recommends that all Member States strengthen routine influenza surveillance. All human infections with non-seasonal influenza viruses are reportable to WHO under IHR (2005).

Relevant Links:

WHO human-animal interface web page

http://www.who.int/influenza/human_animal_interface/en/

Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO

http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/

H5N1 avian influenza: timeline of major events

http://www.who.int/influenza/human_animal_interface/avian_influenza/H5N1_avian_influenza_update.pdf

Avian influenza A(H7N9) information

http://who.int/influenza/human_animal_interface/influenza_h7n9/en/index.html

World Organisation of Animal Health (OIE) web page: Web portal on Avian Influenza

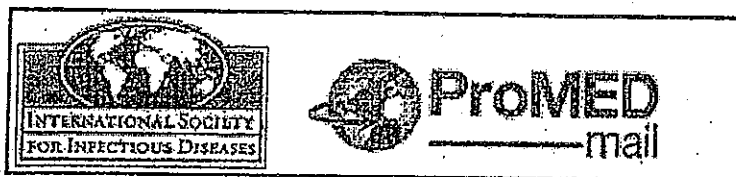
<http://www.oie.int/animal-health-in-the-world/web-portal-on-avian-influenza/>

Food and Agriculture Organization of the UN (FAO) webpage: Avian Influenza

<http://www.fao.org/avianflu/en/index.html>

OFFLU

<http://www.offlu.net/index.html>



Published Date: 2013-06-21 21:57:03
Subject: PRO/AH/EDR> Avian influenza, human (86): Taiwan, H6N1
Archive Number: 20130621.1785829

AVIAN INFLUENZA, HUMAN (86): TAIWAN, H6N1

A ProMED-mail post

<http://www.promedmail.org>

ProMED-mail is a program of the
International Society for Infectious Diseases

<http://www.isid.org>

Date: Sat 21 Jun 2013

Source: Taiwan Centers for Disease Control [edited]

[http://www.cdc.gov.tw/english/info.aspx?](http://www.cdc.gov.tw/english/info.aspx?treeid=BC2D4E89B154059B&nowtreeid=EE0A2987CFBA3222&tid=E36A5E9AB3D3A216)

[treeid=BC2D4E89B154059B&nowtreeid=EE0A2987CFBA3222&tid=E36A5E9AB3D3A216](http://www.cdc.gov.tw/english/info.aspx?treeid=BC2D4E89B154059B&nowtreeid=EE0A2987CFBA3222&tid=E36A5E9AB3D3A216)

On 20 May 2013, the Taiwan Centers for Disease Control (Taiwan CDC) received a report from a hospital concerning a case of human infection with unsubtypeable influenza A virus in a 20-year-old female presenting with mild pneumonia who resides in central Taiwan. The virus isolated from the respiratory specimen from the case was then submitted to Taiwan CDC for further identification. After conducting whole genome sequencing, the National Influenza Center (NIC) at Taiwan CDC identified the virus to be a novel avian-origin influenza A (H6N1) virus. A total of 36 close contacts of the case have been traced for follow-up. Four of them experienced influenza-like illness symptoms, but none of them has been found to be infected with influenza A (H6N1) virus. Taiwan CDC continues to closely monitor influenza activity and advises the public to seek immediate medical attention when experiencing any influenza-like illness with respiratory distress.

According to the epidemiological investigation, the case works at a breakfast shop. She has not traveled out of the country and has not been exposed to any poultry or birds. On [5 May 2013], she developed symptoms, including fever, cough, headache and muscle ache. On [8 May 2013], when her fever persisted and she developed shortness of breath, she sought medical attention at a hospital and was hospitalized for treatment. Her chest X-ray showed mild pneumonia. After administering Oseltamivir, her symptoms improved the next day. On [11 May 2013], she was discharged from the hospital. As of now, she has fully recovered.

Influenza A (H6N1) virus was isolated from the respiratory specimen collected from the case on [7 May 2013]. The case was found to have an antibody titer of 1:20 in the serum specimen collected on [24 May 2013]. Another serum specimen was collected from the case on [8 Jun 2013], and the antibody titer in this specimen was found to be 1:40. Besides testing the respiratory specimen collected from the case for influenza viruses, the NIC also tested the specimen for 23 other common respiratory viruses such as adenovirus, respiratory syncytial virus, coronavirus, enterovirus and rhinovirus, and the specimen tested negative for all these viruses. The agricultural authority has collected specimens from the poultry from the 2 poultry farms located within the one-km perimeter of the case's residence. No avian influenza A (H6N1) virus has been detected in any of the specimens.

Beginning in 1999, besides testing specimens from patients with notifiable infectious diseases such as influenza-related complications, Taiwan CDC regularly collects specimens from patients with influenza-like illness in the community and hospitalized patients with pneumonia of unknown origin to test for influenza viruses and systematically monitors domestic influenza virus activity and the actual clinical situation (patient visits for influenza-like illness). Each year, on average, approximately 10 000 to 20 000 specimens have been tested. As of now, a cumulative total of 250 000 specimens have been tested. 86 000 influenza virus strains have been isolated and identified from the specimens. Besides this H6N1 strain and the imported H7N9 strain detected in 2013, no other non-H1/H3 subtypes of influenza A viruses have been detected in humans in Taiwan.

An expert meeting attended by the agricultural authority and related medical experts and veterinary experts was convened to discuss the case. Based on the results of the epidemiological investigation, the

case presenting with mild pneumonia is a sporadic case, and testing of the close contacts of the cases has not yielded any evidence of human-to-human transmission of this virus in the community. Both the health and agricultural authorities will continue to reinforce avian influenza surveillance in humans, poultry and the environment.

Avian influenza A (H6N1) virus is a low-pathogenic avian influenza virus commonly circulating in the domestic bird population, and the virus had not been previously detected in humans. The genome sequence of the virus isolated from the case is found to be of avian-origin and closely resembles avian influenza A (H6N1) virus in domestic poultry. The virus is also sensitive to influenza antivirals, including Oseltamivir and Zanamivir, according to the sequencing data.

To prevent avian-to-human transmission of avian influenza infections, poultry vendors are advised to take personal precautions and the general public to avoid unnecessary direct contact with poultry and birds or their droppings. In addition, the public is also advised to practice good personal hygiene such as washing hands frequently and consuming only thoroughly cooked poultry and eggs to reduce the risk of infection.

Communicated by:

ProMED-mail

<promed@promedmail.org>

[This report describes a single mild case of pneumonia attributable to infection with avian A/H6N1 influenza virus, a low-pathogenic avian influenza virus commonly circulating in the domestic bird population of Taiwan and not previously detected in humans. The patient had no involvement with poultry, and none of her contacts showed evidence of H6N1 virus infection. No other respiratory viruses appeared to be co-factors. Furthermore, none of 86 000 influenza virus strains that had been isolated and characterised previously by the Taiwan CDC had been identified as avian A/H6N1 influenza virus, and other than this H6N1 strain and the H7N9 virus imported from China in 2013, no other non-H1/H3 subtypes influenza A viruses have been detected in humans in Taiwan.

This case appears to be a unique event and not the precursor to an incipient epidemic. Curiously, the patient displayed a rather poor immune response to infection, and if she had not been not treated with oseltamivir at an early stage, the outcome might have been more severe. The poor immune response may have been a factor also in the victim's susceptibility to H6N1 virus infection.

A significant feature of this report is that the H6N1 isolate was characterised by whole genome analysis, which excludes any ambiguities and may, in the longer term facilitate identification of genetic factors relevant in host switching.

The Taiwan CDC is to be commended for the thoroughness of this analysis. - Mod.CP

A HealthMap/ProMED-mail map can be accessed at: <http://healthmap.org/r/10b5>.]

See Also

Avian influenza, human (58): (Taiwan ex China) H7N9 update [20130424.1669273](#)

Avian influenza, human (30): China (Hong Kong), Taiwan H7N9, NOT: corr. [20130409.1634242](#)

Avian influenza, human (30): China (Hong Kong), Taiwan H7N9, NOT [20130406.1626565](#)
2012

Avian influenza, human (46): (Taiwan) poultry workers, seroprevalence [20120423.1111010](#)
.....mpp/cp/msp/lm

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医薬品 研究報告 調査報告書

識別番号・報告回数	E-13000001	第2報	報告日 2013年05月02日	第一報入手日 2013年04月04日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	1. 乾燥濃縮抗血友病人グロブリン (6343406) 2. 乾燥濃縮抗血友病人グロブリン (6343406) 3. 乾燥濃縮抗血友病人グロブリン (6343406)					
販売名 (企業名)	1. ヘモフィルM 250 (634340612) (Baxter) 2. ヘモフィルM 500 (634340612) (Baxter) 3. ヘモフィルM 1000 (634340612) (Baxter)					
研究報告の概要	2013年3月31日以降、中国および台湾の衛生当局から計135例のH7N9型トリインフルエンザ検査確定症例が報告され、うち44例が死亡の転帰を辿った。これまでに、H7N9型インフルエンザウイルスによるヒト感染例は少数報告されていたが、H7N9型によるヒト感染は今回が初の報告であった。中国の134例は、当初南東沿岸部に限局して発生したが、次第に北部や内陸部に拡大した。台湾の1例は、中国本土からの輸入例であると考えられた。 症状は発熱や咳等の気道感染症症状に始まり、5-7日後に呼吸困難や急性呼吸窮迫症候群を伴う重度の肺炎に至る。このウイルスは抗ウイルス薬アマンタジン、リマンタジンには抵抗性を示したが、早期のノイミニダーゼ阻害薬による治療が有効であった。 H5N1型トリインフルエンザウイルス流行の際には、重症化した鳥類がヒト感染の警告因子となったが、H7N9型トリインフルエンザウイルスは鳥類においては軽微な症状しか示さないか無徴候であり、ヒトにおいて重症化したため、このような予測も不可能であった。H7N9型トリインフルエンザウイルス感染について、自然界で宿主となる動物種や感染経路、ヒトや動物における感染の範囲については、不明な点も多い。しかしながら、生きた家禽の取引市場およびそこから得られた家禽検体中のウイルスとヒト感染例の検体から得られたウイルスとの間に遺伝的相同性が認められたこと、大半のヒト感染例で家禽（多くはニワトリ）への曝露が報告されたこと、生きた家禽の取引市場の閉鎖および一般市民への啓蒙活動を含む公衆衛生対策実施以降に感染例が減少したことより、感染家禽およびそれらに汚染された環境が感染源であると推察された。事実、6月下旬に家禽市場の一部再開した後、7月に入ってから発生した症例が2例報告された。今後気温が下がるとともに、再流行の危険性がある。これまでに患者との接触者20000人以上についての調査が実施されたが、2次感染者はごくわずかであり、家族内のみが4件報告されたのみであった。また、3月および4月の2か月間で、インフルエンザ様症状を呈した患者20000人以上が検査されたが、H7N9型インフルエンザウイルス感染例は6例のみであり、軽症（であるが故に報告されていない）症例が多数存在する可能性はないものと推察された。したがって、持続するヒト-ヒト感染の可能性はごく低いと考えられた。今後、同ウイルスが変異によって高いヒト感染能を獲得する可能性もあるため、同ウイルス感染の再流行時には、充分な注意が必要である。					
報告企業の意見	今後の対応 今後とも注意深く同感染症の発生数や頻度の変化、発生地域の拡大等の動向について情報収集に努める。					
使用上の注意記載状況・その他参考事項等	使用上の注意記載状況・その他参考事項等 自社の血漿由来製剤はすべて米国および欧州における供血から製造されている。 ヘモフィルM：該当ロットなし 本邦において1992年以降輸入実績なし [使用上の注意記載状況] ヘモフィルM：一般的注意（非A非B型肝炎等のウイルス感染症の危険性を完全に否定出来ない）で、観察を十分に行い、肝障害があらわれた場合には適切な処置を行うこと。 [追加・変更箇所] 2013年4月19日に研究報告として完了報告済。 2013年5月2日、ウイルスの特徴や発症症例数、転帰等につき追加情報が得られたため、追加報告をおこなった。					



Influenza at the human-animal interface

Summary and assessment as of 4 July 2013

Human infection with avian influenza A(H5N1) viruses

From 2003 through 3 July 2013, 633 laboratory-confirmed human cases with avian influenza A(H5N1) virus infection, of which 377 died, have been officially reported to WHO from 15 countries.

Since the last update on 4 June 2013, three new laboratory-confirmed human cases with influenza A(H5N1) virus infection were reported to WHO from Cambodia (two) and Indonesia (one). Two of these patients died.

Cambodia reported one recent fatal case in a 6-year-old girl from Kampot province, and one retrospective diagnosis in a 58-year old man from Phnom Penh province who had onset of disease on 4 January 2013. Indonesia reported one new case in a 2-year-old boy from West Java. This was the first case of human infection with influenza A(H5N1) reported from Indonesia in 2013. All cases are considered to be sporadic, with no evidence of community-level transmission.

As influenza A(H5N1) virus is circulating widely in poultry in both countries, additional sporadic human cases or small clusters might be expected in the future.

Table 1: Laboratory-confirmed human cases of avian influenza A(H5N1) virus infection (4 June 2013- 3 July 2013)

Country	Province	Age	Sex	Date of onset	Date of Hospitalisation	Oseltamivir treatment Start date	Date of death	Exposure to
Cambodia	Kampot	6 years	F	24/06/2013	28/06/2013	28/06/2013	28/06/2013	Sick and dead poultry in village
	Phnom Penh	58 years	M	4/01/2013	6/01/2013	NA	NA	unknown
Indonesia	West Java	2 years	M	10/06/2013	17/06/2013	NA	19/06/2013	Live bird market

NA: not applicable or not available

Overall public health risk assessment for avian influenza A(H5N1) viruses: Whenever influenza viruses are circulating in poultry, sporadic infections or small clusters of human cases are possible, especially in people exposed to infected household poultry or contaminated environments. However, currently, this influenza A(H5N1) virus does not appear to transmit easily among people and therefore the risk of community-level spread of this virus remains low. Therefore, the overall public health risk associated with this virus remains unchanged.

Figure 1: Epidemiological curve of avian influenza A(H5N1) cases in humans by country and month of onset

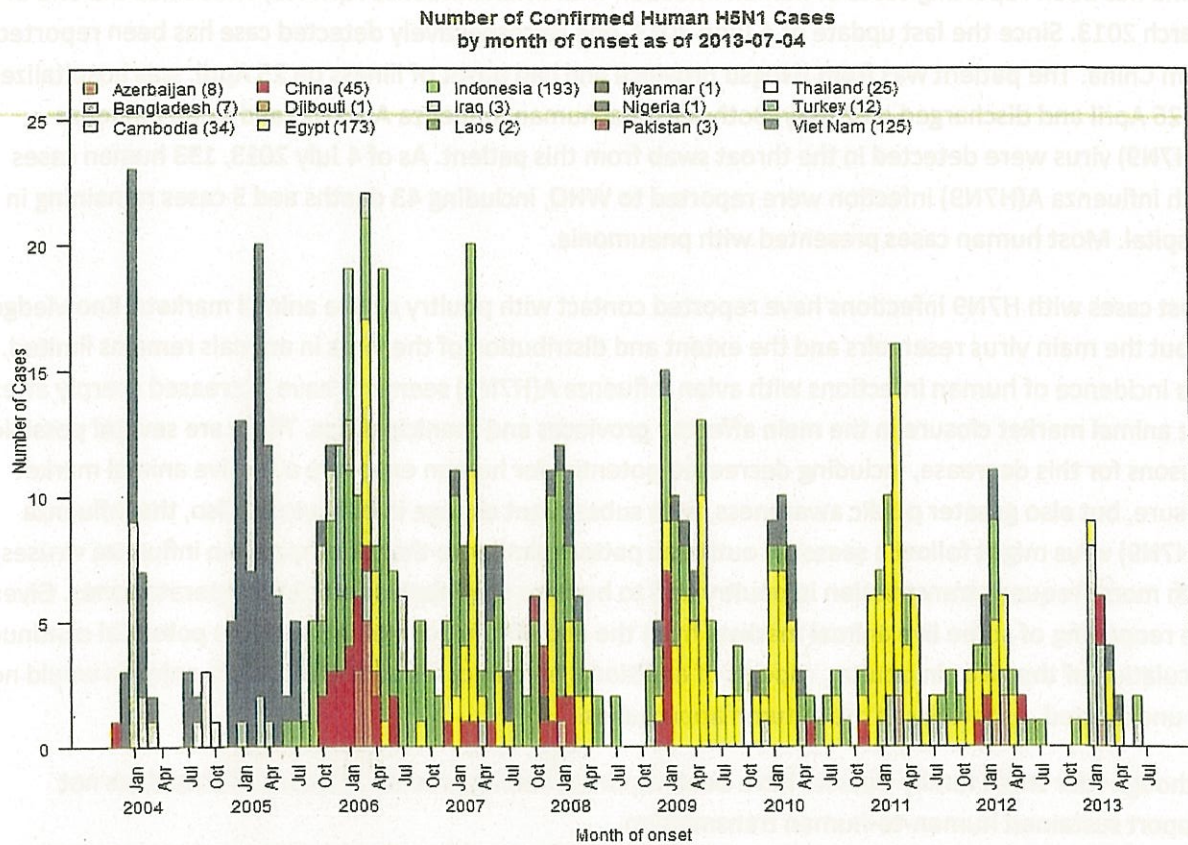
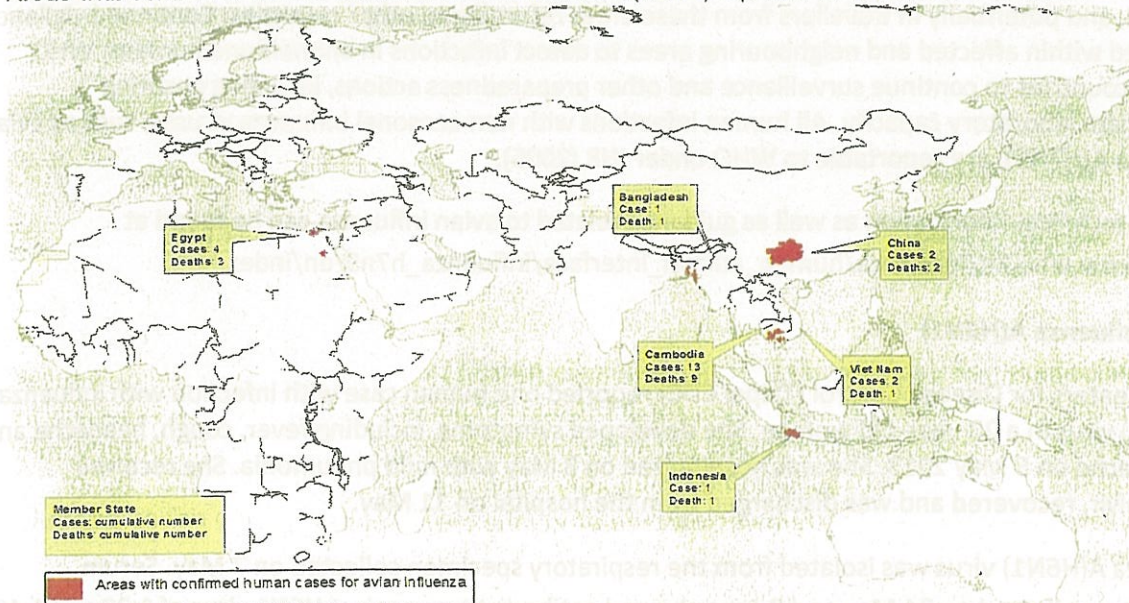


Figure 2: Map of avian influenza A(H5N1) cases in humans in 2013

Areas with confirmed human cases for avian influenza A(H5N1) reported to WHO, 2013- to-date*.



*All dates refer to onset of illness
Data as of 05 July 2013
Source: WHO/GIP

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Human infection with other non-seasonal influenza viruses

Avian influenza A(H7N9)

China has been reporting cases of human infection with avian influenza A(H7N9) virus since the end of March 2013. Since the last update of 4 June 2013, one retrospectively detected case has been reported from China. The patient was from Jiangsu province and had onset of illness on 25 April, was hospitalized on 26 April and discharged on 2 May. Both seasonal human influenza A(H3N2) and avian influenza A(H7N9) virus were detected in the throat swab from this patient. As of 4 July 2013, 133 human cases with influenza A(H7N9) infection were reported to WHO, including 43 deaths and 3 cases remaining in hospital. Most human cases presented with pneumonia.

Most cases with H7N9 infections have reported contact with poultry or live animal markets. Knowledge about the main virus reservoirs and the extent and distribution of the virus in animals remains limited. The incidence of human infections with avian influenza A(H7N9) seems to have decreased sharply after live animal market closure in the main affected provinces and municipalities. There are several possible reasons for this decrease, including decreased potential for human exposure after live animal market closure, but also greater public awareness, with subsequent change in behaviour. Also, the influenza A(H7N9) virus might follow a seasonal outbreak pattern similar to that of other avian influenza viruses, with more frequent transmission in poultry and to humans in winter months in temperate zones. Given the reopening of some live animal markets from the end of June, combined with the potential continued circulation of the virus in poultry, reports of additional human cases and infections in animals would not be unexpected, especially as the autumn approaches.

Although four small family clusters have been reported among previous cases, evidence does not support sustained human-to-human transmission.

Overall public health risk assessment for avian influenza A(H7N9) virus: Sporadic human cases and small clusters would not be unexpected in previously affected and possibly neighbouring areas/countries of China, and potentially in travellers from these areas returning to other countries. Continued vigilance is needed within affected and neighbouring areas to detect infections in animals and humans. WHO advises countries to continue surveillance and other preparedness actions, including ensuring appropriate laboratory capacity. All human infections with non-seasonal influenza viruses, such as avian influenza A(H7N9), are reportable to WHO under IHR (2005).

Current technical information as well as guidance related to avian influenza can be found at http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/index.html

Avian influenza A(H6N1)

Taipei Centers for Disease Control (Taipei CDC) reported one human case with infection with influenza A(H6N1) virus in a 20-year-old woman. She developed symptoms, including fever, cough, headache and muscle ache on 5 May 2013. She was hospitalized on 8 May with mild pneumonia. She received oseltamivir, recovered and was discharged from the hospital on 11 May.

Influenza A(H6N1) virus was isolated from the respiratory specimen collected on 7 May. Serum specimens collected on 24 May and 8 June showed antibody titres against H6N1 virus of 1:20 and 1:40, respectively. The case did not report exposure to poultry or other birds and has no travel history out of

the Taiwan Island. Specimens from poultry from two poultry farms located within a 1-km perimeter of the case's residence tested negative for avian influenza A(H6N1).

A total of 36 close contacts were identified and followed up. Four of these experienced influenza-like illness (ILI); throat swabs and paired serological samples were found to be negative for avian influenza A(H6N1) virus.

Avian influenza A(H6N1) virus is a low-pathogenic avian influenza virus commonly circulating in domestic bird populations¹ in the region and throughout the world. Although previous serological studies have documented antibodies to influenza A(H6) in humans,^{2,3,4} this is the first report of a symptomatic human infection with avian influenza A(H6N1) virus. Taipei CDC reports that the genome sequence of the virus isolated from the case is highly similar to avian influenza A(H6N1) viruses circulating in domestic poultry. The virus is reported to be sensitive to influenza antivirals, including oseltamivir and zanamivir, based on sequencing data.

Overall public health risk assessment for avian influenza A(H6N1) virus: Based on current epidemiological information this seems to be a sporadic human infection with avian influenza A(H6N1) virus. Both health and agricultural authorities continue to enhance influenza surveillance in humans, poultry and the environment in the region, especially in the context of identifying H7N9 virus-associated events. As with other avian influenza virus infections in humans, sporadic cases of human infection with avian influenza H6N1 virus would not be unexpected, as this virus is circulating widely in the local poultry population.

Influenza A(H3N2) variant virus infections in humans

The United States of America (USA) has reported four cases of human infection with influenza A(H3N2)v in the state of Indiana. The first case had onset of symptoms on 18 June. These are the first human cases reported in 2013. The total number of human infections with influenza A(H3N2)v virus reported in the USA was 12 in 2011 and 309 in 2012. Most cases in previous years were associated with exposure to swine at agricultural fairs.

None of the four new cases were hospitalized, no ongoing human to human transmission has been identified, and all four cases reported close contact with swine in the week before onset of illness. One virus sample has been fully characterized by CDC and is 99% similar to H3N2v viruses identified in the 309 human infections that occurred in the United States in 2012. Investigations around cases and contacts are ongoing.

Limited serological studies indicate that adults may have some pre-existing immunity to this virus

¹ Cheung CL et al. Establishment of influenza A virus (H6N1) in minor poultry species in Southern China. *J Virology* 81:10401-412, 2007.

² Shortridge KF. Pandemic influenza: a zoonosis? *Semin Respir Infect* 7:11-25, 1992.

³ Myers P et al. Infection due to 3 avian influenza subtypes in United States veterinarians. *Clinical Infectious Diseases* 45:4-9, 2007.

⁴ Kayali G et al. Evidence of previous avian influenza infection among US turkey workers. *Zoonoses Public Health* 57:265-72, 2010.

but children do not. Seasonal vaccines do not provide cross-protection to influenza A(H3N2)v in adults or children. Three candidate vaccine viruses specific for A(H3N2)v9 have been developed in the USA and could be used to produce an (H3N2)v vaccine if needed.

For more information see: <http://www.cdc.gov/flu/swineflu/h3n2v-cases.htm>.

Overall public health risk assessment for influenza A(H3N2)v viruses: Further human cases and small clusters may be expected, as this virus is circulating in the swine population in the USA and the season of agricultural fairs has begun. Close monitoring of the situation, including continued characterization of the virus to detect any changes, is warranted.

Animal outbreaks with highly pathogenic avian influenza viruses with potential public health impact

Overall, official reports of animal influenza outbreaks are at their expected seasonal levels (http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/WI). Normally, with the onset of summer in the northern hemisphere, there is a downward trend in the number of reports of influenza events in birds. Owing in part to the emergence of avian influenza A(H7N9) virus and infections of humans with this virus in China, there is enhanced surveillance for various subtypes of avian influenza in both humans and animals in China, the countries neighbouring China, and globally. It is therefore expected that more influenza A(H5) and A(H7) events in humans and animals will be detected and reported, as well as identification of infections with a variety of other subtypes and reassortants. Most infections that might be reported in humans will likely be mild and sporadic, and will not change the overall public health risk assessment. However, it is critical that these events be reported through the appropriate channels and viruses be collected and fully characterized in appropriate animal or human health influenza reference laboratories.

Because of the constantly evolving nature of influenza viruses, WHO continues to stress the importance of global monitoring of influenza viruses in animals and people and recommends that all Member States strengthen routine influenza surveillance. All human infections with non-seasonal influenza viruses are reportable to WHO under IHR (2005).

Relevant Links:

WHO human-animal interface web page

http://www.who.int/influenza/human_animal_interface/en/

Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO

http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/

H5N1 avian influenza: timeline of major events

http://www.who.int/influenza/human_animal_interface/avian_influenza/H5N1_avian_influenza_update.pdf

Avian influenza A(H7N9) information

http://who.int/influenza/human_animal_interface/influenza_h7n9/en/index.html

World Organisation of Animal Health (OIE) web page: Web portal on Avian Influenza

<http://www.oie.int/animal-health-in-the-world/web-portal-on-avian-influenza/>

Food and Agriculture Organization of the UN (FAO) webpage: Avian Influenza

<http://www.fao.org/avianflu/en/index.html>

OFFLU

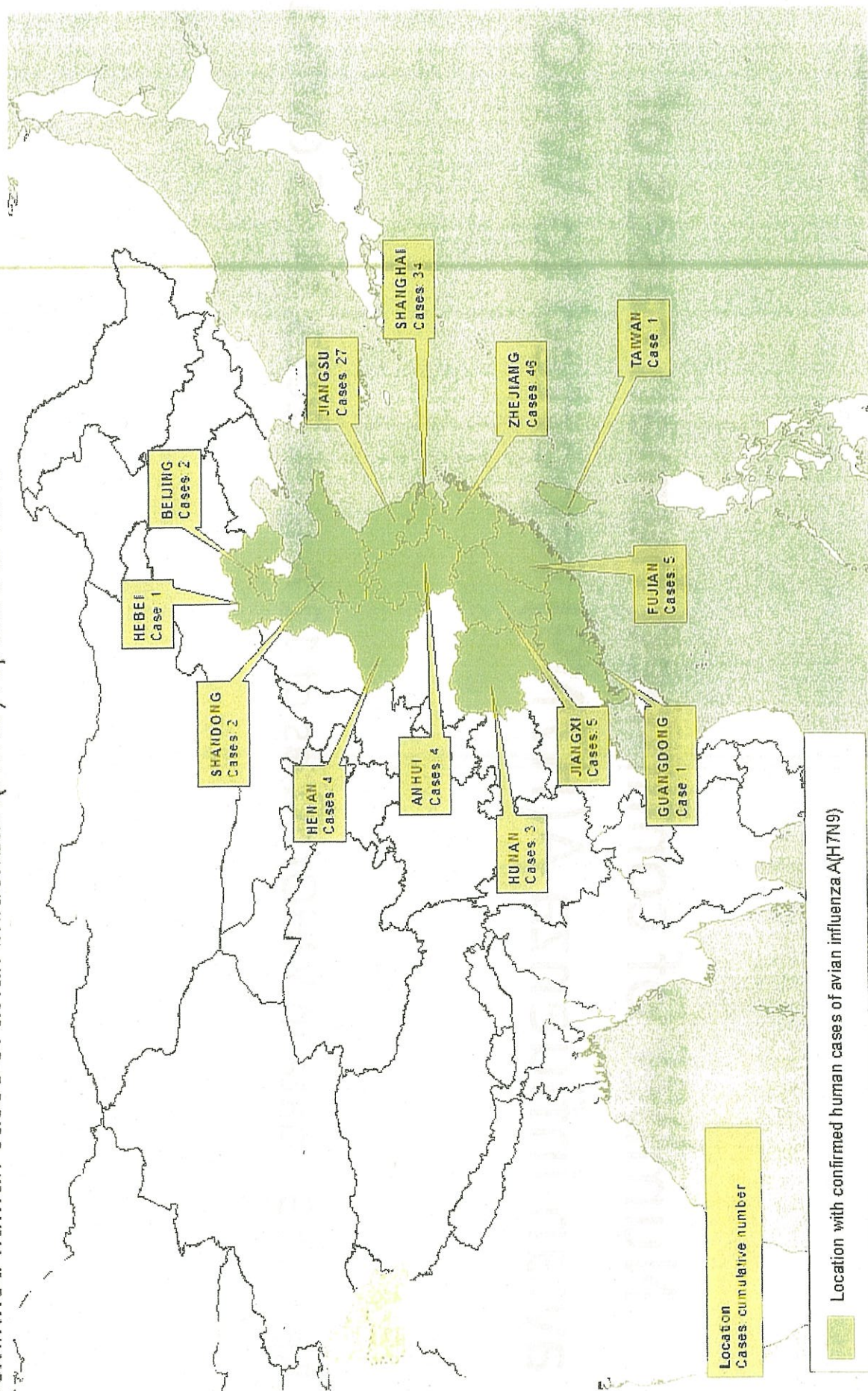
<http://www.offlu.net/index.html>

Number of confirmed human cases of avian influenza A(H7N9) reported to WHO

Report 9 - data in WHO/HQ as of 12 August 2013, 14:45 GMT+1

Geographical location

Confirmed human cases of avian influenza A(H7N9) reported to WHO



The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there is no definite information.

Data as of 12 August 2013, 14:45 GMT+1
Source: WHO/GIP

Cumulative number of confirmed cases of avian influenza A(H7N9) reported to WHO, by month, 2013

	February		March		April		May		June		July		unknown month of onset		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Total	2	2	30	12	88	7	3	0	0	0	2	0	10	23	135	44

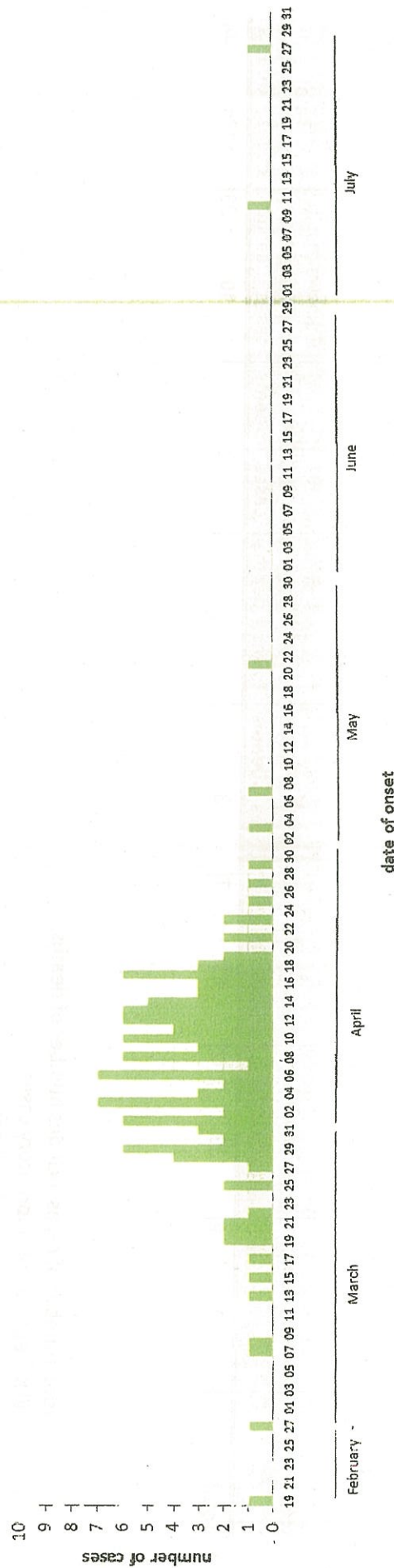
Total number of cases includes number of deaths
WHO reports only laboratory cases
All dates refer to onset of illness

Data in WHO/HQ as of 12 August 2013, 14:45 GMT+1

Source: WHO/GIP

Epidemiological curve of confirmed cases of avian influenza A(H7N9) reported to WHO, by day, 2013

N = 125 confirmed cases for whom date of onset is known



All dates refer to onset of illness

Data in WHO/HQ as of 12 August 2013, 14:45 GMT+1

Source: WHO/GIP



識別番号・報告回数	報告日	第一報入手日 2013年6月21日 該当なし	新医薬品等の区分 該当なし	機構処理欄
一般的名称	研究報告 の公表状 況	報告日	公表国 中国	使用上の注意記載状況・ その他参考事項等 特になし
販売名（企業名）	研究報告 の公表状 況	報告日	公表国 中国	使用上の注意記載状況・ その他参考事項等 特になし
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ORIGINAL ARTICLE

Preliminary Report: Epidemiology of the Avian Influenza A (H7N9) Outbreak in China

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ABSTRACT

BACKGROUND

The first identified cases of avian influenza A (H7N9) virus infection in humans occurred in China during February and March 2013. We analyzed data obtained from field investigations to characterize the epidemiologic characteristics of H7N9 cases in China as of April 17, 2013.

METHODS

Field investigations were conducted for each confirmed case of H7N9 virus infection. A patient was considered to have a confirmed case if the presence of the H7N9 virus was verified by means of real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR), viral isolation, or serologic testing. Information on demographic characteristics, exposure history, and illness timelines was obtained from patients with confirmed cases. Close contacts were monitored for 7 days for symptoms of illness. Throat swabs were obtained from contacts in whom symptoms developed and were tested for the presence of the H7N9 virus testing by means of real-time RT-PCR.

RESULTS

Among 82 persons with confirmed H7N9 virus infection, the median age was 63 years (range, 2 to 89), 73% were male, and 84% were urban residents. Confirmed cases occurred in six areas of China. Of 77 persons with available data, 4 were poultry workers, and 77% had a history of exposure to live animals, including chickens (76%). A total of 17 persons (21%) died after a median duration of illness of 11 days, 60 remain critically ill, and 4 with clinically mild cases were discharged from the hospital; 1 pediatric patient was not admitted to the hospital. In two family clusters, human-to-human transmission of H7N9 virus could not be ruled out. A total of 1251 of the 1689 close contacts of case patients completed the monitoring period; respiratory symptoms developed in 19 of them (1.5%), all of whom tested negative for the H7N9 virus.

CONCLUSIONS

Most persons with confirmed H7N9 virus infection were critically ill and epidemiologically unrelated. Laboratory-confirmed human-to-human H7N9 virus transmission was not documented among close contacts, but such transmission could not be ruled out in two families.

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THE FIRST IDENTIFIED CASES OF HUMAN infection with a novel influenza A (H7N9) virus occurred in eastern China during February and March 2013 and were characterized by rapidly progressive pneumonia, respiratory failure, acute respiratory distress syndrome (ARDS), and fatal outcomes.¹ We analyzed available data from field investigations to characterize the descriptive epidemiology of laboratory-confirmed cases of avian influenza A (H7N9) virus infection in humans reported to the Chinese Center for Disease Control and Prevention (China CDC) as of April 17, 2013. In this report, we summarize the preliminary findings of case investigations and follow-up monitoring of close contacts of persons with confirmed cases of H7N9 virus infection who have been identified to date. This is an ongoing investigation.

METHODS

CASE DEFINITIONS

The case definitions of suspected and confirmed human infection with H7N9 virus were based on the H5N1 case definitions, as recommended by the World Health Organization (WHO) in 2006 (Section S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).² The laboratory test assays for H7N9 virus that we performed have been described previously (Section S2 in the Supplementary Appendix).³

IDENTIFICATION OF CASES

Suspected cases of H7N9 virus infection were identified through the Chinese surveillance system for pneumonia of unexplained origin, which was established in 2004.⁴ Beginning on April 3, 2013, enhanced surveillance was implemented for suspected cases of H7N9 virus infection among persons with mild or moderate illness.⁵ Persons with suspected cases of H7N9 virus infection with mild or moderate illness were identified from the Chinese sentinel surveillance system for influenza-like illness, which has been described previously.⁶ Once each suspected case of H7N9 virus infection was identified, the local CDCs, including prefecture and provincial CDCs, conducted the initial field investigations and obtained respiratory specimens, which were shipped to the National Influenza Center of the China CDC in Beijing for H7N9 laboratory testing. A field investigation team comprising staff

members of the China CDC and or local CDC conducted field investigations of the confirmed cases of H7N9 virus infection.

DATA COLLECTION

It was determined by the National Health and Family Planning Commission that the collection of data from H7N9 case patients and their close contacts was part of a continuing public health investigation of an outbreak and was exempt from institutional review board assessment. Data were collected through a review of medical records and interviews with relatives, contacts, and health care workers who provided medical care for the case patients. We collected information on the dates of illness onset, visits to clinical facilities, hospitalization, and clinical outcomes. Epidemiologic data were collected through interviews and field observations and were reported to the China CDC. Investigators interviewed the relatives of each patient with a confirmed case of H7N9 virus infection to determine exposure histories during the 2 weeks before the onset of the illness, including the dates, times, frequency, and patterns of exposures to poultry or other animals such as swine and wild birds. All epidemiologic information that was collected during the field investigations, including exposure history, timelines of events, and identification of close contacts, was cross-validated, since we were unable to interview any critically ill H7N9 case patients. Households and places that were known to have been visited by the case patients in the 2 weeks before the onset of illness were investigated to assess exposures to poultry and swine, as well as environmental exposures.

IDENTIFICATION AND FOLLOW-UP OF CLOSE CONTACTS

We defined close contacts of patients with confirmed H7N9 virus infection as described previously for H5N1 field investigations⁷ (Section S3 in the Supplementary Appendix); once we identified the close contacts, we monitored them daily for 7 days for symptoms of illness and collected throat swabs from contacts in whom symptoms developed to test for the presence of the H7N9 virus. Antiviral chemoprophylaxis was not provided to close contacts. Paired serum samples were obtained from patients with suspected H7N9 virus infection who did not have respiratory specimens available for H7N9 serologic test-

ing. Oseltamivir treatment was recommended for close contacts in whom symptoms developed (Section 3 in the Supplementary Appendix). Data on demographic characteristics and exposure were collected for close contacts.

STATISTICAL ANALYSIS

We used descriptive statistics to summarize the epidemiologic characteristics and H7N9 testing results for persons with suspected cases of H7N9 virus infection, for those with confirmed cases, and for close contacts of those with confirmed cases. The methods we used for estimating the incubation period have been described previously.⁸

RESULTS

EPIDEMIOLOGIC CHARACTERISTICS OF CONFIRMED CASES

From March 25 through April 17, 2013, respiratory specimens from 664 hospitalized patients with pneumonia of unexplained origin were tested, and 81 patients (12.2%) were confirmed to be infected with the H7N9 virus. Of 5551 respiratory specimens obtained from outpatients with an influenza-like illness through the sentinel surveillance system for influenza-like illness, 1 (0.02%)

tested positive for the H7N9 virus. As of April 17, 2013, a total of 82 laboratory-confirmed cases of H7N9 virus infection and 2 suspected cases had been identified (Fig. 1 and 2); cases were identified in the following provinces: Shanghai (31 confirmed cases and 1 suspected case), Zhejiang (25 confirmed cases), Jiangsu (20 confirmed cases and 1 suspected case), Anhui (3 confirmed cases), Henan (2 confirmed cases) and Beijing (1 confirmed case). The median age of patients with confirmed H7N9 virus infection was 63 years (range, 2 to 89); 38 cases (46%) occurred in persons 65 years of age or older, and 2 (2%) were in children younger than 5 years of age, both of whom had clinically mild upper respiratory illness (see Fig. S1 in the Supplementary Appendix for the age distribution). Most confirmed cases occurred in males (73%), 84% of the case patients were urban residents, and 54 of 71 patients with available data (76%) had underlying medical conditions (Table 1). Among 46 of 54 case patients with sufficient data for a more specific classification of underlying conditions, 40 (87%) were considered to be at increased risk for influenza complications owing to age (<5 years or ≥65 years) or prevalence of certain underlying medical conditions.⁹ Four of the patients with confirmed cases

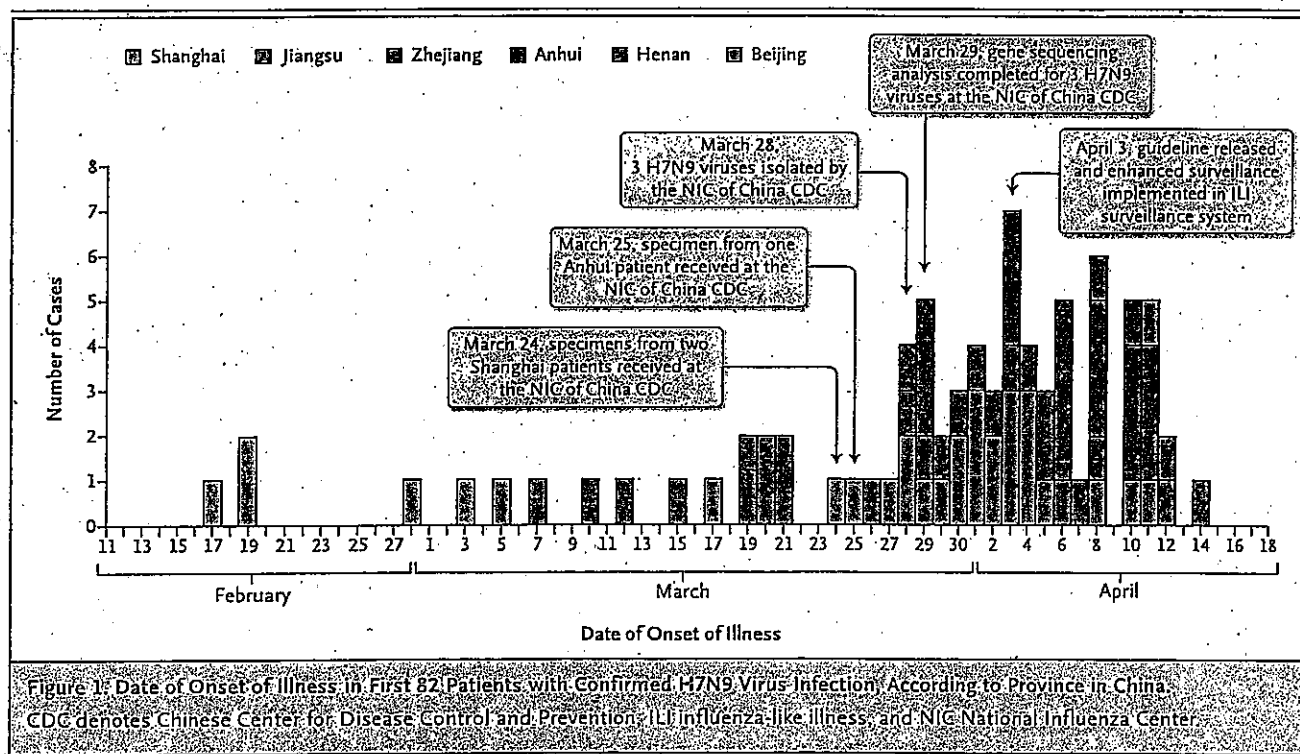
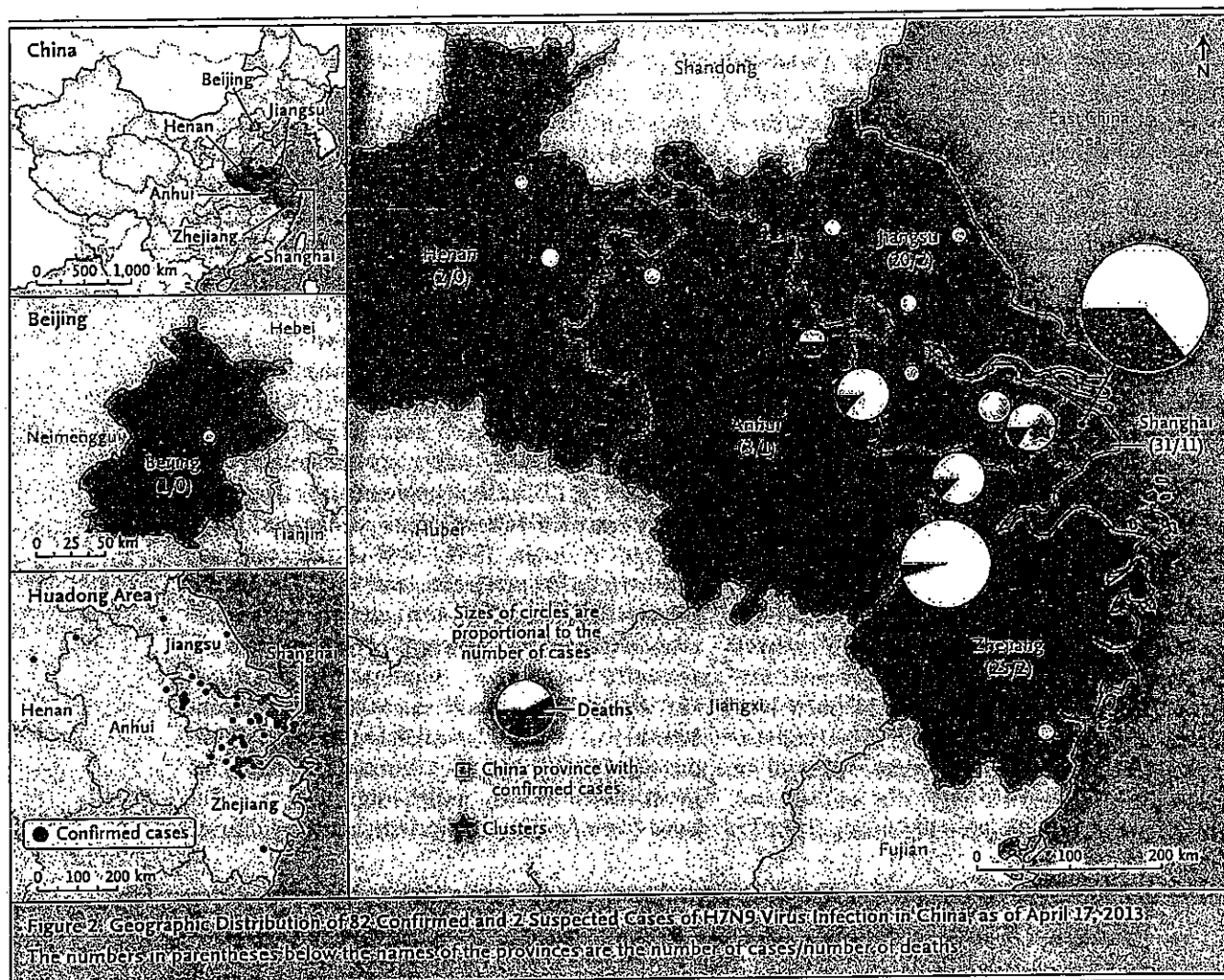


Figure 1. Date of Onset of Illness in First 82 Patients with Confirmed H7N9 Virus Infection, According to Province in China. CDC denotes Chinese Center for Disease Control and Prevention; ILI, influenza-like illness; and NIC, National Influenza Center.



(5%) worked as poultry workers: 3 slaughtered poultry at a live poultry market, and 1 transported live poultry.

A total of 81 of 82 patients with confirmed H7N9 virus infection (99%) were hospitalized. Among the 51 patients with confirmed cases for whom data were available, isolation precautions were instituted for 33 (65%) in an intensive care unit (ICU) because of severe lower respiratory tract disease. As of April 17, a total of 17 patients with confirmed H7N9 virus infection (21%) and 1 patient with suspected infection had died of acute respiratory distress syndrome (ARDS) or multiorgan failure, and 60 patients with confirmed cases and 1 with a suspected case remained critically ill; 4 with clinically mild cases had been discharged from the hospital, and 1 pediatric patient had not been admitted to the hospital. Among 82 confirmed cases of

H7N9 virus infection, 7 (9%) were confirmed by means of virus isolation, 2 (2%) by means of serologic testing, and 73 (89%) by means of nucleic acid detection. Viral culture of 73 respiratory specimens that were confirmed as positive by means of real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assays and diagnostic testing of specimens from suspected cases are ongoing.

Data on recent exposure to animals were available for 77 of the 82 patients with confirmed H7N9 virus infection. Of these, 59 (77%) reported a history of recent exposure to animals, (Table 1): 45 (76%) to chickens, 12 (20%) to ducks, and 4 (7%) to swine; the exposures occurred either while they were working at or visiting a live animal market. Other animals that these 59 patients reported having been exposed to included pigeons, geese, quail, wild birds, pet

Table 1. Epidemiologic Characteristics of 82 Patients with Confirmed H7N9 Virus Infection in China

Characteristic	Patients with Confirmed Cases (N=82)
Age — yr	
Median	63.0
Interquartile range	50–73
Age <5 yr — no. (%)	2 (2)
Age ≥65 yr — no. (%)	38 (46)
Male sex — no. (%)	60 (73)
Type of residence — no. (%)	
Urban	69 (84)
Rural	13 (16)
Poultry worker — no. (%)*	4 (5)
Presence of underlying medical conditions — no./total no. (%)†	54/71 (76)
Exposure to symptomatic case patient within 2 wk before illness onset — no./total no. (%)‡	5/79 (6)
History of exposure to animals — no./total no. (%)§	59/77 (77)
Chickens	45/59 (76)
Ducks	12/59 (20)
Pigeons	8/59 (14)
Quail	1/59 (2)
Geese	1/59 (2)
Pet birds	1/59 (2)
Wild birds	6/59 (10)
Swine	4/59 (7)
Cats	2/59 (3)
Dogs	1/59 (2)
Type of exposure to animals — no./total no. (%)	
Direct contact with poultry	34/59 (58)
Direct contact with swine	2/59 (3)
Visit to live poultry market	38/59 (64)
Method used for diagnosis of H7N9 — no. (%)	
Virus isolation	7 (9)
Nucleic acid detection	73 (89)
Serologic testing¶	2 (2)

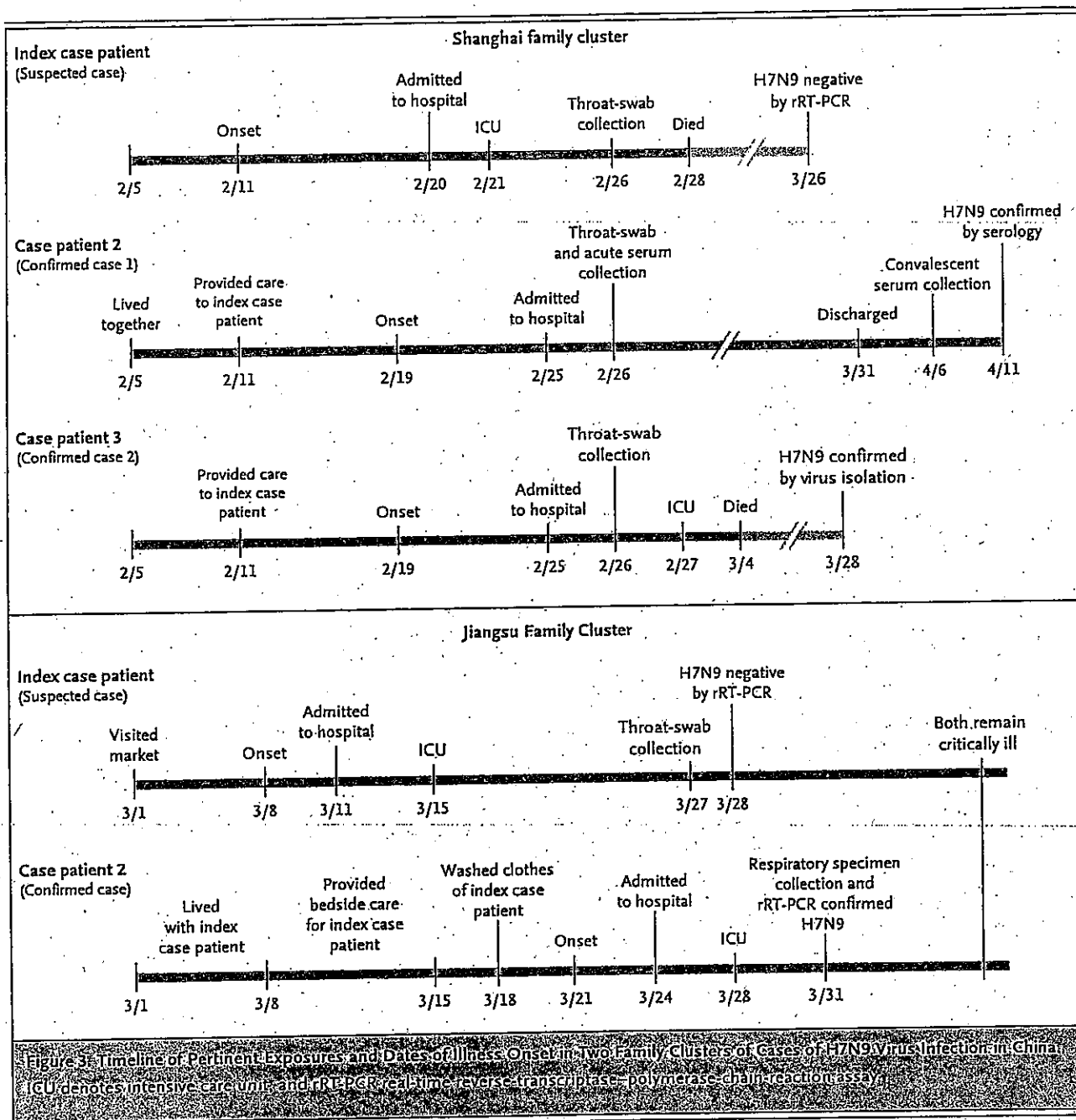
* Three poultry workers slaughtered poultry at a live poultry market, one each in Nanjing, Jiangsu province; Bozhou, Anhui province; and Zhoukou, Henan province. The other poultry worker transported live poultry in Shanghai.

† A total of 54 patients with confirmed H7N9 virus infection reported at least one underlying medical condition, including hypertension (31 patients), diabetes (14), heart disease (12), chronic bronchitis (7), hepatitis (4), smoking (4), rheumatic arthritis (4), rhinitis (2), and obstructive pulmonary emphysema, pulmonary emphysema, asthma, silicosis, cerebral infarction, lymphoma, thyroid cancer, rectal carcinoma, breast cancer, chronic nephropathy, gout, prostatitis, pregnancy, and alcoholism (1 patient each). For 11 of 82 patients with confirmed H7N9 virus infection (13%), collection of information on underlying medical condition is ongoing.

‡ Two family clusters were reported (see Section S4 in the Supplementary Appendix). One cluster included a father (with a confirmed case) and his two sons (one with a confirmed case and one with a suspected case) in Shanghai. Another cluster included a father (with a suspected case) and his daughter (with a confirmed case) in Jiangsu.

§ Among 59 case patients who reported recent exposure to animals, 52 (88%) reported a single exposure to bird or poultry, including chickens, ducks, geese, quail, pigeons, wild birds, and pet birds. The remaining 4 patients (6.8%) had multiple exposures to poultry and swine. Investigation of the history of exposure to animals is ongoing for 5 of 82 case patients (6%).

¶ The brother of the index case patient in the Shanghai family cluster and another patient in Shanghai were confirmed, with the use of a turkey red-cell hemagglutinin inhibition assay, to be seropositive for H7N9 virus antibodies, with an increase by a factor of 4 or more in antibodies in paired serum samples obtained during the acute and convalescent stages of illness.



birds, cats, and dogs. Information on a history of exposure to live animals is unclear for 5 patients with confirmed H7N9 virus infection, since the investigations are still ongoing. The estimated median incubation period in 23 patients with confirmed cases for whom detailed data on animal and environmental exposures was available was 6 days (range, 1 to 10) (Table S1 in the Supplementary Appendix).

FAMILY CLUSTERS

As of April 17, three family clusters had been identified in two provinces. Detailed exposure and timeline information are available for two family clusters that were identified early (Fig. 3, and Section S4 in the Supplementary Appendix); the investigation of one cluster in Shanghai is still ongoing. The first family cluster comprised two persons with confirmed H7N9 virus infec-

tion and one with a suspected infection. The index case patient and his father (confirmed case 2) lived in the same house, and the index patient's brother (confirmed case 1) lived with his wife nearby. After the index case patient (suspected case 1) became ill, his brother (confirmed case 1) and his father (confirmed case 2) had prolonged, close, unprotected contact with him, including eating together, providing care, and accompanying him to seek medical care before his hospitalization (Section S4 in the Supplementary Appendix). None of the three members of the cluster raised poultry or other animals, none brought live poultry into their home, and none had direct contact with sick or dead poultry. The index case patient had visited a live poultry market, purchased a chicken, observed the slaughtering process, brought the freshly killed chicken home, and prepared, cooked, and ate the chicken within 2 weeks before the onset of his illness.

Another family cluster was identified that included one person with confirmed H7N9 virus infection and one person with a suspected case (Fig. 2, and Section S4 in the Supplementary Appendix). After the father (suspected case) became ill, his daughter (confirmed case) had prolonged, close, unprotected contact with him, including eating together, providing care, and accompanying him to seek medical care before his hospital admission. She also provided unprotected bedside hospital care for her father during the period from March 11 through March 15. Diarrhea developed in the father, and the daughter washed her father's diarrhea-soiled underwear on March 18 while wearing gloves. The father had visited a live poultry market 7 days before the onset of his illness. The daughter did not raise poultry or animals at home and had not had any exposures to animals (i.e., had not brought live poultry into the home or visited a live poultry market or had any direct or indirect contact with poultry or pigs).

MEDICAL CARE TIMELINES

Among the 81 patients with confirmed H7N9 virus infection for whom data were available (99% of the 82 patients with confirmed cases), the median time from the onset of illness to the first medical visit was 1 day, and patients were hospitalized a median of 4.5 days after the onset of illness (Table 2). Among 64 of the patients with available data on oseltamivir administration, 41 (64%) received oseltamivir treatment beginning a median of 6 days after the onset of illness. ARDS

Table 2. Clinical Characteristics and Medical Care Timelines for 82 Patients with Confirmed H7N9 Virus Infection in China

Variable	Patients with Confirmed Cases (N=82)
Clinical outcome — no./total no. (%)	
Hospitalization†	81/82 (99)
ICU admission	33/51 (65)
ARDS	19/40 (48)
Death	17/82 (21)
Oseltamivir treatment — no./total no. (%)	41/64 (64)
Time from illness onset to oseltamivir treatment — days	
Median	6.0
Interquartile range	4.0–8.0
Time from illness onset to first medical care — days	
Median	1.0
Interquartile range	0–2.0
Time from illness onset to hospitalization — days	
Median	4.5
Interquartile range	3.0–6.0
Time from illness onset to ICU admission — days	
Median	7.0
Interquartile range	5.0–9.0
Time from illness onset to development of ARDS — days	
Median	8.0
Interquartile range	5.0–10.0
Time from illness onset to death — days	
Median	11.0
Interquartile range	7.0–20.0

* In the case of some characteristics, complete data were not available for all 82 patients with confirmed H7N9 virus infection. Case investigations are ongoing. ARDS denotes acute respiratory distress syndrome, and ICU intensive care unit.

† Four patients with confirmed cases had been discharged as of April 17, 2013. One 2-year-old male child with a confirmed case in Shanghai was not hospitalized, because his illness was mild; he recovered 5 days after the onset of illness.

developed during the course of hospitalization in 19 of 40 patients with confirmed H7N9 virus infection for whom data were available (48%) after a median of 8 days, and 17 patients died a median of 11 days after the onset of illness.

CLOSE CONTACTS

As of April 17, data were available for 1689 close contacts of the 82 patients with confirmed cases in Shanghai (435 contacts), Jiangsu (448), Zhejiang (676), Anhui (100), Henan (28), and Beijing (2). Among the 678 close contacts of 33 patients

with confirmed cases for whom demographic information was available, 422 (62%) were health care workers, 134 (20%) were family member or relatives, and 122 (18%) were social contacts. As of April 17, a total of 1251 of the 1689 contacts had been followed for up to 7 days; among these close contacts (not including those in the family clusters), respiratory symptoms developed during the 7-day surveillance period in 19 (1.5%): 2 household members, 1 medical intern, 1 patient who shared a room with a confirmed case patient, and 15 health care workers (Section S5 in the Supplementary Appendix). All the throat swabs collected from these 19 ill contacts a median of 1 day (range, 0 to 7) after the onset of illness were negative for the H7N9 virus, as assessed by means of real-time RT-PCR.

DISCUSSION

An epidemiologic study of 82 confirmed cases of H7N9 virus infection in China among persons with illness onset during the period from February through April 17, 2013, indicates that the infection affected persons in a wide age spectrum and caused severe lower respiratory tract illness. To date, the mortality is 21%, but since many of patients with confirmed H7N9 virus infection remain critically ill, we suspect that the mortality may increase. Except for one family cluster with 2 confirmed cases, patients with confirmed H7N9 virus infection were epidemiologically unrelated and were identified in six areas of China. Most of the patients with confirmed H7N9 virus infection were considered to be at increased risk for complications from influenza owing to age (<5 years or ≥65 years; median age of patients with confirmed cases, 63 years) or the prevalence of certain underlying medical conditions.

Human infections with influenza A (H7) viruses have been reported sporadically and are usually associated with exposures to poultry.¹⁰⁻¹² Previous human cases of H7 virus infection have been characterized by mild illness (conjunctivitis or uncomplicated influenza) or moderate illness (lower respiratory tract disease) that results in hospitalization.^{10,11,13,14} Only one fatal case of H7 virus infection has been reported previously; that case occurred in an adult with a highly pathogenic avian influenza A (H7N7) virus infection.¹⁵ Many of the confirmed H7N9 case patients had critical and fatal illness, suggesting

that the H7N9 virus is more virulent in humans than are other H7 viruses. The H7N9 case fatality proportion to date is lower than that for reported cases of H5N1 virus infection.¹⁶ However, early surveillance for H7N9 cases was focused on case finding for severe lower respiratory tract illness, and since April 3, expanded testing of outpatients with influenza-like illness has identified some mild cases of illness with H7N9 virus infection. Enhanced surveillance for less severe illness with H7N9 virus infection will help to determine the clinical spectrum of the illness and the total number of cases of H7N9 symptomatic illness and to inform an understanding of the true case fatality proportion. Since this H7N9 virus appears to have emerged recently to infect humans, population immunity is expected to be low, and persons of any age may be susceptible to infection.

Although the source of the H7N9 virus infection in patients with confirmed cases who had exposure to animals cannot be verified without extensive H7N9 testing of animals, we suspect that it is likely to be infected poultry; additional studies are needed. No animal outbreaks were identified in the areas with confirmed H7N9 cases, but 77% of cases with available data occurred in patients who had exposure to live animals such as poultry or swine, including during visits to live animal markets. This raises the possibility of zoonotic H7N9 virus transmission from healthy-appearing swine or poultry to humans through direct or close contact or through exposure to environments that are contaminated with infected swine or poultry. For example, visiting a live poultry market, where avian influenza A viruses can be maintained and amplified, has been identified as a risk factor for H5N1 virus infection in Hong Kong¹⁷ and urban China.^{18,19} However, case-control studies are needed to identify risk factors for H7N9 virus infection. Until the source of H7N9 virus infection is known, implementation of control measures at live poultry markets, such as a ban on the selling of live poultry in market stalls or even market closure, poultry culling, and market disinfection — measures that have been taken to control the spread of H5N1 virus — may be considered in order to help control potential zoonotic transmission of H7N9 virus.

Follow-up prospective investigations of close contacts of patients with confirmed H7N9 virus

infection have not conclusively established human-to-human H7N9 transmission of the virus from one confirmed case to another to date. However, in two family clusters, limited human-to-human transmission of H7N9 virus after close, prolonged, unprotected contact with a symptomatic patient with suspected H7N9 virus infection remains a possibility, because specimens were not available for H7N9 testing from patients with suspected cases; one patient with a suspected case died before a specimen could be obtained, and H7N9 testing of the other patient with a suspected case is still ongoing. Similar family clusters of H5N1 cases that occurred after common poultry exposures or limited human-to-human transmission have been identified.^{7,20,21} Paired serum samples are being obtained during the acute and convalescent stages of illness from contacts of case patients for further assessment of the potential for secondary human-to-human H7N9 virus transmission, including the identification of asymptomatic infections. Although the risk of human-to-human transmission of H7N9 virus appears to be low, the actual risk is currently unknown, and the Chinese national guidelines recommend implementing control measures, such as prompt isolation of the patient, active monitoring of close contacts, and implementation of standard, contact, and droplet precautions by health care personnel in hospitals. In addition, national guidelines recommend that antiviral treatment with oseltamivir should be administered as soon as possible in patients with suspected or confirmed cases of H7N9 virus infection.

The median time from the onset of illness to hospitalization among the 81 of 82 patients with confirmed H7N9 virus infection for whom data on hospitalization were available was 4.5 days, and the median time from the onset of illness to the development of ARDS among the 19 case patients with ARDS (out of 40 patients for whom data on ARDS were available) was 8 days; the corresponding median times among patients with H5N1 virus infection were 7 days and 7.5 days.²² The median duration from the onset of illness to death among the 17 persons with confirmed cases who died was 11 days. The initial findings suggest that H7N9 virus infection can cause critical illness and fatal disease and may affect persons in a wider age range than the H5N1 virus has in China to date (Fig. S1 in the

Supplementary Appendix). Patients with confirmed cases received oseltamivir antiviral treatment a median of 6 days after the onset of illness (median before April 3, 9 days), probably owing to delayed suspicion of influenza. Retrospective observational studies of influenza A (H1N1)pdm09 and H5N1 virus infections suggest that early oseltamivir treatment probably has the greatest clinical benefit but that starting treatment up to 5 days after the onset of illness may still reduce the risk of critical illness and death.²³⁻²⁷ Preliminary data suggest that the H7N9 viruses isolated from humans and analyzed to date are resistant to adamantane antiviral agents and are susceptible to neuraminidase inhibitors. Early clinical suspicion of H7N9 virus infection and early administration of oseltamivir may help to reduce the severity of the disease.

Our study had several limitations. First, we did not collect detailed information from all patients on exposures, such as the times, frequency, intensity, and duration of exposures. Information on exposures is useful for estimating the incubation period after possible exposure to animals or live-animal markets and for evaluating the risk factors for H7N9 virus infection. Second, we may not have identified all the close contacts of case patients and were not able to conduct active follow-up of all contacts. As of April 17, 2013, complete follow-up data were not available for some of the close contacts. Third, we did not have a standard protocol and questionnaire to collect information from all contacts of the first 82 patients with confirmed cases. However, the China CDC has issued a guideline and protocol for field investigations of case patients and close contacts and since April 1 has provided training for personnel at all 31 provincial CDCs. This will help ensure standard data collection. Fourth, specimens were not available for H7N9 testing from some patients with suspected cases. Clinical outcomes in the 82 patients with confirmed H7N9 virus infection are reported as of April 17, 2013, and 60 case patients remain hospitalized. Paired serum samples have not been obtained from some of the contacts; no serologic testing results are available at this time, and given the fact that it is early in the investigation, more time is needed to allow for a humoral immune response in serum obtained during the convalescent period and to allow time for serologic testing to be performed.

In summary, a novel influenza A (H7N9) virus has caused severe and fatal illness in persons in six different areas of China to date. Some clinically mild cases have been identified since the surveillance was widened, suggesting that there is a wide clinical spectrum of H7N9 virus infection. The initial epidemiologic findings suggest that most confirmed H7N9 cases were epidemiologically unrelated. Follow-up investigations of contacts of patients with confirmed H7N9 virus infection suggest that the risk of secondary H7N9 virus transmission, including to health care personnel, is low at this time. However, in two family clusters that include persons with confirmed H7N9 virus infection and persons with epidemiologically linked suspected cases, limited nonsustained human-to-human H7N9 virus transmission could not be ruled out and may have occurred among blood-related family members. Enhanced surveillance for severe and mild human illness with H7N9 virus infection is needed to determine the clinical spectrum of the infection and the total number of symptomatic H7N9 infections. Case-control studies to iden-

tify risk factors and continued investigations of case patients and their contacts are indicated. Data from investigations of potential animal and environmental sources are urgently needed to inform public health control measures.

The views expressed in this article are those of the authors and do not represent the official policy of the Chinese Center for Disease Control and Prevention or the U.S. Centers for Disease Control and Prevention.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix

Preliminary Report: Epidemiology of Avian Influenza A (H7N9) Virus Outbreak in China

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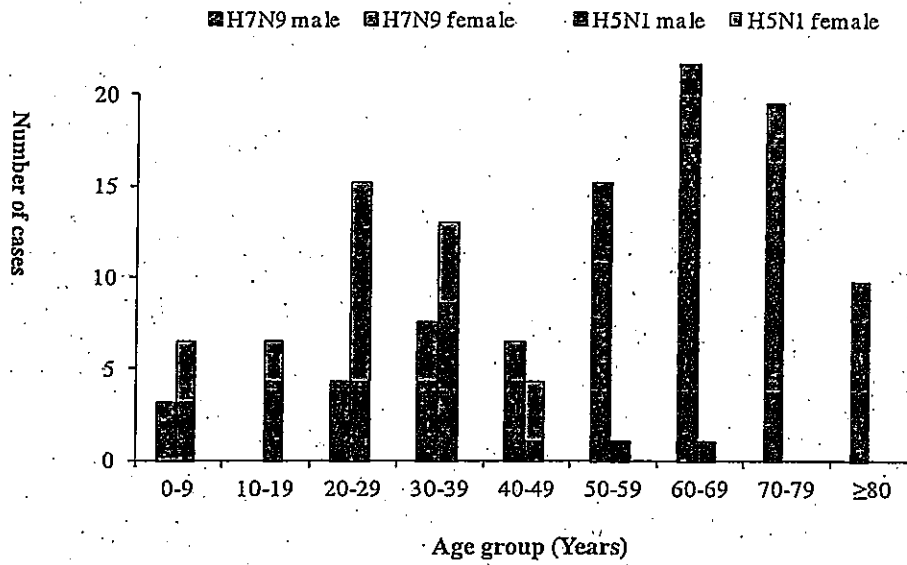
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Figure S1. Age and gender distribution of 82 confirmed H7N9 cases as of April 17, 2013, compared with 44 confirmed H5N1 cases in China*



* Unpublished data, China CDC 2013.

Table S1. Estimated incubation period for 23 human cases of avian influenza A (H7N9) virus infection, China *

Exposure data	Case-patients with exposure to market and poultry	Case-patients with exposure to market only	Case-patients with exposure to poultry only	Case-patients with exposure to suspected case only	All case-patients
No. case-patients with exposures on multiple days	4	1	4	2	11
Overall median incubation period, d (range)	5.5 (5.5-5.5)	5.5	5.5 (5.5-5.5)	5.25 (4-6.5)	5.5 (4-6.5)
Median of minimum incubation period, d (range)	1 (1-1)	1	1 (1-1)	1.5 (0-3)	1 (0-3)
Median of maximum incubation period, d (range)	10 (10-10)	10	10 (10-10)	9 (8-10)	10 (8-10)
No. case-patients with single known exposure	-	10	2	-	12
Overall median incubation period, d (range)	-	6 (1-10)	4 (1-7)	-	6 (1-10)
All case-patients	4	11	6	2	23

Overall median incubation period, d (range)	5.5 (5.5-5.5)	6 (1-10)	5.5 (1-7)	5.25 (4-6.5)	5.5 (1-10)
Overall median of minimum incubation period, d (range)	1 (1-1)	6 (1-10)	1 (1-1)	1.5 (0-3)	1 (0-10)
Overall median of maximum incubation period, d (range)	10 (10-10)	6 (1-10)	10 (1-10)	9 (8-10)	7.5 (1-10)

* The method of estimating incubation periods for H7N9 was based upon methods used for H5N1 as previously described.¹

Section S1. Case definitions

The case definitions for suspected and confirmed human infections with novel avian influenza A (H7N9) virus were based upon the H5N1 case definitions as recommended by the World Health Organization (WHO) in 2006.² A suspected H7N9 case was defined as a person presenting with unexplained acute lower respiratory illness with fever ($\geq 38^{\circ}\text{C}$) and cough, shortness of breath or difficulty breathing or infiltrates or evidence of an acute pneumonia on chest radiograph plus evidence of respiratory failure (hypoxemia, severe tachypnea), and (a) positive laboratory confirmation of an influenza A virus infection but insufficient laboratory evidence for H7N9 virus infection because of lack of specimens or (b) epidemiologically-linked to a confirmed H7N9 case, but without any respiratory specimens available for H7N9 testing. As of April 3rd, 2013, specimens from outpatients with influenza-like illness (ILI) presenting to sites of the Chinese ILI-sentinel surveillance system were tested for H7N9 virus using rRT-PCR assay. A confirmed H7N9 case was defined as a patient with ILI or a suspected case with respiratory specimens that tested positive for H7N9 virus by any of the following: isolation of H7N9 virus or positive results by real-time reverse transcription polymerase chain reaction (rRT-PCR) assay for H7N9, or a fourfold or greater rise in antibody titer for H7N9 virus based on testing of an acute serum specimen (collected 7 days or less after symptom onset) and a convalescent serum specimen collected at least two weeks later (Supplementary Appendix 2)

Section S2. H7N9 laboratory test assays

RNA was extracted from specimens with the RNeasy mini kit (Qiagen, Valencia, CA, USA) as per the manufacturer's protocol and tested by real-time RT-PCR with H7N9-specific primers and probes as previously described.³ The specific sequences have been published on the WHO website at

http://www.who.int/influenza/gisrs_laboratory/a_h7n9/en/. These assays were done in biosafety level (BSL) 3 facilities at Provincial CDC and NIC of China CDC.

Respiratory specimens were inoculated in amniotic cavities of pathogen-free embryonated chicken eggs for viral isolation in enhanced BSL 3 facilities at the NIC of China CDC.

H7N9 serological testing was done by modified hemagglutinin inhibition assay using turkey red-blood-cells in BSL 2 conditions at the NIC of China CDC. Antigens for the assays were produced from the A/Anhui/1/2013(H7N9) virus isolated from the confirmed case in Anhui.³ An individual was considered to be seropositive for H7N9 if a four-fold or greater rise in H7N9 virus antibody was detected by testing paired acute and convalescent sera.

Section S3. Close contact identification and follow-up

We defined close contacts of confirmed H7N9 cases as described previously for H5N1 field investigations.⁴ Close contacts were defined as individuals known to have been within 1 m, or had direct contact with respiratory secretions or faecal material, of a patient with confirmed H7N9 virus infection any time from the day before the onset of illness to when the case was isolated in the hospital. Antiviral chemoprophylaxis was not provided to close contacts. Close contacts were monitored daily for seven days by telephone or in-person for development of fever or any respiratory symptoms after their last known exposure to a confirmed H7N9 case-patient. Throat swabs were collected from close contacts with fever or any respiratory illness symptoms on a median 1 day (range: 0-7) after their illness onset and placed into sterile viral transport media for H7N9 virus testing at the China CDC. Symptomatic close contacts were recommended to receive oseltamivir treatment if oseltamivir was available.

Demographic information and data were collected on use of personal protective equipment, use of oseltamivir, illness symptoms, and potential H7N9 virus exposures (eg, poultry contact, visiting poultry markets, contact with wild or pet birds, and contact with individuals with febrile respiratory symptoms) during the 2 weeks before the last known exposure to an ill confirmed H7N9 case-patient.

Section S4. Two family cluster investigations

One family cluster was identified with two confirmed and one suspected H7N9 cases.

The confirmed cases were a 69-year-old man and his father, an 87-year-old retired man, a resident of Shanghai City. The suspected case, aged 57 years, was the first ill person in the household and developed high fever (maximum temperature 41 °C), cough, sputum production, chills and nausea on Feb 11th (Figure 2). After the suspected case became ill, his brother (confirmed case 1) and the father (confirmed case 2) had close contact with him, including eating together, providing care, and accompanying him to seek medical care before his hospitalization. The suspected case was hospitalized on Feb 20th, diagnosed with pneumonia, placed on mechanical ventilation and isolated in an ICU the next day. He died of ARDS and multi-organ failure on Feb 28th. The throat swab collected from him on Feb 26th tested negative for H7N9, and no specimens were available for H7N9 testing because of his death.

Before his isolation in the hospital, the two confirmed cases had close contact with him without use of any personal protective equipment. Confirmed case 1 developed fever (40 °C), cough, sputum production, nausea and vomiting on Feb 19th, was admitted to hospital on Feb 25th with a diagnosis of pneumonia, and was isolated the next day. A throat swab collected from him on Feb 26th tested negative for H7N9. He improved and was discharged on Mar 13th. Paired serum specimens collected 7 and 46 days after illness onset were tested by turkey red blood cell hemagglutinin inhibition assay and demonstrated a four-fold rise in antibody titer (HAI titer <10 and 80, respectively), indicative of serological confirmation of H7N9 virus infection. The

87-year old father (confirmed case 2) developed cough and sputum production on Feb 19th and had high fever (40.2 °C) on Feb 24th, was admitted to hospital on Feb 25th with a diagnosis of pneumonia, and isolated the next day. A throat swab collected on Feb 26th tested positive for H7N9 virus. He died on Mar 4th. The suspected case and his father (confirmed case 2) lived together and the brother (confirmed case 1) lived with his wife nearby. All three cases did not raise poultry or other animals, and did not bring live poultry into their home. None of the three cases in this family cluster had any direct contact with sick or dead poultry. The suspected case had visited a live poultry market, purchased a well-appearing chicken, observed the slaughtering process, brought the freshly killed chicken home, prepared, cooked and ate the chicken within 2 weeks before his illness onset, but the exact exposure date was unclear because he was severely ill and died before he could be interviewed.

Another family cluster was identified with one confirmed and one suspected case. The confirmed H7N9 case was the daughter of a suspected case and lived together with her father, mother, husband and son in the same house. Her father, the suspected case, was a 60-year-old man with hypertension, who developed fever, cough and shortness of breath on March 8th, and was admitted to hospital three days later and isolated in an ICU on Mar 15th (Figure 2). The daughter, a 32-year-old woman without any underlying medical conditions, developed illness onset on Mar 21st with fever (40 °C) and cough, was hospitalized on Mar 24th, and isolated in an ICU on Mar 28th. Testing of tracheal secretion specimens collected from the daughter on Mar 31st confirmed

H7N9 virus infection. The father's throat swab collected on Mar 27th was positive for influenza A but negative for H7N9. Specimen collection and H7N9 testing is still ongoing. After the father became ill, his daughter (confirmed case) had close contact with him, including eating together, providing care, and accompanying him to seek medical care before his hospital admission. She also provided unprotected bedside hospital care for her father during March 11-15. After isolation, the father developed diarrhea. The confirmed case had washed her father's diarrhea-soiled underwear on March 18th while wearing gloves. The father (suspected case) had visited a live poultry market within 2 weeks before his illness onset but the exact date of exposure is unclear. The confirmed case did not raise poultry or animal at home, and had not had any animal exposures (had not brought live poultry into home or visited a wet poultry market or had any direct or indirect contact with poultry or pigs). Both the father (suspected case) and daughter (confirmed case) remain critically ill in an ICU.

Section S5. Descriptions of 19 ill close contacts during the 7-day surveillance period

As of April 17, 1251 of 1689 close contacts of confirmed H7N9 cases had completed a 7-day observation period. Among them, except for the three family clusters, 19 of 1251 (1.5%) close contacts (two household members, one medical intern, one patient of same ward, and 15 health care workers) developed acute respiratory symptoms or weakness or upper respiratory illness during the 7-day surveillance period. One ill household member, the husband of a confirmed case in Jiangsu, developed fever (37.5 °C) on the same day of the last known exposure to a confirmed case, and recovered on the 10th day after symptom onset. The other one ill household member, niece of a confirmed case in Jiangsu, developed fever (37.3 °C) on the second day of the last known exposure to a confirmed case. The intern was the doctor of a confirmed case in Anhui and provided care for the case in ICU while wearing personal protective equipment, including N95 respirator, gloves and gowns. He developed fever on the last known day of exposure to the confirmed case, and recovered the next day. One patient hospitalized in the same room with a confirmed case in Zhejiang developed slight fever after exposed to the confirmed case. Another 15 health care worker contacts that provided care for a confirmed case in Zhejiang developed cough and sore throat during the 7-day monitoring period. Throat swabs collected from the 19 ill contacts on a median 1 day (range: 0-7) after their illness onset tested negative for H7N9 by real-time RT-PCR.

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一般的名称	新鮮凍結人血漿	研究報告の公表状況	Zhou J, Wang D, Gao R, et al. Nature. 2013 Jul 25;499(7459):500-3. doi: 10.1038/nature12379. Epub 2013 Jul 3.	公表国 中国	使用上の注意記載状況・ その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分 採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)	○新型鳥インフルエンザA(H7N9)ウイルスの生物学的特徴 中国で発生した新型鳥インフルエンザH7N9ウイルスは、遺伝子配列解析により、ヒトに感染する能力を示すいくつかの特徴を備えていることが分かり、パンデミックの脅威が懸念される。新型インフルエンザウイルスがパンデミックを起こす鍵となる第一の過程は、α2,6結合(ヒト型)シアル化グリカンへの優先的な血球凝集受容体結合であることが認められているため、新型H7N9ウイルスの受容体結合能を調べたところ、トリ型(α2,3)及びヒト型(α2,6)双方の受容体に結合した。ヒトの上気道組織と気管には主にα2,6受容体が見られるが、肺組織にはα2,3及びα2,6結合シアル酸が混在する。ヒト気道のこれらの受容体への結合能を調べるため、気管及び肺の細胞にH7N9ウイルスを感染させた。その結果、ヒトの下気道上皮細胞及びH1N1型肺上皮細胞がH7N9ウイルスに感受性を有しており、感染24時間後には透過型電子顕微鏡により細胞外ウイルス粒子も観察され、培養上清中のウイルス量から、効率的なウイルス増殖も証明された。また、H5N1ウイルスの重篤なヒト感染と関連すると考えられているケモカイン及びサイトカインについて、H7N9に感染した7人の急性期血清における濃度を調べたところ、IP-10、MIG、MIP-1β、MCP-1、IL-6、IL-8及びIFN-αの濃度が健常対照群と比較し有意に高かった。 ヒトは新型H7N9ウイルスに感受性があり、現在の季節性インフルエンザワクチンにはH7N9ウイルスに対する保護効果を持たなかった。効率的なヒト-ヒト間感染は発生していないが、H7N9ウイルスはトリからヒトへの感染性は高いと考えられる。パンデミックの可能性を含め、H7N9ウイルスの脅威を過小評価するべきではなく、集中的な監視が必須である。			
研究報告の概要	報告企業の意見 新型鳥インフルエンザH7N9ウイルスは、ヒト型とトリ型受容体の双方に対する受容体結合能を有し、またヒトの気管及び肺の細胞において効率的に増殖することが明らかとなり、これらの特徴からヒトへの感染性は高いと考えられ、パンデミックの可能性を含めた監視が必須であるとの報告である。 今後の対応 日本赤十字社では、問診で発熱などの体調不良者を献血不適としている。また、平成21年5月18日付薬食血発第0518001号「新型インフルエンザの国内発生に係る血液製剤の安全性確保について」に基づき、新型インフルエンザの患者又は罹患者の疑いのある患者と7日以内に濃厚な接触があった人の献血を制限するほか、献血後に新型インフルエンザと診断された場合には当該製剤の回収と医療機関への情報提供を行うこととしていたが、平成21年度第4回血液事業部会運営委員会において、献血血液を通じて新型インフルエンザに感染する可能性が極めて低いことが示されたことから、平成22年3月5日に廃止された。今後も引き続き続き情報の収集に努める。				

LETTER

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Biological features of novel avian influenza A (H7N9) virus

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Human infection associated with a novel reassortant avian influenza H7N9 virus has recently been identified in China¹. A total of 132 confirmed cases and 39 deaths have been reported². Most patients presented with severe pneumonia and acute respiratory distress syndrome^{3,4}. Although the first epidemic has subsided, the presence of a natural reservoir and the disease severity highlight the need to evaluate its risk on human public health and to understand the possible pathogenesis mechanism. Here we show that the emerging H7N9 avian influenza virus poses a potentially high risk to humans. We discover that the H7N9 virus can bind to both avian-type (α 2,3-linked sialic acid) and human-type (α 2,6-linked sialic acid) receptors. It can invade epithelial cells in the human lower respiratory tract and type II pneumonocytes in alveoli, and replicated efficiently in *ex vivo* lung and trachea explant culture and several mammalian cell lines. In acute serum samples of H7N9-infected patients, increased levels of the chemokines and cytokines IP-10, MIG, MIP-1 β , MCP-1, IL-6, IL-8 and IFN- α were detected. We note that the human population is naive to the H7N9 virus, and current seasonal vaccination could not provide protection.

In February 2013, a novel avian influenza H7N9 virus emerged in China and was found to infect humans¹. As of 31 May 2013, a total of 132 human infections and 39 fatal cases have been identified². Most cases have occurred among elders who were considered to be at increased risk for influenza complications because of age immunity and/or underlying medical conditions⁴. In contrast to other previous human H7 infections that mainly manifested mild illness or conjunctivitis, most H7N9 patients were critically ill and presented severe pneumonia and acute respiratory distress syndrome^{1,3,4}. Sequence analysis of the avian influenza A (H7N9) virus indicates that it possesses several genetic features contributing to its ability to infect humans, and raises concerns about the pandemic potential¹. Therefore, to evaluate its risk on human public health and understand the possible pathogenesis mechanism, we investigated the biological features of the H7N9 virus and the host immunity of infected patients as well as the general human population.

It is generally accepted that haemagglutinin-receptor-binding preference to α 2,6-linked (human-type) sialylated glycans is the initial key step for a novel influenza-virus-causing pandemic. First, we tested the receptor-binding property of the novel H7N9 viruses (A/Shanghai/1/2013 (SH1), A/Shanghai/2/2013 (SH2), and A/Anhui/1/2013 (AH1)) using glycan dose-dependent binding assays as described previously⁵. We discovered that the viruses bound to both avian-type (α 2,3) and human-type (α 2,6) receptors, whereas avian influenza H5N1 (A/Anhui/01/2005) and pandemic H1N1 (A/California/07/2009) viruses preferentially bound to α 2,3 and α 2,6 receptors respectively, as expected (Fig. 1). The enhanced

α 2,6-binding of H7N9 may attribute to single or multiple substitutions in haemagglutinin including Ala138Ser in SH1 or Gly186Val and Gln226Leu in both SH2 and AH1, as reported elsewhere^{6–11}. The presence of alanine at residue 160 in haemagglutinin of all three viruses resulted in a glycosylation loss and had been observed to increase the affinity of the H5N1 virus for human-type receptors^{12,13}. Furthermore,

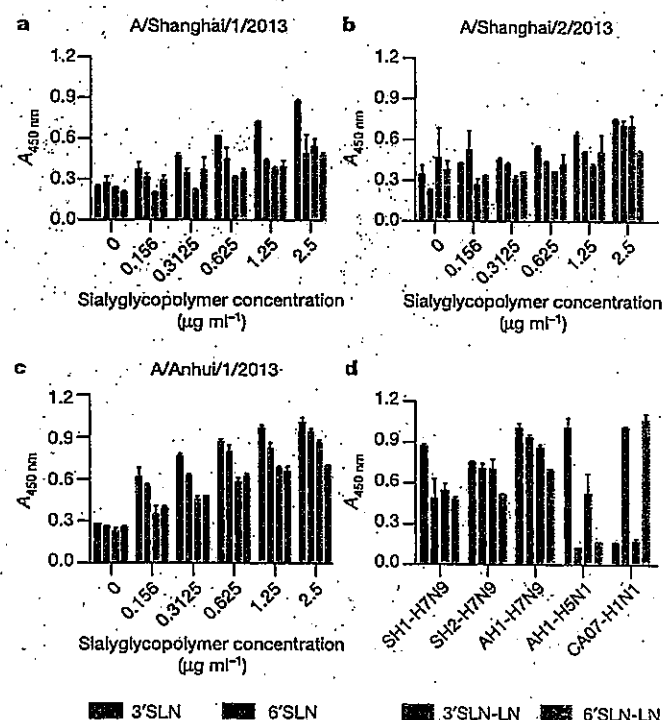


Figure 1 | Receptor binding properties. a–c, Dose-dependent binding of A/Shanghai/1/2013 (a), A/Shanghai/2/2013 (b) and A/Anhui/1/2013 (c) viruses to both α 2,3 and α 2,6 glycans at the concentration of 0, 0.156, 0.3125, 0.625, 1.25 and 2.5 μ g ml⁻¹ sialoglycopolymers. d, The binding pattern of H7N9, H5N1 (A/Anhui/01/2005) and pandemic H1N1 (A/California/07/2009) viruses were exhibited at 2.5 μ g ml⁻¹. H5N1 data were obtained previously, as H5N1 and H7N9 viruses cannot be simultaneously handled owing to biosafety reasons. Results denote mean $\pm$ s.e.m. from two separate assays performed in duplicate. 3'SLN, Neu5Ac α 2-3Gal β 1-4GlcNAc; 3'SLN-LN, Neu5Ac α 2-3(Gal β 1-4GlcNAc β 1-3)₂; 6'SLN, Neu5Ac α 2-6Gal β 1-4GlcNAc; 6'SLN-LN, Neu5Ac α 2-6(Gal β 1-4GlcNAc β 1-3)₂.

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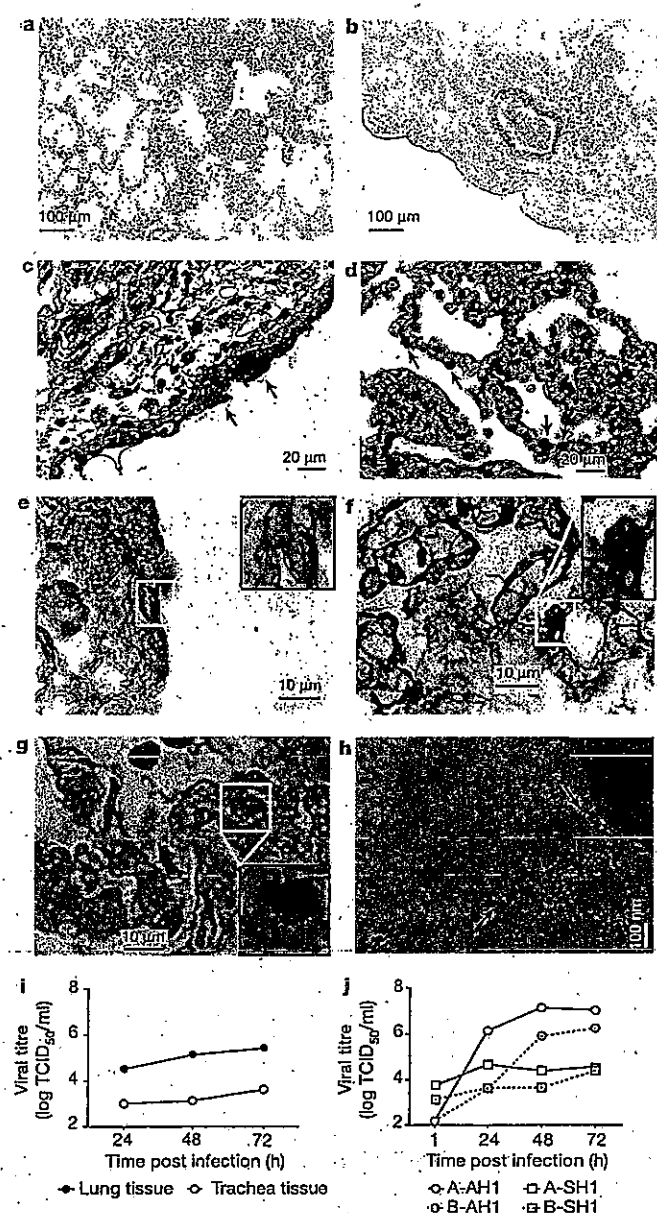


Figure 2 | *Ex vivo* infection of trachea or lung tissue with A/Anhui/1/2013 (AH1) or A/Shanghai/1/2013 (SH1). **a, b**, Trachea (**a**) and lung (**b**) explants without infection. **c, d**, Influenza nucleoprotein (brown, arrows) detected in trachea (**c**) and lung (**d**). **e–g**, Nucleoproteins in trachea epithelia/pneumocytes (**e, f**, red, cytochrome staining) and type II pneumocytes (**g**, red, surfactant staining). **h**, Extracellular viral particles (arrows) in AH1-infected lung detected by transmission electron microscopy. **i**, Viral replication in AH1-infected lung/trachea tissues from one donor (filled/empty circle, respectively). **j**, Viral yields of lung fragments from two additional donors (A/B, solid/dotted line, respectively) infecting AH1 (circle) and SH1 (square). Original magnifications: $\times 10$ (**a, b**), $\times 40$ (**c, d**) and $\times 100$ (**e–g**).

H7N9 viruses bound to long $\alpha 2,6$ glycans ($\alpha 2-6$ SLN-LN) efficiently (Fig. 1). Long $\alpha 2-6$ oligosaccharides with an umbrella-like topology, which are rich in human and swine respiratory epithelial cells, are involved in the adaptation and infection of influenza viruses in these hosts^{5,14,15}. The increased preference of the H7N9 virus for human-type receptors may result in its higher cross-species transmissibility than the H5N1 viruses, which prefer avian-type receptors. This receptor specificity had been considered to be responsible for the major species barrier preventing H5N1 from efficient infection and transmission in humans.

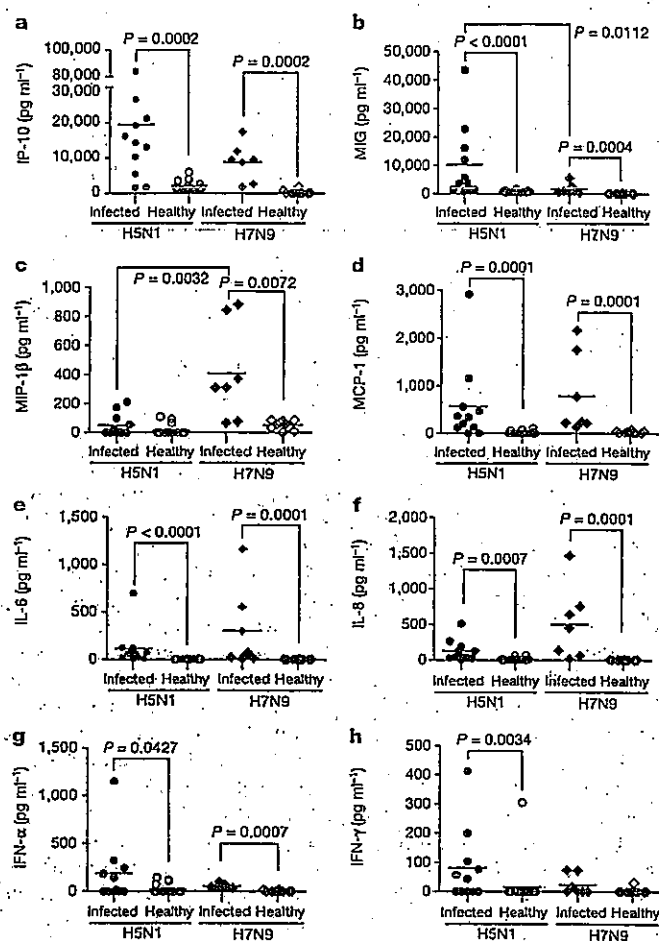


Figure 3 | Sera chemokine/cytokine levels of acutely infected patients by avian H7N9 or H5N1 viruses. **a–h**, Sera concentrations of the chemokines and cytokines IP-10 (**a**), MIG (**b**), MIP-1 β (**c**), MCP-1 (**d**), IL-6 (**e**), IL-8 (**f**), IFN- α (**g**) and IFN- γ (**h**) were measured using cytometric bead-array assays according to the manufacturers' instructions. Results are presented as mean $\pm$ s.e.m. from the specimens of 11 H5N1, 7 H7N9 and 30 close contacts tested in duplicate. P values are shown on the figure; differences between groups were tested using non-parametric Kruskal–Wallis analysis of variance (ANOVA) with Dunn's post-hoc test for multiple comparisons.

Human upper respiratory tissues and trachea contain mainly $\alpha 2,6$ receptors, whereas lung tissues contain a mixture of $\alpha 2,3$ - and $\alpha 2,6$ -linked sialic acid^{5,14}. To assess the effect of dual-receptor binding of the H7N9 virus to human respiratory tracts, sections of tracheal and lung explants were infected. We found that the epithelial cells in the human lower respiratory tract and type II pneumocytes in the alveoli were susceptible for H7N9 virus infections, as evidenced by the expression of viral nucleoprotein (Fig. 2c–g). Extracellular viral particles were also observed in infected lung tissues at 24 hours post infection (h.p.i.) by transmission electron microscopy (Fig. 2h). Productive viral infection in the trachea and lung was demonstrated by increased viral yields in culture supernatants. Notably, the virus titre in lung tissues was about tenfold higher than that in tracheal tissues (Fig. 2i), which was consistent with the results of nucleoprotein staining (Fig. 2c, d). Efficient replication of the H7N9 virus in type II pneumocytes may cause lung function to deteriorate¹⁶, as seen with the H5N1 virus¹⁷. Lower viral yields in the trachea may be associated with the inefficient human-to-human transmission. Although AH1 and SH1 show common high binding preferences to $\alpha 2,3$ glycans, a more productive replication of AH1 in lung tissues was observed, with a peaking titre around 100-fold higher than that of SH1 (Fig. 2j). This indicated that viral replications in respiratory tissues are not solely determined by receptor-binding

Table 1 | Cross-reactive antibody response against the H7N9 virus

Age group	Subjects	Age distribution (years)	Antigen	Increase in antibody titre by a factor of ≥ 4 (%)	Geometric mean titre		Titre ≥ 40 (%)		Titre ≥ 160 (%)	
					Before vaccination	After vaccination	Before vaccination (%)	After vaccination (%)	Before vaccination (%)	After vaccination (%)
Children	30	3–5	CA07	76.7	20.5	272.2	43.3	80.0	10.0	73.3
			Vic361	36.7	55.3	152.8	73.3	93.3	16.7	60.0
			HB158	86.7	36.5	432.1	63.3	96.7	13.3	83.3
			AH1	0	<5	<5	0	0	0	0
Adults	30	18–59	CA07	70.0	14.8	156.3	26.7	76.7	0	60.0
			Vic361	53.3	9.8	38.2	13.3	70.0	0.0	13.3
			HB158	80.0	28.9	259.9	53.3	96.7	10.0	76.7
			AH1	0	<5	<5	0	0	0	0
Older people	30	≥ 60	CA07	46.7	10.5	52.8	10.0	53.3	0	30.0
			Vic361	40.0	25.8	68.1	40.0	90.0	0.0	30.0
			HB158	60.0	33.2	211.1	63.3	93.3	6.7	53.3
			AH1	0	<5	<5	0	0	0	0

Cross-reactive antibody response against the novel H7N9 virus in paediatric and adult recipients of 2012–2013 seasonal trivalent inactivated influenza vaccines. All children received two doses of vaccine, with a 1-month interval. The composition of the trivalent vaccine were A/Christchurch/16/2010 (NIB-74xp) (A/Columbia/07/2009-like), A/Victoria/361/2011 (H3N2) (VR-165 and B/Hubei-Wujiagang/158/2009, AH1, A/Anhui/1/2013 (H7N9); CA07, A/Columbia/07/2009 (H1N1); HB158, B/Hubei-Wujiagang/158/2009; Vic361, A/Victoria/361/2011 (H3N2).

properties, but that other factors, such as viral polymerase and host factors, may also have a role. Moreover, H7N9 isolates demonstrated advanced growth capabilities in human, porcine and canine cell lines that were comparable to that of the pandemic 2009 H1N1 virus (Supplementary Fig. 2).

The dysfunction of the chemokine and cytokine response is considered to be associated with severe human H5N1 infection^{18,19}. Therefore, we measured the concentrations of chemokines and cytokines in acute sera from seven patients infected with H7N9 by cytometric beads array (detailed sampling information shown in Supplementary Table 3). The levels of IP-10, MIG, MIP-1 β , MCP-1, IL-6, IL-8 and IFN- α were significantly higher in patients with H7N9 than in healthy subject controls (Fig. 3). No significant differences were detected between patients with H7N9 and H5N1, with the exception of the MIG and MIP-1 β levels. The MIG response was significantly lower in patients with H7N9 than with H5N1, whereas the MIP-1 β response was significantly higher in patients with H7N9 than with H5N1 (Fig. 3). The hypercytokinaemia in patients with H7N9 may be one of the important contributors to clinical severity, as seen in H5N1 infections²⁰. Notably, most of the severe H7N9 cases were in old adults (≥ 60 years), which might be due to the enhanced expression of chemokine receptor related to ageing or chronic diseases, as observed previously^{21–24}.

Pre-existing immunity in the general population is one of the determining factors for the susceptibility to a novel influenza virus. In the event of H7N9 virus, little is known about the level of pre-existing or cross-reactive immunity in humans. We measured haemagglutination inhibition (HAI) and neutralization antibody responses against the H7N9 virus in different age groups (children, 3–5 years old; adults, 18–59 years old, and old people, ≥ 60 years old) of pre- and post-seasonal influenza vaccination. No pre-existing immunity was detected in serum samples of all age groups. Also, no detectable cross-reactive antibodies against the H7N9 virus were present in all age groups after current seasonal vaccination (Table 1 and Supplementary Table 1). These data demonstrated that the human population is naive to the H7N9 virus, and current seasonal vaccination does not protect against H7N9 infection.

The antiviral drugs M2 ion channel blockers (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir, zanamivir, peramivir and laninamivir) are available at present for the prophylaxis and treatment of influenza virus infection. However, the H7N9 isolates contain one M2 blocker resistance-related mutation (Ser31Asn). Tested by our *in vitro* neuraminidase function assay, current H7N9 viruses are found to be sensitive to both oseltamivir and zanamivir (Supplementary Table 2). However, the antiviral resistance in A/H7N9 viruses should be a concern, as neuraminidase inhibitor-resistance mutants associated with a neuraminidase Arg292Lys substitution were recently observed in two patients infected with H7N9 and correlated with adverse clinical prognosis²⁵.

Although no efficient human-to-human transmission has occurred, biological features we demonstrated here, such as a dual receptor-binding preference and high growth ability, provide the H7N9 virus with higher transmissibility from avian to human. Together with the challenges in the available treatments for H7N9 infection caused by its clinical severity and emerging antiviral resistance, further unpredictable evolution and adaptation of the H7N9 virus and the lack of pre-existing immunity leave the human population at high risk. Thus, the threats of the H7N9 virus with pandemic potential should not be underestimated and intensive surveillance must be undertaken.

METHODS SUMMARY

Viruses and cells. All H7N9 avian influenza viruses were isolated and passed in 9-day-old embryonated specific pathogen-free chicken eggs. All experiments with H7N9 viruses were performed in approved enhanced biosafety level 3 (BSL-3) containment laboratories. Human type II alveolar epithelial (A549) cells were maintained in DMEM, and Madin–Darby canine kidney (MDCK) and porcine kidney (PK15) cells were maintained in MEM, supplemented with 10% FBS, glutamine, HEPES, penicillin and streptomycin.

Direct binding assay with synthetic sialylglycopolymers. As described elsewhere⁵, serial dilutions of sialylglycopolymers in PBS were coated in 96-well flat-bottom polystyrene plates overnight at 4 °C. Thirty-two haemagglutination unit (HAU) live viruses per well, and oseltamivir carboxylate at a final concentration of 17.5 μ M were added, as described previously²⁶. After incubating with appropriate amounts of the primary antibodies, the secondary antibody was added, and bound antibody was detected using tetramethylbenzidine substrate solution.

Ex vivo infection of human lung and tracheal tissue by the H7N9 virus. Tissue fragments were infected with 2×10^7 TCID₅₀ (the tissue culture infectious dose affecting 50% of the cultures) A/Anhui/1/2013 virus for 24 h and 48 h, and the infectivity was assayed by immunohistochemistry staining and morphology examination. For replication tests, the tissue fragments were infected with 10^4 TCID₅₀ A/Anhui/1/2013 or A/Shanghai/1/2013 virus. The viral titres in the supernatants at 1, 24, 48 and 72 h.p.i. were measured in MDCK cells.

Cytokine and chemokine measurement. Sera concentrations of the chemokines and cytokines IP-10, MIG, MCP-1, MIP-1 β , IL-6, IL-8, IFN- α and IFN- γ were measured using cytometric bead-array assays according to the manufacturers' instructions. **Cross-reactive antibody assay.** A total of 90 stored serum samples vaccinated with seasonal influenza vaccines between 2012 and 2013 were tested for antibody responses to the novel H7N9 subtype A/Anhui/1/2013 virus.

Full Methods and any associated references are available in the online version of the paper.

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Supplementary Information is available in the online version of the paper.

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Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to Y.S. (yshu@cnic.org.cn).

METHODS

Cell culture. Human type II alveolar epithelial (A549), Madin-Darby canine kidney (MDCK) and porcine kidney (PK15) cells were obtained from the American Type Culture Collection. A549 cells were maintained in DMEM (Invitrogen) and MDCK and PK15 cells were in MEM (Invitrogen), supplemented with 10% FBS (Invitrogen), glutamine (2 mM; Invitrogen), HEPES (10 mM; Invitrogen), penicillin (100 U ml⁻¹), and streptomycin (100 µg ml⁻¹; Invitrogen).

Virus preparation, titration and infection. In view of the biosafety issues involved in handling H7N9 viruses, the experiments were conducted in approved enhanced biosafety level 3 (BSL-3) containment laboratories. The viruses were propagated in 9-day-old embryonated specific pathogen-free chicken eggs, by a first passage in the H7N9 strain. The allantoic fluid was collected at 48 h.p.i., aliquoted, and stored at -80 °C until use. Infectious titres (TCID₅₀, the tissue culture infectious dose affecting 50% of the cultures) were performed in confluent MDCK cells and confirmed by haemagglutination assay.

Neuraminidase inhibitors. Oseltamivir carboxylate (4-*N*-acetyl-5-amino-3-(1-ethylpropoxy)-1-cyclohexane-1-carboxylic acid), the active metabolite of oseltamivir, was provided by Hoffmann-La Roche. Zanamivir (4-guanidino-Neu5Ac2en) was provided by GlaxoSmithKline.

Direct binding assay with synthetic sialylglycopolymers. Receptor specificity was investigated using biotinylated glycans including 3'-SLN, 3'-SLN-LN, 6'-SLN and 6'-SLN-LN (see legend to Fig. 1), which were obtained from the Consortium of Functional Glycomics through their resource request program. As described elsewhere⁵, serial dilutions of sialylglycopolymers in PBS were coated in 96-well flat-bottom polystyrene plates overnight at 4 °C. Thirty-two haemagglutination unit (HAU) live viruses per well and oseltamivir carboxylate (Hoffmann-La Roche) at a final concentration of 17.5 µM were added, as described previously²⁶. After incubating with appropriate amounts of the primary antibodies, including a panel of rabbit sera against an Eurasian H7N7 virus isolated from the environment, A/California/07/09 (H1N1) and A/Anhui/01/2005 (H5N1) viruses, the secondary antibody was added (horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG; Santacruz Biotechnology), and bound antibody was detected using tetramethylbenzidine substrate solution (R&D Systems). All procedures were performed at 4 °C to avoid the cleavage effects of neuraminidase on the binding.

Growth curves *in vitro*. A549, MDCK and PK15 cells were infected with A/Shanghai/1/2013, A/California/07/2009 or A/Anhui/1/2013 virus at a multiplicity of infection (MOI) of 0.001. One hour after inoculation at 37 °C, at time point 0, the cells were washed once with PBS, and fresh infection medium containing 2 mg l⁻¹ *N*-*p*-tosyl-L-phenylalaninechloromethyl ketone-treated (TPCK) trypsin (Sigma) was added. The supernatants were sampled at 12, 24, 48, 72 and 96 h.p.i. and the viral titres in these supernatants were determined by endpoint titration in MDCK cells as described²⁷.

***Ex vivo* infection of human lung and tracheal tissue by the H7N9 virus.** Human tracheal and lung sections were obtained from one donor, and two additional lung fragments were obtained from another two donors of uninvolved portions of surgical resection samples from patients undergoing removal of lung tissue for possible carcinoma, and regions containing carcinoma or infection were excluded. All patients gave informed consent under a study approved by the Institutional Review Board of National Institute for Viral Disease Control and Prevention, China CDC. The tissues from each donor were cut into multiple 2–3-mm fragments and were infected with A/Anhui/1/2013 at a titre of 2 × 10⁷ TCID₅₀ virus at 37 °C for 24 h and 48 h and the infectivity was assayed by immunohistochemistry staining and morphology examination. As to the replication tests, the tissue fragments were infected with 10⁶ TCID₅₀ A/Anhui/1/2013 or A/Shanghai/1/2013 virus. One hour after inoculation at 37 °C, at time point 0, the tissues were washed three times with 2 ml PBS, and 500 µl fresh infection medium containing 2 mg l⁻¹ TPCK trypsin was added. The supernatants were sampled at 1, 24, 48 and 72 h.p.i. and the viral titres in these supernatants were determined by endpoint titration in MDCK cells.

H&E staining and immunohistochemistry. Routine haematoxylin and eosin (H&E) staining was used to determine the histology of lung and trachea explants. To determine the viral infection in lung or bronchus tissues, immunohistochemical staining was performed on 4-µm-thick deparaffinized sections using monoclonal antibodies against the nucleoprotein of influenza A (VS2525, USCDC) by using a polymer-based colorimetric indirect immunalkaline phosphatase method (Thermo Scientific/Lab Vision). Sequential tissue sections incubated with normal serum pertinent to the primary antibody were used as negative controls. Paraffin-embedded sections were deparaffinized by two washes in xylene and were rehydrated through decreasing concentration of ethanol. After washing with PBS for 5 min at room temperature, sections were treated with 200 µg ml⁻¹ proteinase K for 20 min. After blocking with UV blocking buffer (Thermo Scientific/Lab Vision), the sections were incubated with specific antibodies for 30 min at room temperature. Unbound antibody was removed by 0.5% PBS tween-20 (PBST)

before adding HRP-labelled polymer for 15 min at room temperature (Thermo Scientific/Lab Vision). After washing unbound labelled polymer, peroxidase staining in tissue sections was revealed by DAB solution (Thermo Scientific/Lab Vision). Double-stain immunohistochemistry was performed using peroxidase polymer-labelled antibodies (Thermo Scientific/Lab Vision/Dako) against cytokeratin AE1/AE3 (188132, Invitrogen) as the marker for epithelial cells, surfactant protein A as the marker for type II pneumocytes (ab51891, abcam) on the sections with the nucleoprotein staining, followed by the mouse anti-influenza A nucleoprotein antibody labelled with immunalkaline phosphatase polymer. All staining steps were completed on an automatic staining system (DAKO, Denmark). After stopping the reaction in running water, sections were counterstained by a quick dip in a Mayer's haematoxylin solution. The sections were mounted with DPX and examined by light microscopy (ZEISS M2).

Cytokine and chemokine measurement. Sera concentrations of the chemokines/cytokines IP-10, MIG, MCP-1, MIP-1β, IL-6, IL-8, IFN-α and IFN-γ from seven patients with H7N9 and ten close contacts were measured using cytometric bead-array assays (CBA; Becton Dickinson) according to the manufacturers' instructions. Fifty microlitres of tested sera or sera diluents in assay diluent was incubated with 50 µl capture beads suspension of each flex set kit per test at room temperature for 1.5 h, and then 50 µl phycoerythrin-conjugated detection reagent was added for another 1.5 h. After washing in 1 ml wash buffer, the samples were run and FACS data were collected using a FACSAria I flow cytometer and analysed using BD Cytometric Bead Array 1.4 software. The data together with those of 11 confirmed patients with H5N1 and 20 close contacts previously measured by CBA were analysed.

Cross-reactive antibody assay. We assessed the level of cross-reactive antibody to H7N9 virus in 90 subjects before and after they had been vaccinated with the 2012–2013 seasonal influenza vaccines. The compositions of the trivalent vaccine were A/Christchurch/16/2010 (H1N1), A/Victoria/361/2011 (H3N2) and B/Hubei-Wujiagang/158/2009. The HAI assay was according to standard protocols with 0.5% turkey red blood cells (TRBCs) and a microneutralization assay as the standard protocols were performed^{28,29}. Both HAI and microneutralization titres of 20 or more were defined as seropositive. We tested the samples for antibody responses to the novel H7N9 subtype viruses A/Anhui/1/2013. Before the HAI assay, serum samples were treated with a 1:4 (v/v) dilution of receptor-destroying enzyme (RDE; Denka Seiken) at 37 °C for 18 h, followed by incubation at 56 °C for 30 min. The serum samples for the microneutralization assay were inactivated at 56 °C for 30 min. The acute and convalescent sera of two patients with H7N9 and animal sera against H5N1 or 2009 pdmH1N1 virus were included in the HAI and microneutralization tests as controls. For the HAI assay, 25-µl serial twofold sera diluents were incubated with standard virus of 4 HAU per 25 µl at room temperature for 30 min. Then, 50-µl standardized 0.5% TRBCs were added, mixed well and settled at room temperature for the appropriate 30 min. For the microneutralization assay, 50-µl serial twofold sera diluents were incubated with standard virus of 100 TCID₅₀ per 50 µl at 37 °C, 5% CO₂ for 60 min. Then, 100 µl 1.5 × 10⁶ MDCK cells per well was added for a further 18 h at 37 °C, 5% CO₂. On day 2, the cells were fixed by 80% cold acetone and stained by anti-influenza A nucleoprotein monoclonal antibody pool (MAB8257 and MAB8258, Millipore) and peroxidase polymer-labelled goat anti-mouse IgG (074-1802, Kirkegaard & Perry Laboratories). Bound antibody was detected using freshly prepared *o*-phenylenediamine dihydrochloride and citrate buffer (Sigma). The test dose of virus is acceptable if the back-titration is positive in 5–7 wells containing the lowest dilution of test virus.

Neuraminidase inhibition assay. Fluorimetric neuraminidase activity assays were done according to the manufacturers' instructions of the NA-Fluor™ kit (Life Technologies). In brief, a standardized dose of viruses was incubated with various concentrations of oseltamivir carboxylate or zanamivir in assay buffer containing 2-(*N*-morpholino) ethanesulphonic acid and calcium chloride, for 45 min at 37 °C. Then, substrate methylumbelliferyl-*N*-acetylneuraminic acid (MUNANA) was added as a fluorescent substrate to this mixture. After incubation for another hour at 37 °C, stop solution was added to the mixture to stop the reaction. The fluorescence signal was measured at an excitation wavelength of 360 nm and an emission wavelength of 465 nm, and the 50% inhibitory concentrations (IC₅₀) of antiviral drugs were calculated according to the concentration required for reducing neuraminidase activity by 50% by analysis with GraphPad Prism software, version 5 (GraphPad Software).

Electron microscopic examination. The infected lung tissues with H7N9 virus were processed for transmission electron microscopy at 24 h post infection *ex vivo* as previously described³⁰. The AHI-infected lung tissues were cut into several 1-mm fragments and immersed in fixative (2% paraformaldehyde, 2.5% glutaraldehyde, 0.1% sodium cacodylate buffered, pH 7.2) for 1 h at 4 °C. Tissues were then fixed in 1% osmium tetroxide (0.1% sodium cacodylate buffered, pH 7.2) for 1 h at 4 °C. After several dehydrations and rehydrations, the blocks were infiltrated

with 1:1 (v/v) ethanol/epoxy resin (SPI-Chem low viscosity "Spurr" kits) for 1 h at room temperature following Spurr medium infiltration and embedded in BEEM embedding capsules with Spurr medium. Cured blocks were cut into ultrathin sections with a microtome (LKB Nova) and mounted on copper grids (150 meshes). The sections were stained with 1% uranyl acetate in 50% ethanol for 10 min and 0.2% lead citrate for 5 min, and then observed under transmission electron microscope (FEI Tecnai12) at 80 kV.

Statistical analysis. Statistical significance was determined using non-parametric tests and the GraphPad Prism 5 software package (GraphPad Software). $P < 0.05$ was deemed to indicate statistical significance.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2013. 7. 1	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿	公表国 米国			使用上の注意記載状況・ その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分 採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、 細菌、原虫等の感染、 vCJD等の伝播のリスク
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)	研究報告の公表状況 Centers for Disease Control and Prevention (CDC). MMWR Morb Mortal Wkly Rep. 2013 Jun 28;62(25):513-7.			
研究報告の概要	○ウエストナイルウイルス(WNV)及び他のアルボウイルス疾患-2012年、米国 米国疾病管理予防センター(CDC)が発表した2012年の米国におけるWNV及び他のアルボウイルス疾患が報告された(デング熱を除く)。 米国の3,141郡のうち1,020郡(32%)から5,780症例のアルボウイルス疾患の98%を占め、米国の48州及びワシントンD.C.,プエルトリコの976郡からWNV疾患は5,674例で、報告されたアルボウイルス疾患の98%を占めた。5,199人(92%)が7月～9月に発症し、患者年齢の中央値は56歳で56%が男性であった。2,873人(51%)が神経侵襲性疾患であり、3,491人(62%)が入院し、286人(5%)が死亡した(死亡例の年齢中央値は77歳)。神経侵襲性疾患患者のうち1,615人(56%)は髄膜炎、1,038人(36%)は髄膜炎、220人(8%)は急性弛緩性麻痺(うち、83%が同時に脳炎あるいは髄膜炎を併発)を呈し、270人(9%)が死亡した。神経侵襲性疾患はテキサス州、カルフォルニア州、イリノイ州及びルイジアナ州の4州で、全体の半数以上の症例が報告され、発生率はサウスダコタ州(7.44/10万人)で最も多かった。 他は、ラクロスウイルス78例、東部ウマ脳炎ウイルス15例、ポワッサンウイルス7例、セントルイス脳炎ウイルス3例、ジェームズタウン溪谷ウイルス2例及び非特定のカルポフォルニア血清群ウイルス1例であった。				
報告企業の意見	今後の対応 日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、ウェストナイルウイルス感染の国内発生に備え、平成17年10月25日付血液対策課発事務連絡に基づき緊急対応(献血制限、NAT検査)のほか、厚生労働科学研究「血液製剤の安全性確保と安定供給のための新興・再興感染症の研究」班と共同して対応について検討している。今後引き続き情報の収集に努める。				

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Centers for Disease Control and Prevention

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West Nile Virus and Other Arboviral Diseases — United States, 2012

Arthropod-borne viruses (arboviruses) are transmitted to humans primarily through the bites of infected mosquitoes and ticks. West Nile virus (WNV) is the leading cause of domestically acquired arboviral disease in the United States (1). However, several other arboviruses also cause sporadic cases and seasonal outbreaks of neuroinvasive disease (e.g., meningitis, encephalitis, and acute flaccid paralysis) (1). In 2012, CDC received reports of 5,780 nationally notifiable arboviral disease cases (excluding dengue). A large multistate outbreak of WNV disease accounted for 5,674 (98%) of reported cases, the highest number reported since 2003. Other reported etiologies included Eastern equine encephalitis virus (EEEV), Powassan virus (POWV), St. Louis encephalitis virus (SLEV), and California serogroup viruses such as La Crosse virus (LACV) and Jamestown Canyon virus (JCV). Arboviruses continue to cause serious illness in substantial numbers of persons in the United States. Maintaining surveillance remains important to identify outbreaks and guide prevention efforts.

In the United States, most arboviruses are maintained in transmission cycles between arthropods and vertebrate hosts (typically birds or small mammals). Humans usually become infected when bitten by infected mosquitoes or ticks. Person-to-person transmission occurs rarely through blood transfusion and organ transplantation. The majority of human arboviral infections are asymptomatic. Symptomatic infections most often manifest as a systemic febrile illness and, less commonly, as neuroinvasive disease. Most endemic arboviral diseases are nationally notifiable and are reported to CDC through ArboNET (2,3). In addition to collecting data on human disease cases, ArboNET collects data on viremic blood donors, veterinary disease cases, and infections in mosquitoes, dead birds, and sentinel chickens. Using standard definitions, human cases with laboratory evidence of recent arboviral infection are classified as neuroinvasive disease or nonneuroinvasive disease (2). Because of the substantial associated morbidity, detection and reporting of neuroinvasive disease cases is assumed to be more consistent and complete than for nonneuroinvasive disease cases. Therefore, incidence rate calculations were limited to neuroinvasive disease cases.

In 2012, CDC received reports of 5,780 cases of nationally notifiable arboviral diseases, including those caused by WNV (5,674 cases), LACV (78), EEEV (15), POWV (seven), SLEV (three), JCV (two), and an unspecified California serogroup virus (one). Cases were reported from 1,020 (32%) of the 3,141 U.S. counties; no cases were reported from Alaska or Hawaii. Of the 5,780 total cases, 2,969 (51%) were reported as neuroinvasive disease, for a national incidence of 0.95 per 100,000 population.

A total of 5,674 WNV disease cases, including 2,873 (51%) neuroinvasive cases, were reported from 976 counties in 48 states, the District of Columbia, and Puerto Rico (Table 1). WNV disease cases peaked in late August, with 5,199 (92%) cases having illness onset during July–September. The median age of patients was 56 years (interquartile range [IQR]: 42–68 years); 3,193 (56%) were male. Overall, 3,491 (62%) patients were hospitalized, and 286 (5%) died. The median age of patients who died was 77 years (IQR: 68–84 years).

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TABLE 1. Number and percentage of reported cases of arboviral disease, by virus and selected characteristics — United States, 2012*

Characteristic	Virus							
	West Nile (N = 5,674)		La Crosse (N = 78)		Eastern equine encephalitis (N = 15)		Powassan (N = 7)	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Age group (yrs)								
<18	210	(4)	65	(83)	4	(27)	0	—
18–59	3,124	(55)	11	(14)	4	(27)	4	(57)
≥60	2,340	(41)	2	(3)	7	(47)	3	(43)
Sex								
Male	3,193	(56)	44	(56)	13	(87)	4	(57)
Female	2,481	(44)	34	(44)	2	(13)	3	(43)
Period of illness onset								
January–March	2	(<1)	1	(1)	0	—	0	—
April–June	130	(2)	13	(17)	1	(7)	7	(100)
July–September	5,199	(92)	59	(76)	13	(87)	0	—
October–December	343	(6)	5	(6)	1	(7)	0	—
Clinical syndrome								
Nonneuroinvasive	2,801	(49)	7	(9)	0	—	0	—
Neuroinvasive	2,873	(51)	71	(91)	15	(100)	7	(100)
Encephalitis	1,615	(28)	56	(72)	13	(87)	4	(57)
Meningitis	1,038	(18)	14	(18)	2	(13)	3	(43)
Acute flaccid paralysis†	220	(4)	1	(1)	0	—	0	—
Outcome								
Hospitalization	3,491	(62)	76	(97)	14	(93)	6	(86)
Death	286	(5)	1	(1)	5	(33)	0	—

* Three California serogroup virus disease cases in addition to the La Crosse virus disease cases were reported, including two caused by Jamestown Canyon virus and one unspecified.

† Of the 220 West Nile virus disease patients with acute flaccid paralysis, 183 (83%) also had encephalitis or meningitis. The La Crosse virus disease patient with acute flaccid paralysis also had encephalitis.

Of the 2,873 WNV neuroinvasive disease patients, 1,615 (56%) had encephalitis, 1,038 (36%) had meningitis, and 220 (8%) had acute flaccid paralysis. Among the 220 patients with acute flaccid paralysis, 183 (83%) also had encephalitis

or meningitis. The national incidence of neuroinvasive WNV disease was 0.92 per 100,000 population (Table 2). States with the highest incidence rates included South Dakota (7.44 per 100,000), North Dakota (5.57), Mississippi (3.45), Louisiana

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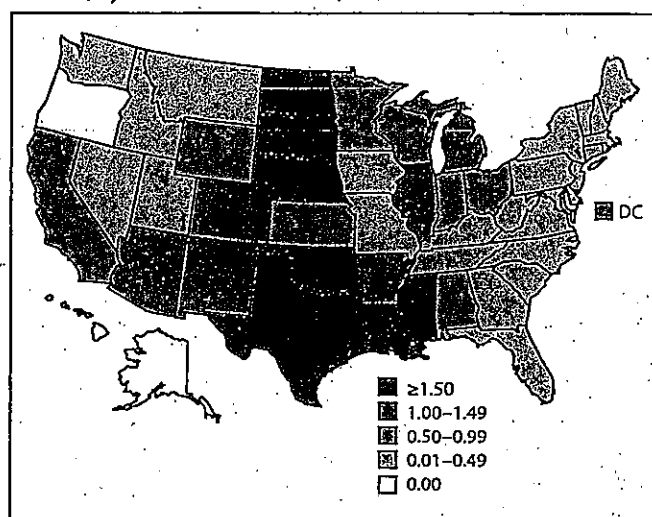
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(3.37), and Texas (3.24) (Figure). Four states reported over half of the WNV neuroinvasive disease cases: Texas (844 cases), California (297), Illinois (187), and Louisiana (155). Neuroinvasive WNV disease incidence increased with age, with the highest incidence among persons aged ≥ 70 years. Among patients with neuroinvasive disease, 270 (9%) died.

The 78 LACV disease cases were reported from 50 counties in 11 states; 71 (91%) were neuroinvasive (Table 1). Dates of illness onset for LACV disease cases ranged from March through October; 13 (17%) had onset during April–June, and 59 (76%) had illness onset during July–September. Forty-four (56%) patients were male. The median age of patients was 9 years (IQR: 6–13 years); 65 (83%) were aged < 18 years. LACV neuroinvasive disease incidence was highest in West Virginia (0.49 per 100,000), North Carolina (0.27), Tennessee (0.14), and Ohio (0.10) (Table 2). Those four states reported 56 (79%) LACV neuroinvasive disease cases. A total of 76 (97%) patients were hospitalized; one (1%) died. Three California serogroup virus disease cases in addition to the LACV cases were reported, including two caused by JCV and one unspecified.

FIGURE. Rate* of reported cases of West Nile virus neuroinvasive disease, by state — United States, 2012



* Per 100,000 population, based on July 1, 2012 U.S. Census population estimates.

TABLE 2. Number and rate* of reported cases of arboviral neuroinvasive disease, by virus, U.S. Census region, and state — United States, 2012

U.S. Census region and state	Virus							
	West Nile		La Crosse		Eastern equine encephalitis		Powassan	
	No.	Rate	No.	Rate	No.	Rate	No.	Rate
United States	2,873	0.92	71	0.02	15	<0.01	7	<0.01
New England	42	0.29	—	—	9	0.06	—	—
Connecticut	12	0.33	—	—	—	—	—	—
Maine	1	0.08	—	—	—	—	—	—
Massachusetts	25	0.38	—	—	7	0.11	—	—
New Hampshire	1	0.08	—	—	—	—	—	—
Rhode Island	2	0.19	—	—	—	—	—	—
Vermont	1	0.16	—	—	2	0.32	—	—
Middle Atlantic	116	0.28	—	—	—	—	1	<0.01
New Jersey	22	0.25	—	—	—	—	—	—
New York	61	0.31	—	—	—	—	1	0.01
Pennsylvania	33	0.26	—	—	—	—	—	—
East North Central	494	1.06	16	0.03	—	—	2	<0.01
Illinois	187	1.45	—	—	—	—	—	—
Indiana	46	0.70	2	0.03	—	—	—	—
Michigan	141	1.43	—	—	—	—	—	—
Ohio	76	0.66	12	0.10	—	—	—	—
Wisconsin	44	0.77	2	0.03	—	—	2	0.03
West North Central	225	1.08	4	0.02	—	—	4	0.02
Iowa	11	0.36	—	—	—	—	—	—
Kansas	20	0.69	—	—	—	—	—	—
Minnesota	34	0.63	4	0.07	—	—	4	0.07
Missouri	17	0.28	—	—	—	—	—	—
Nebraska	42	2.26	—	—	—	—	—	—
North Dakota	39	5.57	—	—	—	—	—	—
South Dakota	62	7.44	—	—	—	—	—	—
South Atlantic	185	0.30	38	0.06	5	<0.01	—	—
Delaware	2	0.22	—	—	—	—	—	—
District of Columbia	8	1.27	—	—	—	—	—	—
Florida	52	0.27	—	—	2	0.01	—	—
Georgia	46	0.46	—	—	1	0.01	—	—
Maryland	25	0.42	—	—	—	—	—	—

See table footnotes on the next page.

TABLE 2. (Continued) Number and rate* of reported cases of arboviral neuroinvasive disease, by virus, U.S. Census region, and state — United States, 2012

U.S. Census region and state	Virus							
	West Nile		La Crosse		Eastern equine encephalitis		Powassan	
	No.	Rate	No.	Rate	No.	Rate	No.	Rate
North Carolina	7	0.07	26	0.27	2	0.02	—	—
South Carolina	20	0.42	1	0.02	—	—	—	—
Virginia	20	0.24	2	0.02	1	0.01	—	—
West Virginia	5	0.27	9	0.49	—	—	—	—
East South Central	173	0.93	10	0.05	—	—	—	—
Alabama	38	0.79	—	—	—	—	—	—
Kentucky	13	0.30	—	—	—	—	—	—
Mississippi	103	3.45	1	0.03	—	—	—	—
Tennessee	19	0.29	9	0.14	—	—	—	—
West South Central	1,146	3.08	3	0.01	—	—	—	—
Arkansas	44	1.49	—	—	—	—	—	—
Louisiana	155	3.37	—	—	—	—	—	—
Oklahoma	103	2.70	—	—	—	—	—	—
Texas	844	3.24	3	0.01	—	—	—	—
Mountain	190	0.84	—	—	—	—	—	—
Arizona	87	1.33	—	—	—	—	—	—
Colorado	62	1.20	—	—	—	—	—	—
Idaho	5	0.31	—	—	—	—	—	—
Montana	1	0.10	—	—	—	—	—	—
Nevada	5	0.18	—	—	—	—	—	—
New Mexico	24	1.15	—	—	—	—	—	—
Utah	3	0.11	—	—	—	—	—	—
Wyoming	3	0.52	—	—	—	—	—	—
Pacific	301	0.59	—	—	—	—	—	—
Alaska	—	—	—	—	—	—	—	—
California	297	0.78	—	—	—	—	—	—
Hawaii	—	—	—	—	—	—	—	—
Oregon	—	—	—	—	—	—	—	—
Washington	4	0.06	—	—	—	—	—	—
Territories	—	—	—	—	—	—	—	—
Puerto Rico	1	0.03	—	—	—	—	—	—

* Per 100,000 population, based on July 1, 2012 U.S. Census population estimates.

Fifteen EEEV neuroinvasive disease cases were reported from six states: Massachusetts (seven cases), North Carolina (two), Vermont (two), Florida (two), Georgia (one), and Virginia (one) (Table 1). Dates of illness onset ranged from June through October, with 13 (87%) occurring during July–September. The median age of patients was 57 years (IQR: 11–68 years); 13 (87%) were male. Fourteen (93%) patients were hospitalized; five (33%) died. The median age of patients who died was 76 years (IQR: 63–79 years).

Seven POWV neuroinvasive disease cases were reported from three states: Minnesota (four cases), Wisconsin (two), and New York (one) (Table 1). Dates of illness onset for all cases were in May or June. All cases occurred in adult patients (median age: 58 years [IQR: 36–73 years]); four were male. Six (86%) patients were hospitalized; none died.

Three SLEV disease cases were reported from Texas; only one was neuroinvasive. Dates of illness onset were in July and August. All cases occurred in adults aged 40–60 years. One of the three SLEV patients was hospitalized; none died.

Reported by

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Editorial Note

A large multistate outbreak of WNV disease occurred in 2012, with more cases reported nationally than in any year since 2003, including the first reported human case from Maine. The 15 EEEV disease cases reported in 2012 were the most reported since 2005, and included the first cases ever reported from Vermont. EEEV disease remained the most severe domestic arboviral disease, with a 33% case-fatality rate. Over 90% of arboviral disease cases occurred during July–September, and most of the remainder occurred during April–June, emphasizing the importance of focusing public health interventions on these periods.

The national incidence of WNV neuroinvasive disease peaked in 2002 (1.02 per 100,000) and 2003 (0.98) (3). During 2004–2011, annual incidence was relatively low (median: 0.31; range: 0.13–0.50) (3–6). In 2012, the national incidence of WNV neuroinvasive disease increased to 0.92 per 100,000. The increase in disease was widespread, with 43 states reporting a higher incidence in 2012 compared with the median for 2004–2011; however, more than half of the neuroinvasive disease cases in 2012 were reported from just four states, and 29% were reported from Texas alone. Of the five states with a lower incidence in 2012 compared with the previous 8 years, four were in the Mountain Region (Montana, Nevada, Utah, and Wyoming). Oregon also reported lower rates compared with recent years; Alaska and Hawaii have never reported a case of WNV disease.

Reported numbers of arboviral disease cases vary from year to year. It is not clear why more WNV activity occurred this year than in recent years. The weather, numbers of birds that maintain the virus, numbers of mosquitoes that spread the virus, and human behavior are all factors that can influence when and where outbreaks occur. Because of this complex ecology, it is difficult to predict how many cases of disease might occur in the future and in what areas.

The findings in this report are subject to at least two limitations. First, ArboNET is a passive surveillance system that relies on clinicians to consider the diagnosis of an arboviral disease and obtain appropriate diagnostic test results and on health-care providers and laboratories to report laboratory-confirmed cases to public health authorities. Second, testing and reporting are incomplete, leading to a substantial underestimate of the actual number of cases (7). Based on previous studies, for every reported case of WNV neuroinvasive disease, there are an estimated 30–70 nonneuroinvasive disease cases. Extrapolating from the 2,873 WNV neuroinvasive disease cases reported, an estimated 86,000–200,000 nonneuroinvasive disease cases might have occurred in 2012. However, only 2,801 (1%–3%) were diagnosed and reported.

Arboviruses continue to cause severe illness in substantial numbers of persons in the United States. However, cases occur sporadically, and the epidemiology varies by virus and geographic area. Surveillance is essential to identify outbreaks and guide prevention efforts aimed at reducing the incidence of these diseases. Health-care providers should consider arboviral infections in the differential diagnosis of cases of aseptic meningitis and encephalitis, obtain appropriate specimens for laboratory testing, and promptly report cases to public health authorities (2). Because human vaccines against domestic arboviruses are not available, prevention of arboviral disease depends on community and household efforts to reduce vector populations (e.g., applying insecticides and reducing mosquito

What is already known on this topic?

West Nile virus (WNV) is the leading cause of domestically acquired arboviral disease in the United States. However, several other arboviruses can cause sporadic cases and outbreaks of neuroinvasive disease, mainly in the summer.

What is added by this report?

A large multistate outbreak of WNV disease occurred in 2012. The 5,674 cases reported nationally were the highest number of cases reported since 2003. Eastern equine encephalitis, although rare, remained the most severe arboviral disease, with a 53% case-fatality rate.

What are the implications for public health practice?

WNV and other arboviruses continue to be a source of severe illness each year for substantial numbers of persons in the United States. Maintaining surveillance remains important to identify outbreaks and guide prevention efforts.

breeding sites), personal protective measures to decrease exposure to mosquitoes and ticks (e.g., use of repellents and wearing protective clothing), and screening blood donors. Updated guidelines for WNV surveillance, prevention, and control are available online from CDC at <http://www.cdc.gov/westnile/resources/pdfs/wnvguidelines.pdf>.

Acknowledgments

ArboNET surveillance coordinators in state and local health departments. Ned Hayes, MD, Div of Vector-Borne Diseases, CDC.

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医薬品 研究報告 調査報告書

識別番号・報告回数	報告日		第一報入手日	新医薬品等の区分	総合機構処理欄
			2013. 5. 16	該当なし	
一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)	インド			
研究報告の概要	○インド東部の部族民における無症候性マラリアの高有病率 熱帯熱マラリア原虫の無症候性感染は、マラリア撲滅の重大な障壁となっている。無症状の感染者は治療を行わず、原虫の保有宿主となるため、公衆衛生上の重大なリスクとなる。無症候性感染者集団を大幅に縮小できればマラリア感染リスクも減少する。 本研究では、西ベンガル州プリア県の部族民1,040人をスクリーニングした。陽性者にはアルデシニンベースとした混合療法(ACT)を行い、42日間追跡した。また、DNAシーケンシングによってマラリア原虫の遺伝子多型についてスクリーニングした。 結果、被験者の相当な割合(8.4%)が熱帯熱マラリア原虫に感染していたにもかかわらず、臨床症状がみられなかった。ACTの有効率は97%であった。全症例で、<i>pfert</i>遺伝子のK76T変異がみられ、<i>pfmdr1</i>遺伝子ではN86Y及びY184F変異がそれぞれ55.5%、11%にみられた。<i>pfdhfr-pfdrps</i>遺伝子には6種類の異なるハプロタイプが確認された。最も重要な点として、分離株の10%から五重変異体が発見され、スルファドキシニープリメタミン耐性の増強が示唆された。この問題の規模を調査するには、インドの他地域でも同様の研究を行う必要がある。				
使用上の注意記載状況・その他参考事項等	新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク				
報告企業の意見	インド東部の部族民における無症候性マラリアについて調査したところ、1,040人の被験者のうち8.4%が熱帯熱マラリアに感染していたにもかかわらず臨床症状がなく、またマラリア原虫の遺伝子には薬剤耐性の発現に關与する可能性のある変異も発見されたとの報告である。				
今後の対応	日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の献血を一定期間延期している(1～3年の延期を行うとともに、帰国(入国)後マラリアを思わせる症状があった場合は、感染が否定されるまで、献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。				

High Prevalence of Asymptomatic Malaria in a Tribal Population in Eastern India

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Asymptomatic infection by *Plasmodium falciparum* is an important obstacle to eliminating malaria. Asymptomatic carriers do not seek treatment for infection, and therefore they become a reservoir for the parasite. For this reason, these carriers pose a real public health risk. The systematic identification and treatment of asymptomatic infections should reduce the parasite reservoir. A large reduction in this pool will lower the chance of transmission of the disease. In this study, we screened a tribal population of 1,040 individuals in the Purulia district of West Bengal by using a dual-antigen rapid diagnostic kit (RDK), microscopy, and species-specific PCR. All positive individuals were treated with artemisinin-based combination therapy (ACT) (artesunate plus sulfadoxine-pyrimethamine) and followed for 42 days. Polymorphisms in candidate genes were screened by DNA sequencing. A significant proportion (8.4%) of the study population was infected with *P. falciparum* but showed no clinical manifestations. The PCR method was more sensitive in detecting infection than the RDK or microscopy. The efficacy of the ACT was 97%. In the *pfprt* gene, the mutation K76T (the mutated amino acid is indicated by bold type) was found in 100% of the cases. In the *pfmdr1* gene, the mutations N86Y and Y184F were noted in 55.5% and 11% of the cases, respectively. Six different haplotypes were identified in the *pfdhfr*-*pfdhps* genes. Most importantly, the quintuple mutant A₁₆I₅₁R₅₉N₁₀₈I₁₆₄S₄₃₆G₄₃₇E₅₄₀A₅₈₁A₆₁₃ was found in 10% of the isolates, which is potentially important for the development of sulfadoxine-pyrimethamine resistance. A significant proportion of the study population harboring *P. falciparum* does not seek treatment and therefore serves as a reservoir for the parasite, maintaining the natural cycle. If the National Vector Borne Disease Control Programme (NVBDCP) of India is to eliminate malaria, then this hidden parasite burden needs to be addressed properly. Similar study in other parts of the country could help to determine the magnitude of the problem.

India is one of the 11 countries in the WHO South-East Asia region and has nearly 980 million people at risk of acquiring malaria. According to the WHO's estimates, India has the largest number of malaria cases outside Africa. India's official statistics suggest that *Plasmodium falciparum* accounts for about 50% of the clinical cases in India. India has a long history of attacking malaria. Organized control programs, which started in the 1940s, used dichlorodiphenyltrichloroethane (DDT) to control mosquitoes. These programs were originally very successful in India and nearly eliminated malaria by 1961. In the subsequent years, however, the disease has reestablished itself in India.

The resurgence of malaria globally and the rising numbers of deaths due to this disease have drawn the attention of the international community. In 1998, the WHO, the World Bank, and several charitable organizations launched the Roll Back Malaria (RBM) Partnership, a global initiative that coordinates actions against malaria. The mission of the RBM Partnership was outlined recently in its global malaria action plan (1). A few of the major goals of the partnership were to reduce the number of global malaria cases from the levels seen in 2000 by 50% by 2010 and by 75% by 2015, to reduce global malaria deaths from the levels seen in 2000 by 50% by 2010 and to near zero by 2015, to eliminate malaria in 8 to 10 countries by 2015, and to achieve eradication of malaria worldwide.

Asymptomatic infection, mainly by *P. falciparum*, is an important obstacle to reaching those goals. Because asymptomatic carriers do not seek treatment for infection, the life cycle of the infection continues. The systematic identification and treatment of individuals with asymptomatic *P. falciparum*, as part of a surveil-

lance intervention strategy, are important for reducing the parasite reservoir and helping to decrease transmission of the disease.

In countries in which malaria is endemic, a significant proportion of *P. falciparum* infections are asymptomatic or subclinical. Microscopy-detected levels of asymptomatic carriage as high as 39% have been reported in other countries (2–6). Similar data from India are not yet available. The present study was designed to determine the prevalence of asymptomatic *P. falciparum* malaria and the epidemiology of antimalarial drug-resistant molecular markers among the prevailing strains of *P. falciparum* in the study population.

MATERIALS AND METHODS

Selection of the study population. This study was conducted in four neighboring villages of the Bundwan block in the Purulia district of West Bengal (22°47'N and 86°30'E), in which malaria is endemic (Fig. 1), and they all have a previous record of malaria transmission. The study villages are situated in a valley surrounded by thick forests and inhabited by a tribal population. The average annual rainfall of Purulia varies between 1,100 and 1,500 mm, and the rainy season is from July to September. Malaria transmission in this area is seasonal with two peaks, the first in

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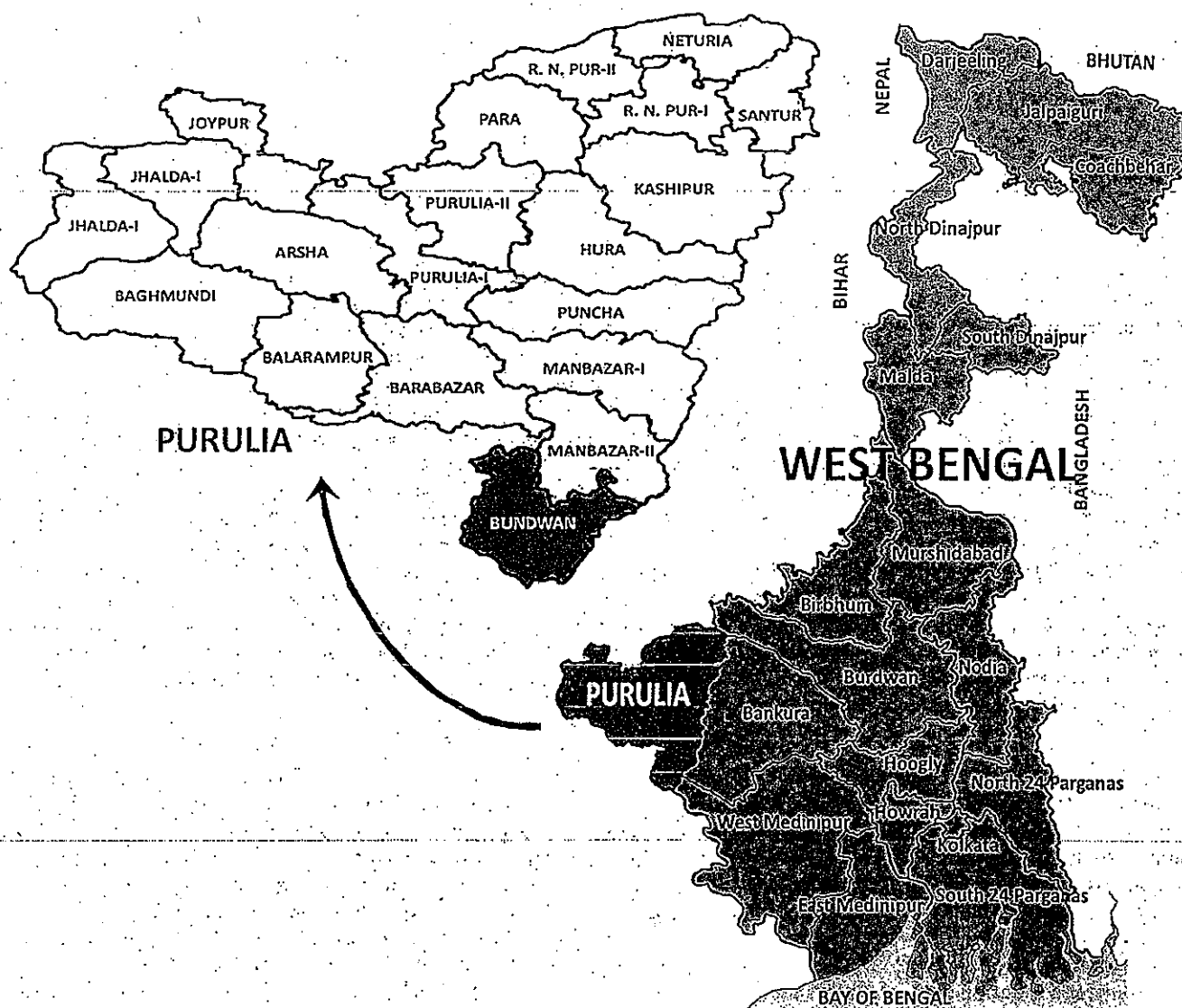


FIG 1 Map of study site.

July through October and the second in January. *P. falciparum* is its predominant malarial species.

Study procedures. At the commencement of the study, 10 field staff members from the study area were trained on the purpose and procedures of the project. The study group was divided into five teams with three members each, headed by a member from the Calcutta School of Tropical Medicine. Each team visited households door-to-door to clinically examine all available individuals and to collect blood samples for the laboratory diagnosis of malarial parasites. Rapid diagnostic testing (RDT) was performed at the field site, and microscopy was performed on the same day at the Bundwan Block Primary Health Centre. All positive individuals were treated with an artemisinin-based combination therapy (ACT), a combination of artesunate (AS) plus sulfadoxine-pyrimethamine (SP), per the National Vector Borne Disease Control Programme (NVBDCP) guidelines. Artesunate at 4 mg/kg body weight was administered once daily for 3 days, a single dose of SP (25/1.25 mg base/kg body weight) was administered on day 0, and a single dose of primaquine (PQ) (0.75 mg/kg) was administered on day 1. Study investigators directly observed and docu-

mented the administration of each dose of medication. In the event of vomiting within 30 min of administration, a full dose was repeated.

The PCR-based diagnosis was done at the Calcutta School of Tropical Medicine. The individuals who tested positive by this method but tested negative by microscopy and RDT were also treated as described above. All microscopy-positive patients were revisited on posttreatment days 1, 2, 3, 7, 14, 21, 28, 35, and 42 for therapeutic efficacy evaluations.

Laboratory evaluations. (i) Microscopy. Both thick and thin blood films were prepared from venous blood, stained with Giemsa stain, and examined by two microscopists for detection of malarial parasites and parasite species.

Parasite counts, performed by an experienced microscopist using a light microscope, were calculated by counting the number of parasites per 200 white blood cells (WBCs). Assuming a healthy WBC count to be 8,000/ μ l of blood, parasitemia was calculated and expressed as the number of parasites per μ l of blood.

(ii) RDT-based diagnosis. RDT was performed with a kit containing a monoclonal anti-*P. falciparum* histidine-rich protein II (HRP-II)-specific

TABLE 1 Prevalence of asymptomatic *P. falciparum* malaria and the sensitivity of three diagnostic methods

Village	Population (n)	Individuals examined (n)	Total positive (n [%])	No. of cases with the following test results:					
				RDT ⁺ PBS ⁺⁺ PCR ⁺	RDT ⁺ PBS ⁻ PCR ⁺	RDT ⁺ PBS ⁺ PCR ⁻	RDT ⁻ PBS ⁺ PCR ⁺	RDT ⁻ PBS ⁺ PCR ⁻	RDT ⁻ PBS ⁻ PCR ⁺
Tharkadaha	250	224	30	20	4	1	2	2	1
Burijhore	305	275	24	14	4	0	2	0	4
Asampani	236	225	25	21	2	0	2	0	0
Karu	249	239	2	2	0	0	0	0	0
Total	1,040	963	81 (8.4)	57	10	1	6	2	5

^a PBS, peripheral blood smear.

antibody and an anti-*Plasmodium vivax* p-LDH-specific antibody (Tulip Group, Goa, India) to detect any malarial parasite infection.

(iii) PCR-based diagnosis. Genomic DNA of the parasite was isolated from the EDTA blood samples using a QIAamp DNA blood minikit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

The *Plasmodium* genus- and species-specific primers were used separately in a two-step procedure for the detection of malarial infection. Three sets of oligonucleotide primers were used in this study. The primer set containing primers L1 (5'-TTAAATTTGTTGCAGTTAAACG-3') and L2 (5'-CCTGTTGTTGCCCTAAACTTC-3') is genus specific and was used to detect the presence of the *Plasmodium* parasite. The species-specific primers for *P. falciparum*, Pf1 (5'-TTAACTGGTTTGGGAAAA CCAATATATT-3') and Pf2 (5'-ACACAATGAAGTCAATCATGACTA CCCGTC-3'), and for *P. vivax*, Pv1 (5'-CGCTTCTAGCTTAATCCACA TAAGTGATAC-3') and Pv2 (5'-ACTTCCAAGCCGAAGCAAAGAAAG TCCTTA-3'), were used together in a single multiplex PCR as described previously (7).

The amplified PCR products were detected on a 1.0% agarose gel, which was subsequently stained with an ethidium bromide solution and visualized under UV light.

(iv) *msp1* and *msp2* genotyping. Genotyping of *P. falciparum* parasites was performed with a nested PCR assay based on the amplification of *msp1* and *msp2* as described in detail elsewhere (8) to differentiate between recrudescence and reinfection.

(v) Polymorphisms in *pfprt*, *pfmdr1*, *pfdhfr*, *pfdhps*, and *pfATPase6*. The region of the *pfmdr1* gene containing codons 86 and 184 was amplified by two pairs of primers as described elsewhere (9). A part of the *pfprt* gene covering the single nucleotide polymorphisms (SNPs) at codons 72 to 76 was amplified as described previously (10). The region of the *pfATPase6* gene spanning codon 769 was amplified as described previously (9). A portion of the *pfdhfr* gene spanning codons 16, 51, 59, 108, and 164 and a portion of the *pfdhps* gene spanning codons 436, 437, 540, 581, and 613 were amplified by two rounds of PCR using the primers described earlier (11, 12).

Direct sequencing of the PCR products was done on an ABI 3130XL genetic analyzer (Applied Biosystems, Foster City, CA). The sequences were analyzed using BioEdit software.

Statistical analysis. The free statistical software program R (version 2.13.1) was used to calculate the Z-test value for the comparison of the sensitivity of the three diagnostic tests used.

Ethical approval. The study protocol was approved by the institutional ethics committee of the Calcutta School of Tropical Medicine.

RESULTS

Prevalence of asymptomatic malaria in the study area. The field study was conducted in June 2012 in four adjoining villages of the Bundwan block of the Purulia district. The total population of the study area was 1,040 (487 male and 553 female). A total of 963 individuals were available in the villages and examined for malarial parasite infection by the three methods. At the field level, 68 people were found to be positive for the *P. falciparum* antigen, and

4 people were positive for *P. vivax*. Out of 68 *P. falciparum*-positive individuals, as determined by RDT, a malarial parasite was detected by microscopy in 58 cases. In addition, the *P. falciparum* parasite was detected in another 8 individuals who were negative according to the dual-antigen rapid diagnostic kit (RDK). The *P. falciparum* parasite was detected in 66 individuals, and of them, 29 were positive for both the ring form and gametocytes, 26 were positive for the ring form only, and 11 were positive for gametocytes only. The average parasite load of the asexual form of *P. falciparum* was 1,718, ranging between 120 and 12,800. The *P. vivax* parasite was detected in all 4 RDT-positive individuals. By PCR, *P. falciparum* infection was demonstrated in 78 individuals, and of them, 73 were positive by either RDT, microscopy, or both. In 5 cases, *P. falciparum* infection was detected by PCR only. It is interesting to note that 3 individuals were positive by RDT or microscopy but were negative by PCR.

A total of 81 individuals were found to be infected with *P. falciparum* (overall prevalence, 8.4%). As microscopy remains the gold standard for diagnostic purposes, 66 parasitologically confirmed individuals were proven asymptomatic carriers (prevalence, 6.8%). The remaining 15 individuals were suspected carriers who were positive by either RDT, PCR, or both (prevalence, 1.5%). Four cases were detected as *P. vivax* mono-infection by both RDT and microscopy.

Among the three diagnostic methods used, PCR was more sensitive (96.3%, 78/81) than RDT (83.9%, 68/81) and microscopy (81.9%, 66/81) (Table 1). The sensitivity of PCR was significantly higher than that of RDT ($Z = 2.684$, $P = 0.0036$) and that of microscopy ($Z = 2.681$, $P = 0.0036$). No significant difference between the sensitivities of RDT and microscopy ($Z = 0.401$, $P = 0.344$) was detected.

Efficacy of artesunate plus sulfadoxine-pyrimethamine in asymptomatic *P. falciparum* malaria. All 81 *P. falciparum*-positive individuals were treated by AS plus SP as per the government of India's national guidelines for the treatment of malaria. Sixty-six microscopically positive true asymptomatic individuals were followed for 42 days posttreatment. The demographic parameters of these cases are listed in Table 2. Splenic enlargement was noted in 5 (7.6%) cases, and hepatic enlargement was seen in 2 (3.03%) cases. Out of these 66 cases, recurrent parasitemia was recorded in 2 cases which were classified as late parasitological failure (LPF) by *msp1* and *msp2* genotyping (efficacy rate, 97% [64/66]).

Prevalence of polymorphisms in marker genes associated with antimalarial drug resistance. We attempted to study the polymorphisms of the *pfprt*, *pfdhfr*, *pfdhps*, *pfmdr1*, and *pfATPase6* genes in 30 randomly selected isolates. The mutant South American haplotype CVIET was found in the *pfprt* gene of all isolates.

TABLE 2 Baseline characteristics of parasite-positive patients

Characteristic (n = 66)	Result
Sex (no. [%])	
Male	40 (49.4)
Female	41 (50.6)
Age category (no. [%])	
0–4 yr	10 (12.3)
5–8 yr	13 (16.1)
9–14 yr	10 (12.3)
Adult	48 (59.3)
Liver size (cm)	
Mean	0.189
Range	0–5
SD	0.876
95% CI*	0–0.482
Spleen size (cm)	
Mean	0.365
Range	0–5
SD	0.976
95% CI	0.039–0.691
Parasite count (no./ μ l)	
Mean	1,718
Range	120–12,800
SD	2,733.1
95% CI	1,045.5–2,390

* CI, confidence interval.

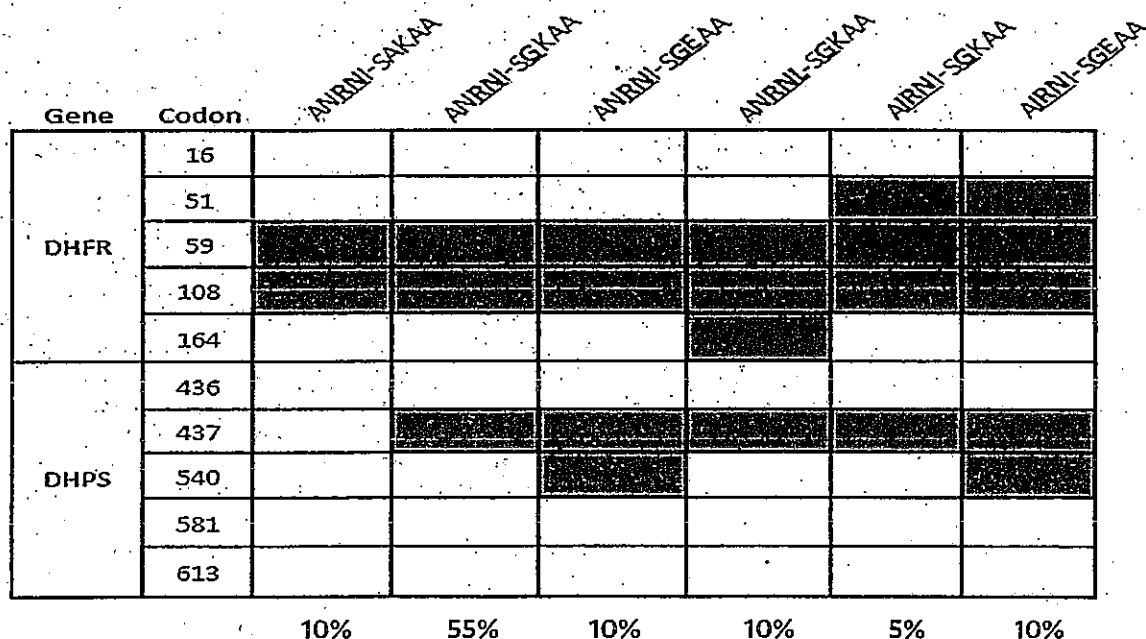
The *pfmdr1* gene was sequenced successfully in 27 isolates. The mutation N86Y was observed in 15 isolates (55.5%), and the Y184F mutation was demonstrated in 3 (11%) isolates only (the mutated amino acids are indicated by bold type). No S769N mutation in the *pfaTPase6* gene was observed in the prevailing para-

site population. The *pfdhfr* gene was sequenced successfully in 24 isolates that covered codons 16, 51, 59, 108, and 164. An N51I mutation at codon 51 was found in 12.5% of the isolates, and C59R and S108N mutations at codons 59 and 108, respectively, were found in 100% of the isolates. Three different haplotypes were recorded, among which the double mutant A₁₆N₅₁R₅₉N₁₀₈I₁₆₄ was the most prevalent (77%). A triple mutant, A₁₆I₅₁R₅₉N₁₀₈I₁₆₄, was recorded in 12.5% of the isolates. Sequencing of the *pfdhps* gene was successful in 26 isolates. Mutations at codons 437 (A437G) and 540 (A540E) were noted in 92.3% and 15.3% of the isolates, respectively. Among the three different haplotypes, S₄₃₆G₄₃₇K₅₄₀A₅₈₁A₆₁₃ was the most prevalent (80%).

Both the *pfdhfr* and the *pfdhps* genes were sequenced in 20 isolates. The triple mutation haplotype A₁₆N₅₁R₅₉N₁₀₈I₁₆₄-S₄₃₆G₄₃₇K₅₄₀A₅₈₁A₆₁₃ was found in 55% of the isolates. A quintuple mutation, A₁₆I₅₁R₅₉N₁₀₈I₁₆₄-S₄₃₆G₄₃₇E₅₄₀A₅₈₁A₆₁₃, with triple *pfdhfr* and double *pfdhps* was recorded in 10% of the cases (Fig. 2). This particular mutant haplotype has been found to be associated with SP resistance.

DISCUSSION

Jalpaiguri and Purulia are the two districts in West Bengal which have recorded the largest numbers of *P. falciparum* malaria cases during the last few years. A focus of chloroquine (CQ)-resistant (resistance level III [R-III] type) *P. falciparum* malaria was first reported from Purulia (13). Malaria transmission in this part of the state is seasonal. The prevalence of symptomatic malaria during the dry season is relatively low or even zero. The present study was done during the month of June, with temperatures ranging as high as 47°C to 49°C. Malaria transmission during that period was very low due to reduced vector population owing to the high atmospheric temperature. Under the existing conditions, an overall prevalence of 8.4% *P. falciparum* infection was detected without any signs or symptoms of malaria, of which 6.8% were parasitologically confirmed to be true carriers and 1.6% were suspected

FIG 2 Prevalence of *pfdhfr*-*pfdhps* haplotypes in the study population (n = 20).

carriers. It has been observed that *P. falciparum* infection can persist asymptomatically in semi-immune individuals for more than 18 months when the possibility of reinfection is excluded (14). Similar to the observation of our present study, the persistence of asymptomatic *P. falciparum* infection interseasonally, in areas with seasonal transmission (15), has been reported in African countries (12, 16, 17). Such findings have driven presumptive intermittent antimalarial treatment strategies for asymptomatic carriers, regardless of their infection status, to reduce the disease burden in African countries.

Age is one of the most important factors that correlate with protective immunity in areas in which malaria is endemic. Infection among nonimmune individuals invariably results in clinical manifestations and often leads to death, particularly in young children if left untreated, as they have not yet acquired immunity (18). Adults and older children have a lower prevalence of infection and a lower incidence of clinical symptoms (19), as these individuals acquire immunity from several episodes of malaria during the early part of their lives (20). As a result, an adolescent or adult is more likely to develop uncomplicated or asymptomatic malaria (21). In our present study, we observed that out of 81 *P. falciparum*-positive cases, only 10 cases were within the <4-year age group. Similarly, the prevalence of asymptomatic carriage has been reported to be the greatest in adolescents and children. In a study in the Gambia, the prevalence of asexual parasitemia (61%) was highest in individuals aged 5 to 15 years (5).

Identification and management of asymptomatic carriers have become new and important challenges for malaria control programs. One aspect of these challenges, in particular, is the systematic diagnosis of asymptomatic cases. A PCR-based method can detect low-level parasitemia which is below the microscopic potency level. In the present study, 5 cases were diagnosed by PCR only, but in the field and for mass screening, it is impossible to perform PCR for all cases.

An artemisinin-based combination therapy (ACT), which is advocated as the first line of antimalarial treatment, has been reported to be effective in reducing even submicroscopic levels of gametocytes (22–24). In the present study, we used an ACT, a combination of AS and SP, to treat all positive cases, and we observed that this combination was highly effective in the study population against the *P. falciparum* strain (efficacy, 97%). As a higher level of resistance to this combination has been reported from the Jalpaiguri district of the state (25), the efficacy of this combination should be monitored periodically.

The K76T mutation in the *pfcr* gene and the N86Y mutation in the *pfmdr1* gene were widely distributed, indicating a high potential for resistance against chloroquine in the prevailing parasite population. A mutant quadruple or quintuple haplotype with mutations at codons 51, 59, and 108 in the *pfdhfr* gene and codon 437, with or without 540 point mutations, in the *pfdhps* gene were detected in 10% of the isolates. This figure indicates the enhancing resistance power of *P. falciparum* strains to SP in the study area.

A significant proportion of our study population was harboring *P. falciparum* infection without any signs or symptoms of malaria. Naturally, this population does not tend to visit a diagnostic facility or seek antimalarial treatment. Thus, these individuals become reservoirs within the population for parasites, which enables further transmission of the disease. The parasite strains of the study area are resistant to CQ, as evidenced by the prevailing mutant *pfcr* and *pfmdr1* molecular markers. However, these strains

are sensitive to AS plus SP, an ACT recommended by the National Drug Policy of India, as evidenced by the *in vivo* efficacy study of the combination as well as the prevailing *pfdhfr*, *pfdhps*, and *pfATPase6* molecular markers. If malaria control programs are going to be successful in eliminating malaria, this hidden parasite burden needs to be addressed properly. This can be achieved by either active mass screening of the entire population or mass drug administration. Similar studies in other parts of the country might be helpful to determine the magnitude of the problem.

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We have no conflicts of interest concerning the work reported in this article.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2013年7月17日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	3D化人血清アルブミン (131I) 放射性3D化人血清アルブミン注射液 (富士フイルムRIファーマ株式会社)	研究報告 の公表 状況	ProMED-mail RABIES - INDIA (06): (MADHYA PRADESH): CANINE, HUMAN-TO-HUMAN TRANSMISSION SUSPECTED Published Date: 2013-07-06 Archive Number: 20130706.1810755	公国 インド	使用上の注意記載状況・その他参考事項等 特になし
販売名 (企業名)					
研究報告の概要	要約: 6歳男児が2013年1月に狂犬病の犬に襲われ、犬は翌日死亡、男児は3日後に狂犬病の症状を呈し、6月に死亡するまでに家族5人(父親、母親、祖母、叔父、叔母)を噛んだ。叔母によると男児に噛まれた後全員が狂犬病予防ワクチンを投与したが、男児の叔父は現在狂犬病の兆候を示している。 曝露後のタイムリーなワクチン投与にもかかわらず、6歳男児から一人の家族へ、噛まれたことによる狂犬病伝播が疑われた報告。				
報告企業の意見	今後の対応 本研究報告は、ヒト血液を原料とする血漿分画製剤とは直接関連がないことから、現時点で当該生物由来製品に 関し、措置等を行う必要はないと判断する。				
報告企業の意見	本報告は2013年1月に6歳男児が狂犬病の犬に襲われ、その3日後に狂犬病の症状を呈し、6月に死亡するまでに家族5人を噛み、その後家族全員が曝露後のワクチン投与をしたものの、男児の叔父が狂犬病の兆候を示したという、噛まれたことによるヒトからヒトへの狂犬病伝播が疑われた報告である。本報告はニュースサイトからの情報であり、症例の詳細や症状経過、この2例の狂犬病感染の根拠についても不明確なままであるが、狂犬病の患者に噛まれたヒトが狂犬病を発症したという報告はこれまでに無く、重大な感染症かつ新規感染経路に該当すると考えられ、感染症定期報告の対象と判断する。				



Published Date: 2013-07-06 21:20:24

Subject: PRO/AH/EDR> Rabies - India (06): (MP) canine, human-to-human transmission susp.

Archive Number: 20130706.1810755

RABIES - INDIA (06): (MADHYA PRADESH): CANINE, HUMAN-TO-HUMAN TRANSMISSION SUSPECTED

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Date: Fri 5 Jul 2013

Source: Bhaskar News [edited]

<http://daily.bhaskar.com/article/MP-OTC-uncle-bitten-by-six-year-old-rabid-boy-in-agar-road-in-ujjain-battles-for-life-4311555-NOR.html>

After being bitten by a 6-year-old rabid boy, 5 members of a family are battling for their lives. The boy was attacked by a dog in January [2013] in Agar Road in Ujjain [Madhya Pradesh state]. The rabies-affected dog died a day after biting the boy. According to family members, the 6-year-old boy developed rabies and bit 5 of them before his death in June [2013]. His uncle is said to have developed symptoms of rabies.

According to a source, he [the boy] died because he was treated by a quack. The boy developed symptoms of rabies 3 days after the dog bit him. He was rushed to MY Hospital when his condition became worse. However, the doctors refused to treat him because he was very critical.

The boy had bitten his father, mother, grandmother and an uncle and aunt before his death. The boy's aunt said, "We all received rabies inoculations after the boy's death. But my brother fell sick and we think he is also affected by rabies now. I don't know if anybody else in the family has developed rabies or not."

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[An unusual account of suspected transmission of rabies from a 6-year-old boy to a family member (by bite) despite timely administration of post-exposure prophylaxis to all family members bitten by the sick child. The story lacks independent corroboration, and time frames are confusing. In particular, it was not unequivocally confirmed that the boy actually died as a result of rabies virus infection. The diagnosis seems to be based on the biting behavior of the sick child before his death. Likewise it has not been established that the boy's sick uncle has actually contracted rabies virus infection. Further information is awaited.]

Ujjain is an ancient city in Malwa region in central India, on the eastern bank of the Kshipra River, today part of the state of Madhya Pradesh. It is the administrative centre of Ujjain District and Ujjain Division (<http://en.wikipedia.org/wiki/Ujjain>). - Mod:CP

A HealthMap/ProMED-mail map can be accessed at: <http://healthmap.org/r/7wFC>, <http://healthmap.org/r/7wFC>.]

See Also

Rabies - India (05): (MN) canine, human [20130618.1779367](#)

Rabies - India (04): (JK) canine, human, comment [20130326.1604680](#)

Rabies - India (03): (JK) canine, human [20130325.1602945](#)

Rabies - India (02): (TN) canine, human [20130315.1588809](#)

Rabies - India: (GA) canine, human [20130211.153897](#)

.....cp/je/dk

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Iodinated Human Serum Albumin-¹³¹I Injection

日本薬局方ヨウ化人血清アルブミン(¹³¹I)注射液

本剤は、貴重な血液を原料として製剤化されたものです。問診、感染症関連の検査等の安全対策を講じていますが、血液を原料としていることによる感染症の伝播等の危険性を完全に排除することはできないことから、疾病の診断上の必要性を十分に検討の上、必要最小限の使用にとどめるようお願いいたします。

【組成・性状】

1 バイアル中

容量	0.5mL
ヨウ化人血清アルブミン(¹³¹ I) (検定日時)	18.5MBq
添加物	リン酸一カリウム 2mg 水酸化ナトリウム 適量
外観	無色～淡黄色澄明な液体
pH	7.0～8.5
浸透圧比 (0.9%生理食塩液に対する比)	約0.3

本剤の成分である「ヨウ化人血清アルブミン(¹³¹I) (生物由来製品指定成分名)」には人血清アルブミン(採取国：日本、献血)が使用されている。

【効能又は効果】

1. 循環血漿量の測定
2. 循環血液量の測定
3. 血液循環時間の測定
4. 心拍出量の測定

【用法及び用量】

1. 循環血漿量の測定

本品0.185～0.74MBqを静注し、10～15分後採血し血漿中の放射能を計測する。注射全放射能と10～15分後血漿中放射能から希釈法の原理に従って次式により循環血漿量を算出する。

$$\text{○循環血漿量(mL)} = \frac{\text{注射液を希釈したもの1mLあたりの計数値}}{\text{注射後血漿1mLあたりの計数値}} \times \text{希釈倍数} \times \text{注射量(mL)}$$

2. 循環血液量の測定

循環血漿量を求めたのち、ヘマトクリット値(Ht)から次式により算出する。

$$\text{○循環血液量(mL)} = \frac{\text{循環血漿量}}{100 - \text{Ht}(\%)} \times 100$$

3. 血液循環時間の測定

本品0.185～1.85MBqを可及的速やかに注射し、ガンマカメラ又は指向性シンチレーション検出器を測定しようとする部位にあて、放射能の出現までに要する時間を測定する。

4. 心拍出量の測定

本品0.185～1.85MBq静注後、ガンマカメラ又は指向性シンチレーション検出器を心臓部にあて、放射能を連続記

録する。注射5分後に採血し、体外計数値を校正する。得られた希釈曲線をもとにして算出する。

○心拍出量(mL/分)

$$= \frac{(\text{注入した総計数値}) \times 60}{(\text{血液中の平均計数値}) \times (\text{第一次循環に要する時間(秒)})}$$

【使用上の注意】

1. 重要な基本的注意

- (1) 血液循環時間の測定又は心拍出量の測定における本品の使用は、本品より被曝の少ない薬剤が入手し得ない場合に限る。
- (2) 診断上の有益性が被曝による不利益を上回ると判断される場合にのみ投与することとし、投与量は最小限度にとどめること。

2. 高齢者への投与

一般に高齢者では生理機能が低下しているため、患者の状態を十分に観察しながら慎重に投与すること。

3. 妊婦、産婦、授乳婦等への投与

妊婦又は妊娠している可能性のある婦人及び授乳中の婦人には、原則として投与しないことが望ましいが、診断上の有益性が被曝による不利益を上回ると判断される場合にのみ投与すること。

4. 小児等への投与

小児等に対する安全性は確立していない(現在までのところ、十分な臨床試験成績が得られていない)。

5. 適用上の注意

前処置：本品を投与することにあたっては放射性ヨウ素が甲状腺に摂取されることを防止するため、投与前日から試験後も数日無機ヨウ素1日20mg以上を投与し、甲状腺ヨウ素摂取能を抑制しておく必要がある。

6. その他の注意

(社)日本アイソトープ協会医学・薬学部会放射性医薬品安全性専門委員会の「放射性医薬品副作用事例調査報告」において、まれに血管迷走神経反応、発熱、アレルギー反応などがあらわれることがあると報告されている。

【薬物動態】

本品は静注した場合、その放射能を血液たん白に転荷することではなく、体内では血漿成分と同じ挙動を示す。静注後10分以内に循環血液と完全に混合し、1時間後にその90%は血漿中に存在し、血液及びリンパ組織内に7～13時間とどまる。その後ヨウ化人血清アルブミン(¹³¹I)は分解されて¹³¹Iは主として尿中に排泄され、その量は1日に5～15%であり、¹³¹Iの約5%が甲状腺に摂取されるが、遊離された¹³¹Iが更に体内でヨウ化人血清アルブミン(¹³¹I)に合成されることはない。

Ⓐ 注意－医師等の処方せんにより使用すること。

【臨床成績】

ヨウ化人血清アルブミン (^{131}I) による循環血漿量、循環血液量、血液循環時間、心拍出量の測定に関しては、次のような臨床試験が報告されている。

1. 循環血漿量、循環血液量、心拍出量の測定

疾患名	症例数
僧帽弁狭窄症	20
僧帽弁閉鎖不全症	15
僧帽弁狭窄閉鎖不全症	10
大動脈弁閉鎖不全症	5
心房中隔欠損症	50
心室中隔欠損症	50
Fallot四徴症	13
肺動脈狭窄症	6
単純性甲状腺腫	73
甲状腺機能亢進症	80
甲状腺機能低下症	8

2. 血液循環時間の測定

疾患名	症例数
高血圧症	15
僧帽弁狭窄症	9
僧帽弁逆流症	13
左室不全症	12

【吸収線量】

吸収線量は次のとおりである²⁾。

臓器	吸収線量 ($\mu\text{Gy}/185\text{kBq}$)
全身	440
血液	870
生殖腺	440

【有効成分に関する理化学的知見】

1. ヨウ化人血清アルブミン (^{131}I)

・分子量 約69,000

2. ^{131}I の核物理学的特性

- (1) 物理的半減期 8.02070日
- (2) 主な γ 線エネルギー 364keV (81.7%)
- (3) 主な β 線エネルギー 606keV (89.9%)
- (4) β 線組織内飛程 2mm
- (5) 減衰表

経過日数 (日)	残存放射能 (%)	経過日数 (日)	残存放射能 (%)	経過日数 (日)	残存放射能 (%)
-3	129.6	8	50.1	19	19.4
-2	118.9	9	45.9	20	17.8
-1	109.0	10	42.1	21	16.3
0	100	11	38.7	22	14.9
1	91.7	12	35.5	23	13.7
2	84.1	13	32.5	24	12.6
3	77.2	14	29.8	25	11.5
4	70.8	15	27.4	26	10.6
5	64.9	16	25.1	27	9.7
6	59.5	17	23.0	28	8.9
7	54.6	18	21.1	29	8.2

【包装】

18.5MBq/0.5mL/バイアル

【主要文献】

- 1) 日本公定書協会監修：第十六改正日本薬局方解説書，廣川書店，東京 2011：C5036-5037
- 2) 飯尾正宏監修：核医学診断マニュアルインピボ編，テクノ，東京 1978：V-361

【文献請求先】**

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医薬品 研究報告 調査報告書

識別番号・報告回数	報告日		第一報入手日 2013. 5. 20	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿			公表国 WHOほか	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」120(日本赤十字社) 新鮮凍結血漿-LR「日赤」240(日本赤十字社) 新鮮凍結血漿-LR「日赤」480(日本赤十字社)		研究報告の公表状況 ProMED 20130518.1721873		
研究報告の概要	OMERSコロナウイルス 東地中海沿岸地域-2013年5月17日付 WHOによる概要報告 ※国際ウイルス分類委員会のコロナウイルス研究グループは、新型コロナウイルスを中東呼吸器症候群(MERS)コロナウイルスと いう新たな呼称にするよう提案した。 2012年4月からの累計で、MERSコロナウイルスに感染した確定患者数は合計40人となった。中東ではヨルダン、サウジアラビア、アラブ首長国連邦、カタール、欧州ではフランス、ドイツ、英国において患者が確認されている。フランスと英国では中東への渡航歴がなく、最近中東から帰国した渡航者との濃厚接触に起因するとみられる症例が発生している。性別が判明している患者の39人中31人が男性であり、年齢は24～94歳(中央値56歳)であった。報告された臨床症状は、ARDS、腎不全、DIC及びびん膜炎で、多くの患者に下痢を含む胃腸症状も見られた。40人の患者のうち20人が死亡した。 このウイルスは感染経路は不明であるが、動物から散発的にヒトに感染すると考えられている。また一方、ヒト-ヒト間の感染も起こり得ることは明白である。				
使用上の注意記載状況・ その他参考事項等	新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分 採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク				
報告企業の意見	今後の対応 日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としているほか、発熱などの体調不良者を献血不適としている。また、重症急性呼吸器症候群(SARS)患者または罹患者の疑いがある場合や既往がある場合は献血不適とし、SARS患者または罹患者の疑いのある者と接触した場合は、発熱等の症状の有無にかかわらず、最後に接触した日から3週間は献血不適としている。今後引き続き情報の収集に努める。				
MERSコロナウイルスのWHOによる概要報告である。					



Published Date: 2013-05-18 15:24:35
Subject: PRO/AH/EDR> MERS-CoV - Eastern Mediterranean (02): WHO summary, ECDC risk assessments
Archive Number: 20130518.1721873

MERS-COV - EASTERN MEDITERRANEAN (02): WHO SUMMARY; ECDC RISK ASSESSMENTS

A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

- In this update:
- [1] WHO Summary statement 17 May 2013
 - [2] ECDC risk assessment
 - [3] Newswire

[1] WHO summary statement 17 May 2013
Date: 17 May 2013
Source: WHO Global Alert and Response [edited]
http://www.who.int/csr/disease/coronavirus_infections/update_20130517/en/index.html

Novel coronavirus summary and literature update - as of 17 May 2013

Since April 2012, there have been 40 laboratory-confirmed cases of human infection with novel coronavirus (nCoV) [note there are presently 41 confirmed cases as mentioned in ProMED-mail post MERS-CoV - Eastern Mediterranean: Saudi Arabia, new case, RFI 20130518.1721601, earlier today 18 May 2013] - Mod.MPP]. Several countries in the Middle East have been affected, including Jordan, Saudi Arabia, the United Arab Emirates (UAE), and Qatar. Cases have also been reported by 3 countries in Europe: France, Germany, and the United Kingdom. All of the European cases have had a direct or indirect connection to the Middle East. However, in France and the United Kingdom, there has been limited local transmission among close contacts who had not been to the Middle East but had been in contact with a traveler recently returned from the Middle East.

The most recent case reported had onset on 10 May 2013. Most patients are male (79 percent; 31 of 39 cases with sex reported), and range in age from 24 to 94 years (median 56 years). All of the laboratory confirmed cases had respiratory disease as part of the illness, and most had severe acute respiratory disease requiring hospitalization. Reported clinical features include acute respiratory distress syndrome (ARDS), renal failure requiring hemodialysis, consumptive coagulopathy, and pericarditis. Many patients have also had gastrointestinal symptoms including diarrhea during the course of their illness. One patient, who was immunocompromised, presented with fever, diarrhea and abdominal pain but had no respiratory symptoms initially; pneumonia was identified incidentally on a radiograph. 20 of the 40 patients have died.

Since [6 Apr 2013], 21 cases of infection have been confirmed and reported in the region of Al-Ahsa in the Eastern Province of Saudi Arabia (16 males and 5 females, median age 56 years) [22 cases including the newly reported case of today, 18 May 2013 - Mod.MPP]. Nine of these have died, and 6 remain critically ill. Most patients were reported to have at least one comorbidity. The majority of the initial cases were associated with a single health care facility in Al-Ahsa. Additional cases have subsequently been identified who were not patients at the facility. Three family members of cases linked to the facility and 2 health care workers not associated with the Al-Ahsa facility but who had contact with laboratory confirmed cases have become infected. Two additional cases have been identified in the community that did not have any links with other cases from the Al-Ahsa healthcare facility. Although investigations are still ongoing into the source of this outbreak, early information indicated that only a small minority of these cases had contact with animals in the time leading up to their illness.

Since 8 May 2013, 2 cases have been reported by France. The 1st case became ill after a 9-day vacation to Dubai, UAE. The 2nd case, reported on 12 May 2013, is a patient who shared a room at a health care facility with the 1st case. Investigations to look for additional cases among fellow travelers of the 1st case and close contacts of both cases are currently underway, but no further cases have been identified. Of note, the patient's initial nasopharyngeal swab was

negative, but a bronchoalveolar lavage was found to be positive for nCoV [newly named MERS-CoV].

All clusters reported to date have occurred among family contacts or in a health care setting. Human-to-human transmission occurred in at least some of these clusters; however, the exact mode of transmission is unknown. So far, no evidence of sustained transmission beyond the clusters into the community has been observed.

Recent peer-reviewed papers published since the last update:

The Coronavirus Study Group of the International Committee on Taxonomy of Viruses has published a proposed new designation for the novel coronavirus, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Reference: De Groot RJ, et al. Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Announcement of the Coronavirus Study Group. J Virol. Published ahead of print 15 May 2013. doi:10.1128/JVI.01244-13. [available at: <http://jvi.asm.org/content/early/2013/05/08/JVI.01244-13.abstract>].

Summary assessment

The nCoV [MERS-CoV] is thought to be of animal origin and to be sporadically transmitted to humans through an as yet unknown route. However, it is clear that the virus can also be transmitted between humans. So far, human-to-human transmission has only been observed in health care facilities and close family contacts, and sustained transmission in the community has not been observed. The continued appearance of cases that are not part of larger clusters, and who do not have a history of animal contact, increases concerns about possible community transmission. This possibility is being investigated by authorities in Saudi Arabia.

The infection of 2 health care workers who had contact with infected patients and other examples of nosocomial transmission re-emphasize the need for meticulous adherence to appropriate infection control measures when nCoV is suspected, beginning with initial patient triage. Current infection control recommendations can be found at: http://www.who.int/csr/disease/coronavirus_infections/en/.

The large number of cases with reported co-morbidities suggests that increased susceptibility from underlying medical conditions may play a role in transmission. In addition, it has now been demonstrated that nCoV [MERS-CoV] infection may present atypically, and initially without respiratory symptoms, in immunocompromised individuals.

Limited evidence suggests that the use of nasopharyngeal swabs for diagnosis may not be as sensitive as the use of lower respiratory specimens. Lower respiratory specimens should be used for diagnosis in addition to nasopharyngeal swabs when they are available. If [a] nasopharyngeal swab tests negative, consider retesting using lower respiratory specimens such as sputum, endotracheal aspirate, or bronchoalveolar lavage. Clinicians should take care to follow strict infection prevention and control guidelines when collecting respiratory specimens of any kind.

The recent increase in cases may in part be related to increased awareness among the medical community; however, the demonstrated ability of this virus to transmit between humans and to cause large outbreaks has increased concerns about the possibility of sustained transmission. Countries in the Middle East in particular should maintain a high level of vigilance and a low threshold for testing of suspect cases. Current surveillance recommendations can be found at: http://www.who.int/csr/disease/coronavirus_infections/en/.

WHO expects that more cases will be identified. Control of the disease will require urgent multisectoral investigations aimed at identifying the source of the virus and the exposures that result in infection. It is critical for member states to report these cases and related information urgently to WHO, as required by the International Health Regulations, to inform effective international alertness, preparedness and response.

Communicated by:
ProMED-mail
<promed@promedmail.org>

[Important points to be highlighted in the above summary include the identification of 2 cases in the Eastern Province in Saudi Arabia that are presently considered to have been community acquired and not directly related to the outbreak in the health care facility. Another key point is the recommendation to use lower respiratory tract specimens rather than nasopharyngeal swabs for laboratory diagnosis of the MERS-CoV infection, an observation that may explain why some earlier contacts of confirmed cases who did have respiratory illnesses temporally consistent with infection, but were milder illnesses and had nasopharyngeal swabs taken that were negative for the MERS-CoV (see prior ProMED-mail report Novel coronavirus - Saudi Arabia (11): clin. lab. & epi. investigations 20121004.1324712).

[2] ECDC Risk Assessment

Date: 17 May 2013

Source: ECDC Rapid Risk Assessment [edited]

<http://www.ecdc.europa.eu/en/publications/Publications/risk-assessment-middle-east-respiratory-syndrome-coronavirus-MERS-CoV-17-may-2013.pdf>

Summary:

- As of 14 May 2013, 38 cases of Middle East respiratory syndrome coronavirus (MERS-CoV) have been reported worldwide, including 20 deaths. All cases remain associated (including indirect association following secondary person-to-person transmission in the UK and France) with transmission in the Arabian Peninsula and Jordan. The age of cases ranges from 24 to 94 years (N=34 cases), with a mean of 55.5 years and a male/female ratio of 1:0.2.
- The report of 19 new infections in Saudi Arabia in the past 2 weeks -- including one infection with the novel coronavirus [now known as EMC-CoV] acquired in the United Arab Emirates and later imported to Europe -- indicate that there is an ongoing source of infection and risk of transmission to humans in the Arabian Peninsula and Jordan.
- The most recent imported case, which resulted in a nosocomial transmission, originated in the United Arab Emirates and then moved to France. Both patients had underlying conditions and a degree of immunosuppression. One of the transmissions in the UK also affected an immunosuppressed person. These underlying conditions may be increasing vulnerability and the risk of transmission.
- The 1st French case raises the possibility that presentations may not include respiratory symptoms initially, especially in those with immunosuppression or underlying chronic conditions. This needs also to be taken into account when revising case-finding strategies.
- The confirmed infection in France of a patient who shared a hospital room with a patient returning from the United Arab Emirates indicates the risk of nosocomial transmission. This is the 2nd nosocomial transmission in Europe. The 1st one took place when an imported case in the UK visited a relative in the hospital in February 2013.
- These conclusions should be seen in the light of the many uncertainties that still continue with the investigation of cases in the Arabian Peninsula and Jordan. It is unusual to have such a degree of uncertainty at this stage in an outbreak.

Recommendations

- Healthcare workers in the EU should be vigilant in identifying patients that may require further investigation; they should also follow ECDC and national guidance for case finding. Patients developing severe respiratory infections and who have been in the Arabian Peninsula or neighbouring countries in the preceding 10 days should be investigated rapidly. Special attention should be given to medical evacuated patients from the Arabian Peninsula and neighbouring countries.
- Patients with chronic underlying conditions who develop severe infections (not just respiratory infections) should also be investigated rapidly for novel coronavirus if they have been in the Arabian Peninsula or neighbouring countries in the preceding 10 days.
- Since routine microbiological sampling (nasopharyngeal swabs) may give misleading negative results in persons later shown to be infected with the coronavirus, tests should be repeated with deeper respiratory sampling if a person fits into a category that requires investigation, especially if their condition is worsening.
- As demonstrated by a case with dual influenza and novel coronavirus infections, there is a possibility of co-infection; and this should be considered by healthcare personnel. Identification of one causative agent should not exclude testing for novel coronavirus where indicated.
- Mapping of international routes of medical evacuation or emergency medical care from the Arabian Peninsula and neighbouring countries to the EU could be considered in order to determine the most vulnerable centres in the EU where these cases might arrive.
- Companies undertaking medical evacuations from affected areas should be reminded of the risk of transferring infections across borders and of their obligations to protect staff engaged in the transfer; the same holds true for the staff of institutions which receive patients.
- Healthcare workers caring for patients under investigation for MERS-CoV should exercise standard infection control measures following national or international guidance.
- Close contacts of confirmed cases must be monitored for symptoms for 10 days after the last exposure; and should be tested, and should be informed what to do should they become ill. This should be carried out according to guidance, such as that developed by Public Health England UK (See "Sources of additional information" below).
- Healthcare workers caring for confirmed cases should be monitored for early symptoms of infection and advised to seek testing and thereafter self-isolate if they become unwell.
- Clusters of severe acute respiratory infections in the community or in healthcare settings, either among patients or healthcare workers, should always be reported rapidly and investigated for a range of pathogens, regardless of where in the world these infections occur.
- ECDC does not currently consider a need for testing individual patients with unexplained pneumonias or other respiratory symptoms unless they fall under one of the above categories.
- Any probable or confirmed case being diagnosed in the EU/EEA should be reported to national authorities through the Early Warning and Response System (EWRS) and to WHO under the International Health Regulations (2005). Reporting through EWRS qualifies as IHR notification and avoids double reporting. Patients still under investigation do not need to be reported internationally before confirmation, but information on outcomes of such testing exercises should be shared with ECDC.
- ECDC supports the WHO travel advice which imposes no travel or trade restrictions in relation to novel coronaviruses. However, EU citizens travelling to the Arabian Peninsula and neighbouring countries need to be aware of the presence of MERS-CoV in this geographical area and of the small risk of infection. Member States may consider active information efforts for travellers to areas most at risk.
- Although the reservoir of infection in the Middle East is unknown, other novel coronaviruses are zoonoses and have come from animal sources. Travellers should, therefore, follow standard good hygiene practices and avoid contact with animals or their waste products.

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Communicated by:
 ProMED-mail Rapporteur Kunihiro Iizuka

[For those readers who are interested in an excellent review of the current state of knowledge on the epidemiology and laboratory aspects of the MERS-CoV, reading the full report of the ECDC risk assessment, available at <http://www.ecdc.europa.eu/en/publications/Publications/risk-assessment-middle-east-respiratory-syndrome-coronavirus-MERS-CoV-17-may-2013.pdf> is highly recommended. There is an excellent epicurve by month of onset available on page 5 of the report, along with a mapping of cases by location of presumed exposure in the Arabian Peninsula available on page 6.

Reviewing the epicurves, this moderator is very much struck by the similarities with the initial stages of the SARS outbreak in 2002/2003. In the early stages, the cases appeared to be sporadic, until amplification in the healthcare setting occurred. One difference in the epicurves is seen in the X-axis. In the SARS outbreak, the epicurve was plotted by week of onset, whereas in the MERS-CoV, the plotting is by month of onset, probably related to the absence of information on exact dates of onset of many of the earlier confirmed cases.

The concerns raised in the above risk assessment for Europe are similar to those that would be raised elsewhere: concerns of importations of cases either through apparently healthy travellers (at the time of crossing international borders) and/or those of ill travellers seeking medical care outside of the country where the illness began. - Mod.MPP]

[3] Newswire

Date: 18 May 2013

Source: CTV News / Canadian Press [edited]

<http://www.ctvnews.ca/health/who-warns-saudi-coronavirus-may-be-spreading-seeks-urgent-search-for-source-1.1287364#ixzz2TeiGnbft>

The World Health Organization has issued a blunt assessment of the coronavirus outbreak in Saudi Arabia, acknowledging for the 1st time that there are concerns the virus may be spreading from person to person, at least in a limited way.

The statement called for urgent investigations to find the source of the virus and how it is infecting people. And it reminded countries they have a duty to the international community to rapidly report cases and related information to the WHO.

The worrying appraisal of the situation was echoed in a revised risk assessment issued Friday [17 May 2013, see below] by the European Centre for Disease Control. It warned hospitals in Europe to be on the lookout for coronavirus cases coming in by air ambulance, saying the numbers of such patients may rise if the public in affected countries are afraid to seek care in their own hospitals.

The warnings come as health leaders from around the world are gathering in Geneva for the World Health Assembly, the annual general meeting of the WHO. The 8-day meeting begins Monday [20 May 2013].

Though other nations have not publicly pressed leaders of coronavirus-affected countries for more transparency to date, it is likely that concerns about the virus and the opaque way investigations into it are being handled will be aired during the meeting.

"There is no formal agenda for novel coronavirus, but I would be surprised if it didn't come up," WHO spokesman Gregory Hartl said in an interview.

Saudi Arabia, after all, is the home of Mecca. The holy site draws roughly 3 million Muslims from around the world every year to the Hajj, a mass pilgrimage Muslims are meant to perform at least once in their lifetimes. As well, nearly one million international tourists travel to Mecca during Ramadan -- the Muslim month of fasting -- to take part in another pilgrimage called Umrah.

Ramadan starts in the 2nd week of July this year [2013]. And the 2013 Hajj will take place in mid-October [2013], only 5 months from now. Experts watching the coronavirus situation are already worried about the potential for spread of the new virus, both within Saudi Arabia and internationally.

"I don't think anyone necessarily knows for certain what is or isn't happening," said Dr. Kamran Khan, an infectious diseases specialist at St. Michael's Hospital in Toronto. In his research, Khan tracks global travel patterns as a tool to predict and interpret spread of diseases.

"I'm not sure that there's an easy way to get at this information unless it's more forthcoming from within Saudi Arabia or any of the partners that are working with Saudi Arabia inside the country."

To date, the WHO has been notified of 40 confirmed infections with the virus [see ProMED-mail post MERS-CoV - Eastern Mediterranean: Saudi Arabia, new case, RFI 20130518.1721601 for a report on the 41st case reported earlier today (18 May 2013). - Mod.MPP], which has been recently named MERS, for Middle Eastern respiratory syndrome coronavirus. Of those cases, 20 have been fatal.

The bulk of the infections have occurred in Saudi Arabia, which is investigating a large and potentially ongoing outbreak in

the eastern part of the country, near the Persian Gulf.

In its statement, the WHO said 2 of the cases in that outbreak have no links to either other coronavirus patients or a hospital where some transmission is known to have occurred. These unconnected cases suggest 2 possibilities. They could have contracted the virus from its as-yet unidentified reservoir, which is thought to be one or more animal species. Or these cases could be a signal that undetected human transmission is happening there.

"The continued appearance of cases that are not part of larger clusters, and who do not have a history of animal contact, increases concerns about possible community transmission. This possibility is being investigated by authorities in Saudi Arabia," the WHO statement revealed.

Other countries that have reported cases are Jordan, Qatar, the United Arab Emirates, Germany, Britain and France. But the infections in the European countries all had their origins in the countries on the Arabian Peninsula.

The statements from the WHO and the ECDC both underscored how little the world has learned about the new virus since its existence was 1st publicly announced last September [2012; see ProMED-mail post Novel coronavirus - Saudi Arabia: human isolate 20120920.1302733 for the 1st public announcement. - Mod.MPP].

"It is unusual to have such a degree of uncertainty at this stage in an outbreak," the European health agency's statement noted. It called the information that has been shared about the cases, including the current Saudi outbreak, "insufficient."

Illustrating the problem, Saudi Arabia's Ministry of Health changed the count on its coronavirus website on Friday [17 May 2013], adding another case, which brings the global total to 41. The ministry revealed only that an infected person had been found in the eastern region and was receiving care.

The ECDC statement also said that at this point, it cannot exclude the possibility that the virus is acting in a SARS-like manner, especially given that outbreaks in hospitals have now occurred in several instances.

Hospitals played a key amplifying role during the 2003 SARS outbreak, with undetected cases infecting other patients, visitors and health-care workers. The MERS virus is from the same family as the SARS virus:

The criticisms and concerns embedded in the 2 statements might appear mild, but in the world of public health diplomacy, they are unusually frank.

One expert called the WHO statement a shot across the bow for the countries that have been the sources of MERS infections.

"When you see those words, you realize that this is not being handled," said Michael Osterholm, director of the Centre for Infectious Diseases Research and Policy at the University of Minnesota.

"The WHO statement and the ECDC's, both, they're really important statements." But even at that, Osterholm fears the time for carefully worded warnings may be running out.

"You've got to kind of call the code," he said. "Either there is going to be an intensive effort made to understand what's going on in the Middle East and appropriate control measures brought to bear, or potentially we're going to be asking a lot of questions one day as to why we didn't do more."

Neither the WHO nor the ECDC is currently calling for the type of travel advisory the WHO levied on Toronto during the SARS outbreak. At the height of SARS, the WHO urged world travellers to stay away from afflicted areas, including Toronto.

But the ECDC said European travellers to the Arabian Peninsula and surrounding countries should be informed of the infection risk. And companies that operate medical evacuations should be reminded of their responsibility to try to prevent transmission of infections across borders.

It suggested that mapping the medical evacuation routes from that region would help to identify which centres in Europe might be at greatest risk of receiving unidentified coronavirus cases:

[Byline: Helen Branswell]

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[New information contained in the above documents includes the 1st good epicurve and mapping of reported cases thus far (in the ECDC risk assessment document) and the mention by WHO that 2 of the cases of MERS-CoV associated illness in the Eastern Province of Saudi Arabia did not have a history of exposure related to the ongoing nosocomial outbreak associated with a single health care facility (HCF) in the province. Specific information on these non-HCF cases was not

provided in the above documents.

The newswire report raises the potential concerns about upcoming pilgrimages to Saudi Arabia, the Umrah during Ramadan, (approximately 10 Jul 2013 - 10 Aug 2013, and involves approximately one million incoming pilgrims from around the world) and the Hajj (13 Oct 2013 - 18 Nov 2013, although 1st date for arrival of pilgrims in Saudi Arabia begins on 7 Sep 2013, and involves approximately 3 million incoming pilgrims from around the world <http://dobuy.co.uk/cbhuk/?p=3462>). For the map of Saudi Arabia, showing the location of Mecca, see http://commons.wikimedia.org/wiki/File:Mecca_Saudi_Arabia_locator_map.png.

If one looks closely at the map of confirmed MERS-CoV cases in the middle east, one sees that where location of exposure is known, the cases occurred in the eastern regions of the Saudi Arabian Peninsula, including Riyadh, and the Eastern Province in Saudi Arabia, Jordan, the UAE (Dubai and Abu Dhabi) and Qatar. Working under the presumption of a non-human animal intermediate host, this mapping suggests this intermediate host is more likely found in the eastern portion of the Saudi Arabian peninsula.

As is well stated in all 3 of the above reports (the WHO, ECDC and the newswire reports), more information on the findings of epidemiologic, laboratory, and environmental investigations of these cases is eagerly awaited. - Mod.MPP]

See Also

MERS-CoV - Eastern Mediterranean: Saudi Arabia, new case, RFI [20130518.1721601](#)
Novel coronavirus - Eastern Mediterranean (29): MERS-CoV, ICTV nomenclature [20130516.1717833](#)
Novel coronavirus - Eastern Mediterranean (28): Saudi Arabia, WHO [20130516.1716274](#)
Novel coronavirus - Eastern Mediterranean (27): Saudi Arabia, WHO, RFI [20130515.1715334](#)
Novel coronavirus - Eastern Mediterranean (26): Saudi Arabia new cases, RFI [20130514.1712370](#)
Novel coronavirus - Eastern Mediterranean (25): Saudi Arabia deaths, WHO [20130512.1708475](#)
Novel coronavirus - Eastern Mediterranean (24): France, 2nd case [20130512.1707305](#)
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Novel coronavirus - Eastern Mediterranean (21): Saudi Arabia [20130509.1701527](#)
Novel coronavirus - Eastern Mediterranean (20): France ex UAE, WHO, Saudi Arabia [20130508.1700034](#)
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Novel coronavirus - Eastern Mediterranean (18): Saudi Arabia [20130505.1693290](#)
Novel coronavirus - Eastern Mediterranean (17): Saudi Arabia [20130503.1688355](#)
Novel coronavirus - Eastern Mediterranean (16): Saudi Arabia, RFI [20130502.1686948](#)
Novel coronavirus - Eastern Mediterranean (15): camel exposure [20130405.1623188](#)
Novel coronavirus - Eastern Mediterranean (14): Germany ex UAE, WHO, fatal [20130326.1604564](#)
Novel coronavirus - Eastern Mediterranean (13): Germany ex UAE [20130326.1603038](#)
Novel coronavirus - Eastern Mediterranean (12): KSA, UK fatality, RFI [20130323.1600113](#)
Novel coronavirus - Eastern Mediterranean (11): UK, pers to pers transm [20130316.1588808](#)
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Novel coronavirus - Eastern Mediterranean (05): UK, 3rd case, [20130215.1544940](#)
Novel coronavirus - Eastern Med. (04): UK, pers to pers trans susp [20130213.1541531](#)
Novel coronavirus - Eastern Med. (03): Saudi comment [20130212.1540011](#)
Novel coronavirus - Eastern Med. (02): UK ex Saudi Arabia, Pakistan [20130212.1539086](#)
Novel coronavirus - Eastern Mediterranean: bat reservoir [20130122.1508656](#)
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Novel coronavirus - Eastern Mediterranean (05): WHO, transmission route [20121223.1465597](#)
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Novel coronavirus - Eastern Mediterranean (02): diagnostics [20121207.1442473](#)
Novel coronavirus - Eastern Mediterranean: WHO, Jordan, conf., RFI [20121130.1432498](#)
Novel coronavirus - Saudi Arabia (18): WHO, new cases, cluster [20121123.1421664](#)
Novel coronavirus - Saudi Arabia (17): 4th case, RFI [20121121.1418018](#)
Novel coronavirus - Saudi Arabia (16): whole genome sequence [20121114.1409556](#)
Novel coronavirus - Saudi Arabia (15): new case [20121104.1391285](#)
Novel coronavirus - Saudi Arabia (14): KSA MOH [20121022.1358297](#)
Novel coronavirus - Saudi Arabia (12): RFI [20121019.1353615](#)
Novel coronavirus - Saudi Arabia (11): clin. lab. & epi. investigations [20121004.1324712](#)
Novel coronavirus - Saudi Arabia (10): WHO, revised case def. [20120930.1315960](#)
Novel coronavirus - Saudi Arabia (09): real-time RT-PCR, addition [20120929.1315725](#)
Novel coronavirus - Saudi Arabia (08): real-time RT-PCR assay [20120928.1314254](#)

Novel coronavirus - Saudi Arabia (07): Eurosurveillance reports [20120928.1313337](#)
Novel coronavirus - Saudi Arabia (06): [20120927.1311743](#)
Novel coronavirus - Saudi Arabia (05): WHO, case def., nomenclature [20120926.1309747](#)
Novel coronavirus - Saudi Arabia (04): RFI, Jordan, April 2012 [20120925.1308001](#)
Novel coronavirus - Saudi Arabia (03): UK HPA, WHO, Qatar [20120923.1305982](#)
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Novel coronavirus - Saudi Arabia: human isolate [20120920.1302733](#)
.....mpp/mpp/mpp

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識別番号・報告回数		報告日	第一報入手日 2013年08月12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	①②抗HBs人免疫グロブリン ③④乾燥抗HBs人免疫グロブリン ⑤ポリエチレングリコール処理抗HBs人免疫グロブリン ①抗HBs人免疫グロブリン筋注200単位/1mL「日赤」 (日本血液製剤機構) ②抗HBs人免疫グロブリン筋注1000単位/5mL「日赤」 (日本血液製剤機構) ③へブスブリン筋注用200単位 (日本血液製剤機構) ④へブスブリン筋注用1000単位 (日本血液製剤機構) ⑤へブスブリンIH静注1000単位 (日本血液製剤機構)		研究報告の 公表状況 The Lancet Infectious Diseases, Early Online Publication/2013/08/09		公表国 スペイン; オマーン	
販売名 (企業名)						
研究報告の概要 2012年、新種のベータコロナウイルスー中東呼吸器症候群コロナウイルス (MERS-CoV) ーが中東の重症呼吸器疾患患者で確認された。2013年8月2日現在、46人の死亡を含む94の確定症例がWHOに報告されている。 MERS-CoV感染と関連した病気は、主に軽度から重度の呼吸器系の訴えによって特徴付けられ、大部分は急性呼吸困難 (窮迫) 症候群のために病院への入院を必要としている。併存症と免疫抑制は感染や重症に罹りやすくするよう、未発表の血清学的調査は無症候性感染が起こることを示唆する。 これまでに報告された全ての症例は、ヨルダン、カタール、サウジアラビア及びアラブ首長国連邦からのものであった。ヒトヒト感染は特に医療施設で報告されているが、利用可能な根拠に基づき基本再生産数 (R_0) は低いと考えられ、ウイルスが容易に感染しないことを示唆する。従って、MERS-CoVの主なりザーバーはおそらく動物である。異なるコロナウイルスは野生動物、家畜、家禽、ペット、そしてヒトを含む様々な宿主が持っている。幾つかのヒトコロナウイルスの起源で見られる様に、コロナウイルスは新しい宿主種に適応することができる。ヒトコロナウイルスOC43は、最近ウシコロナウイルスと共通の祖先を持っている。キクガシラコウモリ (Rhinolophid bats) は中間の肉食宿主を経由し、2002-2003年にヒトで明らかになった重症急性呼吸器症候群コロナウイルス (SARS-CoV) のもとともらしいザーバーとして特定された。分子クローリング解析は、SARS-CoVと密接に関連したウシウイルスのコロナウイルスとジャコウネコの株が、発生前の数年間に唯一分岐したことを示した。ヒトコロナウイルス229Eは、ガーナのキクガシラコウモリ (Hipposideros spp bats) で見つかったコロナウイルスと共通の祖先を持っている。 様々なコウモリの細胞系及び系統発生解析において複製可能なMERS-CoVは、ヨーロッパとアジアの小型コウモリ (Pipistrellus spp bats) からのベータコロナウイルス系統C型ウイルスと密接に関連していることを示す。ヒトMERS-CoVの疫学的に関連していない分離株の分子クローリング年代推定は、2012年後期に分岐した東部アラビア半島からの分離株のクラスターと共に、2011年中期における共通の祖先からのそれらの分岐を推定した。この知見は、ヒトでのMERS-CoVの多様性が中東での多様な独立した、地理的に体系化された人獣共通感染の事象の結果であることを示唆できた。 可能な動物のザーバーは、MERS-CoVの循環がどのように維持されているかを決定し、そして感染の連鎖を破るために特定する必要がある。MERS-CoVはヒトとコウモリを含む数種類の細胞に感染できる。機能的なレセプターは種の間で維持され、レセプターの使用が異種間感染に重要な障壁ではないことを示唆している。患者の暴露履歴のデータが不足しているが、ヒトコブラクダとヤギを含む家畜との接触を示唆している。2011年の食糧農業機構データは、ウシ、ヤギ、ヒツジとヒトコブラクダがヨルダン、サウジアラビア及びアラブ首長国連邦における肉とミルクの主要な源であることを示す。 血清学的調査は動物集団のスクリーニングに最も適しているが、幾つかの方法がヒトの抗体を検査するために記述されているにもかかわらず、動物でのMERS-CoVの報告はまだされていない。特異性のために、WHOは組換え型スパイクたん白質によるスクリーニング分析、及び中和へブスブリン						
使用上の注意記載状況・その他参考事項等 代表としてへブスブリンIH静注1000単位の記載を示す。 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抗体陰性であることを確認している。更に、プーリ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高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。						

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法による確認検査の組み合わせの使用を勧告する。ここでは、地域の内外の群れから集めたサンプルを用いて、中東における MERS-CoV の疫学に関連する可能性がある主要な家畜種からの血清サンプルの抗体プロファイリングを説明する。

血清サンプル収集方法

我々はカナリア諸島の 2 つの群れから、105 頭のヒトコブラクダ (*Camelus dromedarius*) の集団をサンプリングした。50 頭はオス、55 頭はメス、88 頭は大人、9 頭は年齢 3-4 歳、7 頭は年齢 2 歳、1 頭は年齢 3 ヶ月であった。両方の群れは、群れの間で頻繁に交換が行われ、同じ所有者が持っていた。一つの群れは他に家畜がない沿岸の砂丘生息地からのもの、もう一方の群れはーフルーソウモリを引き付けることができた一特定のマンゴとパパイヤーの熱帯フルーツ農園の近くの内陸溪谷にあり、近くに (約 500m) それぞれ 25 頭のウマと 300 匹のヤギの農場があった。ラクダはモロッコから輸入された 3 頭の大人を除いてカナリア諸島で生まれ、観光産業に使われている。80 の血清は 2012 年 4-6 月に、9 つの血清は 2013 年 5 月に採取された、そして 16 対の血清は 2012 年と 2013 年の 4-6 月にサンプリングされた (全て日常の獣医科用)。サンプルは頸静脈穿刺により得られた。

オマーンからの 50 頭のメスのヒトコブラクダは、2013 年 5 月にサンプリングされた。ラクダは年齢 8-12 歳で、別々の場所から異なる所有者に属していた。ラクダは競技用ラクダを引退し、現在繁殖用で、血液はブルセラ症の日常スクリーニングのために頸静脈穿刺により取得された。

血清は、オランダの 2 頭のラクダ (*Lama glama*)、6 頭のアルパカ (*Vicugna pacos*)、2 頭のフタコブラクダ (*Camelus bactrianus*) から、チリのブイン動物園の 2 頭のフタコブラクダ、18 頭のアルパカ、5 頭の野生のラクダ (*Lama guanicoe*) から獣医科用に集められた。ウシ ($n=40$)、国内のヤギ ($n=40$) とヒツジ ($n=40$) からの血清は、オランダの動物保健サービスへの定期提出物からであった。スペインの国内ヤギ ($n=40$) からの血清は、2011 年に結核対策用の提出物から Recursos Cinegeticos 研究所 (Ciudad Real, Spain) によって提供された。全ての血清は動物疾患立法に関してローカル規制とオランダの輸入規制に合意して得られた。前述したように、マイクロアレイに使用される 3 つの抗原に対する陽性ヒトコントロール血清を採取した。全てのサンプルは試験まで -20°C で保存された。

実験手順

(省略)

結果

血清は、たんぱく質マイクロアレイにおける MERS-CoV、SARS-CoV、及びヒトコロナウイルス OC43 S1 抗原と反応する IgG 抗体について検査した (図 1)。ヒトコロナウイルス OC43 は、血清学的に (19 世紀末に分離した) ウシコロナウイルス S1 は入手できないため、ヒトコロナウイルスは、ウシ、ヒツジ、ヤギ、そして旧世界と新世界のラクダで循環する。ウシコロナウイルス S1 は入手できないため、ヒトコロナウイルス OC43 S1 抗原が代用として使われた。3 頭のラクダ、4 頭のアルパカ、1 頭の野生のラクダ、そして 2 頭のフタコブラクダからの血清は、ヒトコロナウイルス OC43 抗原と反応した。105 頭のスペインのヒトコブラクダの内 16 頭 (15%) からの血清で反応したように、1 頭のウシと 1 頭のヤギの血清がヒトコロナウイルス OC43 抗原と反応した。ウシ、ヒツジとヤギからの全ての血清は MERS-CoV 抗原に陰性反応であったが、15 頭 (14%) のスペインのラクダからの血清は MERS-CoV 抗原と反応した (図 1)。反応性は、同じ血清は SARS-CoV 抗原と結合しなかったが、陽性対照標本に結合し、高い特異性があった。MERS-CoV 抗原とヒトコロナウイルス OC43 抗原との血清の反応性の間に相関が存在しなかった (図 2)。MERS-CoV 抗原と反応した一つの血清サンプル以外は、大人の動物からのものであった。一つの反応性血清は 2 歳の動物からであった。

スペインのラクダ血清中の MERS-CoV 特異的 IgG の存在を確認するために、我々はマイクロアレイによる MERS-CoV とヒトコロナウイルス OC43 抗原との反応性の異なる程度を持つ 49 頭のラクダの血清のサブセットを検査するために MERS-CoV 中和法を使用した。9 頭のスペインのラクダは 1/20~1/320 の間で力価変化する MERS-CoV 中和抗体を持っていた (表 1)。12 の血清の内 3 つが MERS-CoV スパイク抗原と反応したが、非中和エピソードの認識の可能性が最も高い MERS-CoV を中和しなかった。全ての MERS-CoV 中和血清はマイクロアレイ上の MERS-CoV 抗原と (殆ど) 飽和反応性を持っていた、一方ヒトコロナウイルス OC43 抗原との反応性は飽和反応性の 0~50% まで変化した (表 1)。可変ヒトコロナウイルス OC43 のシグナルは、MERS-CoV がヒトコロナウイルス OC43、或いはウシコロナウイルス抗原と一般的に交差反応しなかったこと

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を示唆する。MERS-CoV 中和抗体を持つ全ての9頭のラクダ(7頭はメス、2頭はオス)は、カナリア諸島で生まれ育った。8頭のラクダは大入で、1頭は2歳であった。

マイクロアレイによるヒトコロナウイルス OC43 抗原を持つラクダ血清の反応性がウシコロナウイルス IgG の存在に起因したことを示すために、更にウシコロナウイルス抗体により交差反応によって生じる MERS-CoV 中和活性を除外するために、我々は MERS-CoV とウシコロナウイルスのブラーク除去中和検査の比較において十分な血清サンプルが残っているサンプル (n=15) を有するラクダを検査した (図2、表2)。全てのラクダの血清はウシコロナウイルスを中和した。マイクロアレイでの OC43 のための 20,000RFU より低いカットオフを示した (図1)。5頭のラクダはウシコロナウイルス (マイクロアレイ上のヒトコロナウイルス OC43 抗原に対して 50,000RFU より大きい平均シグナル強度) に対して高い中和抗体価を持っていたが、MERS-CoV 中和に対して陰性であった (この方向での交差反応は起こらなかった、そして MERS-CoV 中和活性はウシコロナウイルス中和抗体の存在に起因していなかった) ことを示唆している。MERS に罹った患者からの血清サンプルは、高い力価 (1/640) を持つ MERS-CoV を中和したが、ウシコロナウイルスは効果的に中和しなかった (力価 1/80)。後者の知見は九分通り以前のヒトコロナウイルス OC43 への感染に起因していた。この患者はヒトコロナウイルス OC43 組換えスパイク免疫蛍光抗体染色法で高力価 (1/5120) を持ち、マイクロアレイでのヒトコロナウイルス OC43 抗原との飽和シグナルを持った。ヒトコロナウイルス OC43 に対して陽性の2つのヒト血清サンプルは、(1/80)の力価でウシコロナウイルスを中和した (表2)。

我々はマイクロアレイによる1/20の希釈及び MERS-CoV 中和検査でオマーンのヒトコブラクダからの50の血清を検査した。全ての血清は、マイクロアレイ上で MERS-CoV 抗原と飽和反応性を、SARS-CoV 抗原反応性はなく、そしてヒトコロナウイルス OC43 抗原反応性は陰性 (20,000RFU のカットオフ以下) と飽和シグナルの間で変化を示した (図1、表1)。1/20の初期希釈で両方の抗原に対する飽和反応性を持つ2つの血清の連続希釈は、MERS-CoV 抗原反応性が1/5120のカットオフ以上にまでであることを、逆にヒトコロナウイルス OC43 抗原反応性は1/80~1/320までの希釈でカットオフを下回ったことを示した (図2D)。マイクロアレイデータと一致して、全ての血清は1/320 (50 サンプルの内7) ~1/2560、或いはより以上 (16、或いは50 サンプル) の間で変化する力価を持つ、高 MERS-CoV 中和能力を持っていた。

論議

本調査では、我々は MERS-CoV と関係した地域 (オマーン) と関係していない地域 (カナリア諸島) の両方のヒトコブラクダにおける MERS-CoV の中和抗体の存在を認めた。オマーンのヒトコブラクダからの全ての血清とスペインのヒトコブラクダからの一部の血清は、MERS-CoV 受容体結合領域 S1 と特異的 IgG 反応性を持っていた。我々は別のベータコロナウイルス-ウシコロナウイルス-ウシコロナウイルス-ウシコロナウイルスがこれらのラクダで循環していたとすると我々の予想を確認した。しかしながら、全てのオマーンのラクダと9頭 (9%) のスペインのラクダは、ウシコロナウイルス抗体によって明らかに交差反応に起因していなかった MERS-CoV に対して特異的中和抗体を持っていた。

我々の調査は、それらの動物で MERS-CoV に特異な抗体の存在について検査した最初である。動物のスクリーニングは MERS-CoV の疫学を理解するために必要である。現時点では、コウモリは SARS-CoV 同様に幾つかの確立したヒトコロナウイルスの最終的なリザーバーであると考えられる。従って、系統解析は MERS-CoV がアブラコウモリ属コウモリで見つかっていたベータコロナウイルス系統 C 型ウイルスと関連していることを示している。しかしながら、コウモリからヒトへの MERS-CoV の直接感染はありそうもない。おそらくヒトとの濃厚接触が可能な中間宿主 (例えば、家畜) の確認が緊急に必要とされた。中東における共通の家畜種はヒトコブラクダであるが、ウシ、ヒツジ、ヤギも含まれる。入手可能なデータに基づいて、我々は MERS (これらの種での種での関連したコロナウイルス) の循環を排除することができないため、血清は疫学的に関連した地域から入手できなかった。

オマーンからのヒトコブラクダの MERS-CoV 中和抗体の高い陽性率は、この集団での MERS-CoV、或いは密接に関連したウイルスの循環を示唆する。しかしながら、パンコロナウイルスと特異的なベータコロナウイルス 2C PCR 法を用いたスペインのラクダの血清及び糞便サンプルにおけるウイルスの配列を同定する試みは、糞便サンプルのデリン配列決定の後に増幅を目標にできなかった (未発表データ) として、成功しなかった (未発表データ)。これらの結果は、ラクダがサンプリング時に積極的なウイルスを排出していなかったことを意味している。カナリア諸島の動物の10%未満で、1/320までの力価で MERS-CoV 中和血清を持っていた。この低い抗体陽性率は、他の推定リザーバーへの動物の曝露は稀であるか、ウイルスが約2000頭の動物のこの切り離された集団では存在しないかのどちらかを意味する。我々は、集団がサンプリング時までに流行があったかたもれないが、抗体価が減少し且つウイルスの新たな伝播が起きなかったことを否定できない。

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い。 ラクダは野生のげっ歯類、ウサギ、ハトと野生の小鳩、そしておそらくコウモリとも接触していた。3つの小型コウモリ属を含む7つの食虫コウモリの種はカナリア諸島原産である、一方エジプトのフルツコウモリ (<i>Rousettus aegyptiacus</i>) が持ち込まれた。異なる所有者と場所からのオマーンのラクダの強力飼育者を持つ 100%の抗体陽性率は、MERS-CoV、或いは密接に関連したウイルスの広範な循環で、中東における異なる状況を示唆している。増加した動物の感染性とヒト感染で、疫学のこの差は中東で循環しているウイルスがスペインで循環しているウイルスと異なるためかもしれない。その上、オマーンのラクダはかつて競走用ラクダで、現在は繁殖用として飼われ、ウイルス感染を保持する環境を維持する可能性がある。ウシの場合は、関係は呼吸器疾患、管理医療、そして動物の輸送の発生率と影響の間で確立されている。我々の知る限りでは、オマーンとカナリア諸島のラクダ集団は繋がっていない。カナリア諸島のラクダは、もともと労働と輸送のためにアフリカのホーンから 15 世紀に輸入された。今日では、アフリカからの動物の輸入は中絶疫のリスクのために禁止されている。我々の調査では3頭のラクダだけが、18 年以上前に最初にモロッコから輸入された。MERS-CoV の最も近い親類はアフリカの <i>Neoromicia zuluensis</i> コウモリでごく最近特定されたため、MERS-CoV、或いは一部のアフリカのラクダの集団における関連したウイルスの伝来は数十年前に起こることができた、カナリア諸島のラクダの MERS-CoV 抗体に対する可能な説明を与えている。中東では、膨大な数のラクダが肉の需要を満たすためにアフリカから輸入されている。上位 5 つのラクダ繁殖国は全てアフリカであり、サウジアラビアとアラブ首長国連邦は上位 5 つのラクダの肉生産国である。免疫学的に無感作の動物のより頻繁な流入を通して、カナリア諸島と比べて中東における動物のこの増加した回転率はウイルスの疫学に影響を及ぼすこともできた。 目標を絞った調査は、MERS-CoV のヒト症例に関して我々の知見とそれらの可能な関連を確認するために必要である。疫学的背景情報が入手できるラクダからの組織的な、そしてより最近のサンプルの比較抗体陽性率検査は、抗体陽転している動物からのサンプルのウイルス学的評価と同様に、この MERS-CoV 関連のウイルスを同定し、そして特徴描写するために必要である。その間に、我々はラクダやそれらの製品がヒト感染の潜在的な感染源であるかどうかを確認するために、動物製品を含むどんな動物に暴露したかの見直し、そして目標を絞った、前向きな血清調査により、MERS-CoV に罹った人の詳しい症例履歴を推奨する。	<table border="1"> <tr> <th data-bbox="1005 560 1053 2161">報告企業の意見</th><th data-bbox="1053 560 1332 2161">今後の対応</th></tr> <tr> <td data-bbox="1005 560 1053 2161"> 「Middle east respiratory syndrome coronavirus : MERS-CoV (マーズコロナウイルス)」と命名された新種のコロナウイルスである。コロナウイルスは、コロナウイルス科 (Coronaviridae) コロナウイルス亜科 (Coronavirinae) に属する直径 80~220nm の円形或いは楕円形のエンベロープを有する 1 本鎖の RNA ウイルスで、万一原料血漿にコロナウイルスが混入したとしても、Bovine viral diarrhoea virus (BVDV) をモデルウイルスとしたウイルススクリーニング試験成績から、本剤の製造工程において不活化・除去されると考えている。 </td><td data-bbox="1053 560 1332 2161"> 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。 </td></tr> </table>	報告企業の意見	今後の対応	「Middle east respiratory syndrome coronavirus : MERS-CoV (マーズコロナウイルス)」と命名された新種のコロナウイルスである。コロナウイルスは、コロナウイルス科 (Coronaviridae) コロナウイルス亜科 (Coronavirinae) に属する直径 80~220nm の円形或いは楕円形のエンベロープを有する 1 本鎖の RNA ウイルスで、万一原料血漿にコロナウイルスが混入したとしても、Bovine viral diarrhoea virus (BVDV) をモデルウイルスとしたウイルススクリーニング試験成績から、本剤の製造工程において不活化・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。
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Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study



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Summary

Background A new betacoronavirus—Middle East respiratory syndrome coronavirus (MERS-CoV)—has been identified in patients with severe acute respiratory infection. Although related viruses infect bats, molecular clock analyses have been unable to identify direct ancestors of MERS-CoV. Anecdotal exposure histories suggest that patients had been in contact with dromedary camels or goats. We investigated possible animal reservoirs of MERS-CoV by assessing specific serum antibodies in livestock.

Methods We took sera from animals in the Middle East (Oman) and from elsewhere (Spain, Netherlands, Chile). Cattle (n=80), sheep (n=40), goats (n=40), dromedary camels (n=155), and various other camelid species (n=34) were tested for specific serum IgG by protein microarray using the receptor-binding S1 subunits of spike proteins of MERS-CoV, severe acute respiratory syndrome coronavirus, and human coronavirus OC43. Results were confirmed by virus neutralisation tests for MERS-CoV and bovine coronavirus.

Findings 50 of 50 (100%) sera from Omani camels and 15 of 105 (14%) from Spanish camels had protein-specific antibodies against MERS-CoV spike. Sera from European sheep, goats, cattle, and other camelids had no such antibodies. MERS-CoV neutralising antibody titres varied between 1/320 and 1/2560 for the Omani camel sera and between 1/20 and 1/320 for the Spanish camel sera. There was no evidence for cross-neutralisation by bovine coronavirus antibodies.

Interpretation MERS-CoV or a related virus has infected camel populations. Both titres and seroprevalences in sera from different locations in Oman suggest widespread infection.

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Introduction

In 2012, a new betacoronavirus—Middle East respiratory syndrome coronavirus (MERS-CoV)—was identified in patients with severe respiratory disease in the Middle East. As of Aug 2, 2013, 94 laboratory-confirmed cases, including 46 deaths, have been reported to WHO.¹ Illness associated with MERS-CoV infection is characterised primarily by mild-to-severe respiratory complaints, most requiring hospital admission for acute respiratory distress syndrome. Comorbidities and immunosuppression seem to predispose for infection and severe disease,^{2,4} and unpublished serological studies suggest that asymptomatic infections occur.⁷

All cases reported so far have been linked to Jordan, Qatar, Saudi Arabia, and United Arab Emirates. Human-to-human transmission has been reported, particularly in health-care settings, but on the basis of available evidence the basic reproduction number (R_0) is thought to be low, suggesting that the virus is not transmitted readily.^{6,8} Therefore, the primary reservoir of MERS-CoV is probably animals. Different coronaviruses have various hosts including wildlife, livestock, poultry, pets, and

human beings. Coronaviruses can adapt to new host species, as shown by the zoonotic origin of several human coronaviruses.⁹ Human coronavirus OC43 has recent common ancestry with bovine coronaviruses.¹⁰ Rhinolophid bats were identified as a likely reservoir for severe acute respiratory syndrome coronavirus (SARS-CoV), which emerged in people in 2002–03, through intermediate carnivorous hosts.¹¹ Molecular clock analysis¹² showed that bat and civet strains of viruses closely related to SARS-CoV only diverged a few years before the outbreak. Human coronavirus 229E has a common ancestor with coronaviruses found in Ghanaian *Hipposideros* spp bats.¹³

MERS-CoV is able to replicate in various bat cell lines¹⁴ and phylogenetic analyses show that it is closely related to betacoronavirus lineage C viruses from *Pipistrellus* spp bats in Europe and Asia.^{15–18} Molecular clock dating of epidemiologically unlinked isolates of human MERS-CoV estimated their divergence from a common ancestor in mid-2011,^{4,19} with a cluster of isolates from the eastern Arabian peninsula diverging in late 2012.⁴ This finding could suggest that the diversity of

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MERS-CoV in people is the result of multiple independent, geographically structured, zoonotic events in the Middle East.^{4,19}

Possible animal reservoirs need to be identified to determine how circulation of MERS-CoV is maintained and to break the chain of transmission.²⁰ MERS-CoV can infect cells of several species, including human beings and bats.¹⁴ The functional receptor is conserved between species, suggesting that receptor use is not an important barrier to cross-species transmission.²¹ Data for exposure history of patients are scarce, but suggest contact with livestock, including dromedary camels and goats.^{24,5} Food and Agriculture Organization data from 2011 show that cows, goats, sheep, and dromedary camels are the main sources of meat and milk in Jordan, Saudi Arabia, and United Arab Emirates.²⁴

Serological studies are best suited to screen animal populations, but have not yet been reported for MERS-CoV in animals, although several methods have been described for testing antibodies of people.^{22,23} For specificity, WHO recommends use of a combination of screening assays with recombinant spike protein, and confirmatory testing by neutralisation assays. Here, we describe antibody profiling of serum samples from major livestock species that might be relevant to the epidemiology of MERS-CoV in the Middle East, using samples collected from herds inside and outside the region.

Methods

Serum sample collection

We sampled a cohort of 105 dromedary camels (*Camelus dromedarius*) from two herds on the Canary Islands. 50 were male, 55 were female, 88 were adults, nine were age 3–4 years, seven were age 2 years, and one was age 3 months. Both herds had the same owner, with frequent exchange of animals between the herds. One herd is from a coastal dune habitat with no other livestock, while the other herd is in an inland valley close to a tropical fruit farm, in particular mango and papaya—which could attract fruit bats—and nearby (roughly 500 m) to horse and goat farms with 25 and 300 animals, respectively. The camels were born in the Canary Islands except for three adults, which were imported from Morocco.²⁵ The camels are used in the tourist industry. 80 sera were taken April–June, 2012, nine in May, 2013, and 16 paired sera were taken in these months in 2012, and 2013, all for routine veterinary purposes. Samples were obtained by jugular puncture.

50 female dromedary camels from Oman were sampled in March, 2013. The camels were aged 8–12 years and belonged to different owners from separate locations. The camels are retired racing camels now used for breeding, and blood was taken by jugular puncture for routine screening for brucellosis.

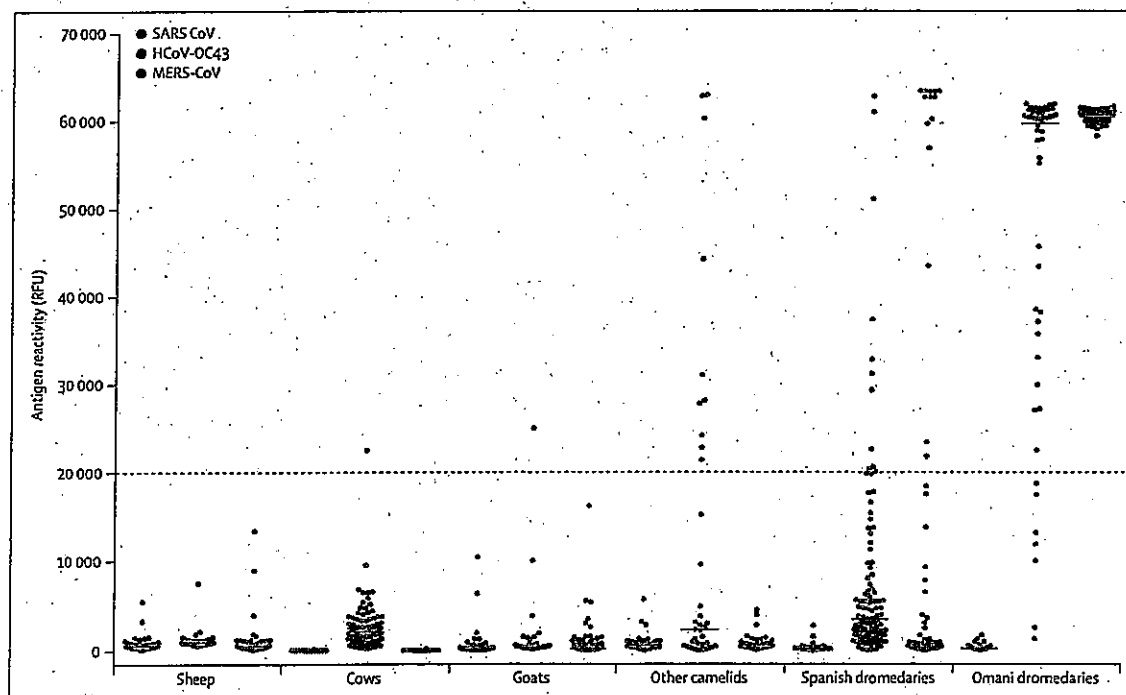


Figure 1: Reactivity of livestock sera with three coronavirus S1 antigens

Fluorescent intensities per antigen at a serum dilution of 1/20. Black lines indicate median. Dashed line is cutoff of the assay. RFU=relative fluorescence units. SARS-CoV=severe acute respiratory syndrome coronavirus. HCoV=human coronavirus. MERS-CoV=Middle East respiratory syndrome coronavirus.

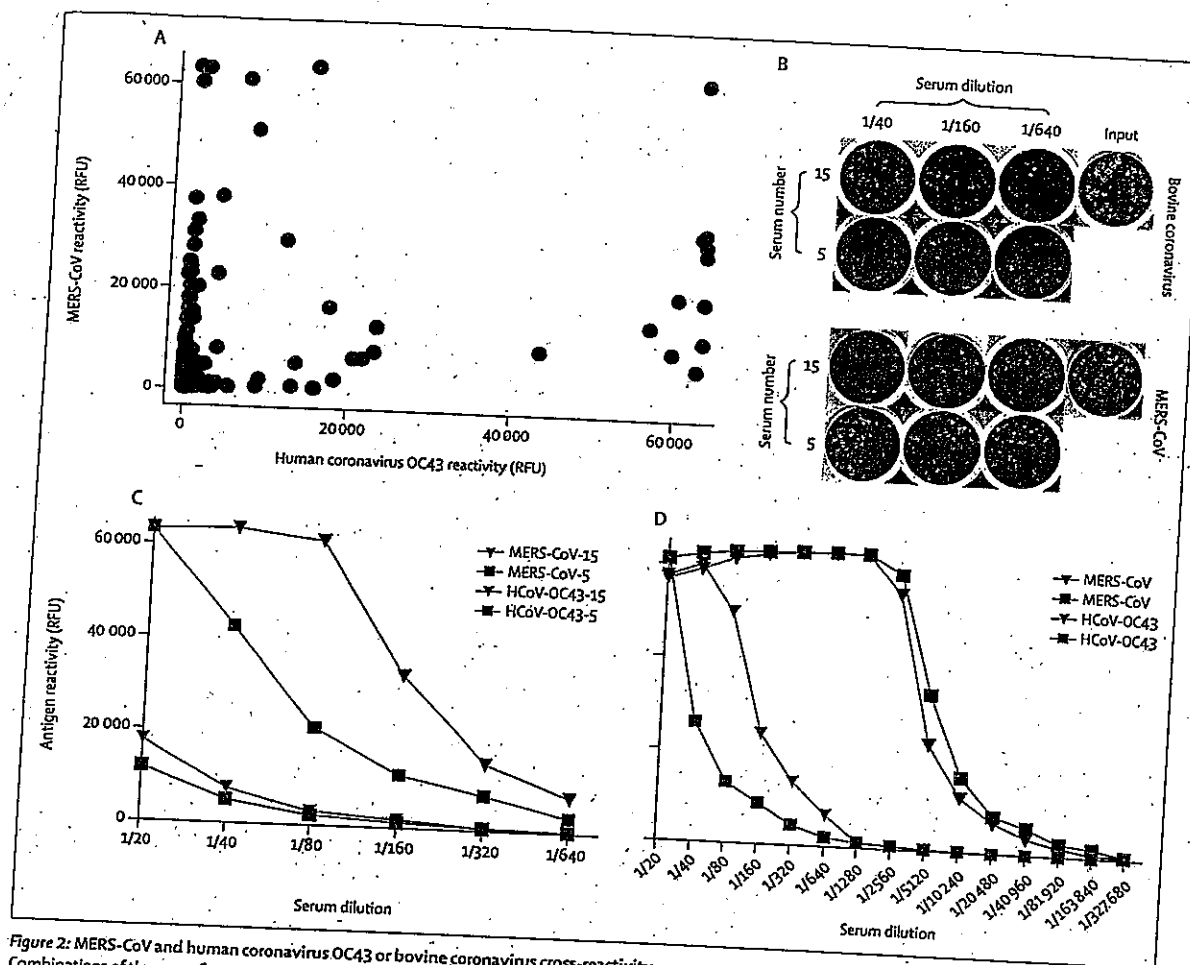


Figure 2: MERS-CoV and human coronavirus OC43 or bovine coronavirus cross-reactivity
Combinations of the mean fluorescent intensities of reactions of sera with MERS-CoV and human coronavirus OC43 antigens from 105 Spanish dromedary camels (A), plaque reduction neutralisation tests for bovine coronavirus and MERS-CoV (B); two representative sera are shown (numbers 15 and 5, corresponding to camel ID numbers in table 2) in dilutions of 1/40, 1/160, and 1/640 as well as the virus input control. All samples were tested in duplicates (only one well shown) and titres were expressed as the serum dilution resulting in a plaque reduction of at least 90%. IgG reactivity of both camel sera to MERS-CoV antigen and human coronavirus OC43 antigen in a two-step dilution series in the microarray (C). IgG reactivity of two two-step serially diluted Omani dromedary camel sera with human coronavirus EMC antigen and human coronavirus OC43 antigen in the microarray (D). RFU=relative fluorescence units. MERS-CoV=Middle East respiratory coronavirus.

Sera were collected for veterinary purposes from two llamas (*Lama glama*), six alpacas (*Vicuña pacos*), and two Bactrian camels (*Camelus bactrianus*) in the Netherlands. Sera were collected for veterinary purposes from two Bactrian camels, 18 alpacas, five llamas, and two guanaco (*Lama guanicoe*) in Buin Zoo in Chile. Sera from cattle (n=40), domestic goats (n=40), and sheep (n=40) were from routine submission to the Dutch Animal Health Service. Sera from Spanish domestic goats (n=40) were provided by the Instituto de Investigación en Recursos Cinegéticos (Ciudad Real, Spain) from submissions for tuberculosis control in 2011. All sera were obtained in agreement with local regulations and Dutch import regulations with regard to animal disease legislation. Positive human control sera for the three antigens used on the microarray were taken as described previously.²⁴ All samples were stored at -20°C until testing.

Laboratory procedures

We tested the sera for the presence of IgG antibodies reactive with MERS-CoV, SARS-CoV, and human coronavirus OC43 S1 antigens in a protein microarray. The receptor-binding domains, which contain the S1 subunit of spike proteins of MERS-CoV (residues 1-747), SARS-CoV (residues 1-676), and human coronavirus OC43 (residues 1-760) were expressed, purified, and spotted on glass slides. Slides were incubated with serum and species-specific conjugates, as previously described.²⁴ Goat sera were incubated with Alexa Fluor 647-conjugated rabbit anti-goat IgG Fc fragment (Jackson Immuno Research; West Grove, PA, USA); cow sera with Alexa Fluor 647-conjugated goat anti-bovine IgG (H+L; Jackson Immuno Research); sheep sera with Alexa Fluor 647-conjugated donkey anti-sheep IgG (H+L; Millipore, Temecula, CA, USA); and camelid sera with Dylight

650-conjugated goat anti-llama IgG (H+L; Agrisera, Vännas, Sweden). Fluorescence signals were quantified as described previously.²⁴ We tested the sera for IgG reactivity at a dilution of 1/20 and set an arbitrary cutoff at an average signal intensity of 20 000 relative fluorescence units (RFU). This high cutoff was chosen to reduce cross-reactive false positives.²⁴ We present results as RFU for each set of quadruplicate spots per antigen. Negative fluorescent intensities (caused by background correction) were assigned to 0. Analyses were done with GraphPad Prism (version 6.02).

Sera were heat-inactivated before virus neutralisation by incubation for 30 min at 56°C. Two-fold serial dilutions of sera were prepared using 96-well plates, starting dilution 1/10. MERS-CoV was diluted in Iscove's modified Dulbecco's medium (IMDM) supplemented with clemizole penicillin (penicillin G), streptomycin, and 1% fetal bovine serum, to a dilution of 2000 tissue culture infective dose₅₀ per mL. 50 µL virus suspension was added to the plates and the plates were incubated at 37°C for 1 h. The mixtures of virus and serum were then incubated on 96-well plates containing Vero cells for 1 h followed by washing with phosphate buffered saline and incubation with IMDM and 1% fetal bovine serum for 3–4 days, after which endpoint titres were measured. All tests were repeated twice independently.

We tested neutralisation activity of sera against MERS-CoV (Erasmus MC isolate) and bovine coronavirus (Nebraska strain) by plaque reduction neutralisation test (90% plaque reduction) with African green monkey kidney cells (cell line Vero B4; DSMZ ACC 33) or bovine kidney cells (cell line PT; CCLV-R1E11) in a 24-well plate format. Virus (30–60 plaque-forming units) and heat-inactivated sera (diluted from 1/40 to 1/640) were pre-incubated in 200 µL of serum-free OPTipro medium (Life Technologies, Karlsruhe, Germany) at 37°C for 1 h. Virus adsorption was done at 37°C for 1 h. Supernatants were removed and overlaid with Avicel resin (FCM BioPolymer, Brussels, Belgium).²⁵ Assays were stopped after 3 days by fixation with 8% paraformaldehyde for 30 min. All samples were tested in duplicate and titres were expressed as the serum dilution resulting in a plaque reduction of at least 90%.

Role of the funding source

The sponsors had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Sera were tested for IgG antibodies reactive with MERS-CoV, SARS-CoV, and human coronavirus OC43 S1 antigens in a protein microarray (figure 1). Human coronavirus OC43 is serologically closely related to bovine coronavirus,²⁶ diverging at the end of the 19th century.¹⁰ Bovine coronavirus circulates in cows, sheep, goats, and Old and New World camelids.^{27–29} Because bovine coronavirus S1 was not available, human coronavirus OC43 S1 antigen was used as a proxy. Sera from three llamas, four alpacas, one guanaco, and two Bactrian camels reacted with human coronavirus OC43 antigen. One cow and one goat serum reacted with human coronavirus OC43 antigen as did sera from 16 of 105 (15%) Spanish dromedary camels. All sera from cattle, sheep, and goats tested negative for MERS-CoV antigen, but sera from 15 Spanish camels (14%) did react with MERS-CoV antigen (figure 1). The reactivity was highly specific—the same sera did not bind to SARS-CoV antigen but a positive control specimen did. No correlation existed between the reactivity of sera with MERS-CoV antigen and human coronavirus OC43 antigen (figure 2). All but one serum sample that reacted with MERS-CoV antigen were from adult animals. One reactive serum was from a 2-year-old animal.

To confirm the presence of MERS-CoV specific IgG in the Spanish camel sera, we used a MERS-CoV neutralisation assay to test a subset of 49 camel sera with different degrees of reactivity with MERS-CoV and human coronavirus OC43 antigen according to microarray. Nine Spanish camels had MERS-CoV neutralising antibodies with titres varying between 1/20

	Number of serum samples	Positive MERS-CoV neutralisation titre (n%)	Titre range
Spanish samples (geographic link)			
MERS-CoV antigen array signal (RFU)			
<10 000	31	0 (0%)	–
10 000–20 000	3	0 (0%)	–
20 000–30 000	3	0 (0%)	–
30 000–40 000	0	0 (0%)	–
>40 000	12	9 (75%)	1/20 to 1/320
Human coronavirus OC43 antigen array signal (RFU)			
<10 000	26	1 (4%)	–
10 000–20 000	8	3 (38%)	–
20 000–30 000	5	2 (40%)	–
30 000–40 000	6	3 (50%)	–
>40 000	4	0 (0%)	–
Omani camel samples (geographic link)			
MERS-CoV antigen array signal (RFU)			
0–40 000	0	0 (0%)	–
>40 000	50	50 (100%)	1/320 to 1/2560
Human coronavirus OC43 antigen array signal (RFU)			
<10 000	3	3 (100%)	–
10 000–20 000	4	4 (100%)	–
20 000–30 000	4	4 (100%)	–
30 000–40 000	5	5 (100%)	–
>40 000	34	34 (100%)	–

RFU, relative fluorescence units; MERS-CoV, Middle East respiratory syndrome coronavirus.

Table 1. Results of neutralising assay for MERS-CoV from Spanish and Omani camel serum samples

and 1/320 (table 1). Three of the 12 sera reacted with MERS-CoV spike antigen but did not neutralise MERS-CoV, most likely because of recognition of non-neutralising epitopes. All MERS-CoV neutralising sera had (almost) saturating reactivity with MERS-CoV antigen on the microarray, whereas reactivity with human coronavirus OC43 antigen varied from negative to 50% of saturating reactivity (table 1). The variable human coronavirus OC43 signals suggest that MERS-CoV did not generally cross-react with human coronavirus OC43 or bovine coronavirus antigens. All nine camels with MERS-CoV neutralising antibodies were born and raised on the Canary Islands; seven were female, two were male. Eight camels were adults, one was 2 years old.

To show that the reactivity of the camel sera with human coronavirus OC43 antigen according to the microarray was caused by the presence of bovine coronavirus IgG and to further exclude MERS-CoV neutralising activity caused by cross-neutralisation by the bovine coronavirus antibodies, we tested camels that had sufficient serum left ($n=15$) in a comparative MERS-CoV and bovine coronavirus plaque reduction neutralisation test (figure 2, table 2). All camel sera neutralised bovine coronavirus, but with varying titres, suggesting a lower cutoff than 20 000 RFU for OC43 in the microarray (figure 1). Five camels had high neutralising antibody titres against bovine coronavirus (and a mean signal intensity of greater than 50 000 RFU for human coronavirus OC43 antigen on microarray) but were negative for MERS-CoV neutralisation, suggesting that cross-neutralisation in this direction did not occur and that the MERS-CoV neutralising activity was not caused by the presence of bovine coronavirus neutralising antibodies. A serum sample from a patient who had MERS, neutralised MERS-CoV with a high titre (1/640) but neutralised bovine coronavirus less efficiently (titre 1/80). The latter finding was most probably caused by previous infection with human coronavirus OC43—this patient had a high titre (1/5120) in a human coronavirus OC43 recombinant spike immunofluorescence assay and a saturating signal with human coronavirus OC43 antigen in the microarray. Two human serum samples positive for human coronavirus OC43 did not neutralise MERS-CoV, one of which neutralised bovine coronavirus at a titre of 1/80 (table 2).

We tested 50 sera from dromedary camels in Oman at a dilution of 1/20 by microarray and MERS-CoV neutralisation test. All the sera showed saturating reactivity with MERS-CoV antigen on the microarray, no SARS-CoV antigen reactivity, and human coronavirus OC43 antigen reactivity varying between negative (below the cutoff of 20 000 RFU) and saturating signals (figure 1, table 1). Serial dilution of two sera with saturating reactivity for both antigens at the initial dilution of 1/20 showed that MERS-CoV antigen reactivity was still above the cutoff at 1/5120, whereas human coronavirus OC43 antigen reactivity fell below the cutoff at dilutions of 1/80 to 1/320 (figure 2D). Consistent with the microarray data,

	PRNT	PRNT	Microarray (RFU)	
	MERS-CoV	BCoV	MERS-CoV	HCov-OC43
Camel				
1	Negative	>1/640	7848	51147
2	Negative	1/320	23 235	8164
3	Negative	1/160	1273	20 064
4	Negative	>1/640	3725	37 972
5	Negative	>1/640	6493	61 046
6	Negative	1/160	1321	63 015
7	Negative	1/640	62 748	62 837
8	Negative	1/40	18 421	2376
9	1/80	1/160	62 775	6554
10	1/40	1/80	59 729	9726
11	1/40	1/160	63 433	29 333
12	1/40	1/640	63 377	31 207
13	Negative	1/160	13 806	5483
14	1/160	1/640	63 438	19 775
15	1/160	1/320	63 402	12 029
Human				
MERS-CoV	1/640	1/80	64 353	63 437
HCov-OC43 (13 DPI)	Negative	1/80	2848	>55 000
HCov-OC43 (10 DPI)	Negative	1/40	2826	>55 000

PRNT, plaque reduction neutralisation test; MERS-CoV, Middle East respiratory syndrome coronavirus; BCoV, bovine coronavirus; HCov, human coronavirus; DPI, days post-infection; RFU, relative fluorescence units; titration endpoint; AOC, 1/250 dilution; figure 1 to 10.

Table 2. Protein microarray and PRNT results from dromedary camels and three people

all sera had high MERS-CoV neutralising capacity, with titres varying between 1/320 (seven of 50 samples) and 1/2560 or more (16 of 50 samples).

Discussion

In this study we describe the presence of MERS-CoV neutralising antibodies in dromedary camels both in a MERS-CoV linked (Oman) and unlinked regions (Canary Islands). All the sera from dromedary camels from Oman and some from Spain had specific IgG reactivity with the MERS-CoV receptor binding domain S1. We confirmed our expectation that another beta-coronavirus—bovine coronavirus—circulated in these camelids.^{29,30} However, all Omani camels and nine Spanish camels (9%) had specific neutralising antibodies against MERS-CoV that were clearly not caused by cross-neutralisation by bovine coronavirus antibodies.

Our study is the first in which animals have been tested for the presence of antibodies specific to MERS-CoV (panel). Animal screening is necessary to understand the epidemiology of MERS-CoV. At present, bats are thought to be the ultimate reservoirs for several established human coronaviruses as well as SARS-CoV. Accordingly, phylogenetic analysis has shown that MERS-CoV is related to betacoronavirus lineage C viruses found in

Panel Research in context

Systematic review

We searched PubMed for novel coronavirus EMC/2012-MERS-CoV, we identified 11 reports in English linked to the Middle East respiratory syndrome coronavirus (MERS-CoV), published before July 22, 2013. None of these reports described a serological study for MERS-CoV-specific antibodies in animals.

Interpretation

Our report describes the first MERS-CoV serological study of major livestock relevant to the Middle East. Our study shows that MERS-CoV-related virus has infected dromedary camel populations. Both titre levels and seroprevalences in sera from different locations in Oman suggest widespread infection of camels with MERS-CoV or a closely related virus. Targeted studies are needed to confirm our findings and that possible relevance to human cases of MERS-CoV. Comparative seroprevalence testing of historical and more recent samples from animals from different regions for which epidemiological background information is available, as well as virological assessment of samples from seroconverting animals are needed to identify and characterise this MERS-CoV-related virus. In the meantime, we recommend a detailed case history of confirmed MERS-CoV cases, with review of any animal exposures, including animal products, and targeted, prospective serosurveys to establish whether camels or their products are a potential source of human infections.

Pipistrellus spp. bats.^{15,16} However, direct transmission of MERS-CoV to people from bats seems unlikely.¹¹⁹ The identification of possible intermediate hosts that are probably in closer contact with people (eg, livestock) is urgently needed. Common livestock species in the Middle East include dromedary camels but also cattle, sheep, and goats. Based on the available data, we cannot rule out circulation of a MERS-related coronavirus in these species—sera were not available from epidemiologically linked regions.

The high prevalence of MERS-CoV neutralising antibodies in dromedary camels from Oman suggests circulation of MERS-CoV or a closely related virus in this population. However, attempts to identify viral sequences in Spanish camel sera and faecal samples using pancoronavirus and specific betacoronavirus 2C PCR methods^{15,31,32} were unsuccessful (unpublished data), as was untargeted amplification followed by deep sequencing of faecal samples (unpublished data). These results imply that the camels were not actively shedding the virus at the time of sampling.

Less than 10% of the animals in the Canary Islands had MERS-CoV neutralising sera with titres up to 1/320. This low seroprevalence means either that exposure of the animals to other putative reservoirs is rare³³ or that the virus is absent in this closed-off population of roughly 2000 animals.³⁵ We cannot rule out that the population might have once had an outbreak but that by the time of sampling, antibody titres had waned and no new introductions of the virus had occurred. The camels have contact with wild rodents, rabbits, pigeons, and doves and possibly also with bats. Seven insectivorous bat species, including three *Pipistrellus* spp, are native to the

Canary Islands, while Egyptian fruit bats (*Rousettus aegyptiacus*) have been introduced.³⁴

The 100% seroprevalence with high titres in Omani camels from different owners and locations suggests a different situation in the Middle East, with widespread circulation of MERS-CoV or a closely related virus. This difference of epidemiology might be because the virus circulating in the Middle East is different to that circulating in Spain, with increased animal transmissibility and human infections.³⁵ In addition, the Omani camels were once racing camels now held for breeding and might be kept in circumstances that favour virus transmission. For cattle, a relation has been established between the incidence and effects of respiratory diseases, management practices, and animal transport.^{36,37}

To our knowledge, the camel populations in Oman and the Canary Islands are not connected. Camels on the Canary Islands were originally imported in the 15th century from the Horn of Africa for labour and transport. Nowadays, import of animals from Africa is banned because of the risk of foot-and-mouth disease. Only three camels in our study were originally imported from Morocco, more than 18 years ago. Because the closest relatives of MERS-CoV were identified very recently in *Neoromicia zuluensis* bats from Africa,³⁸ the introduction of MERS-CoV or related viruses into some African camel populations could have occurred decades ago, giving a possible explanation for MERS-CoV antibodies in camels from the Canary Islands. In the Middle East, huge numbers of camels are imported from Africa to meet the demand for meat. The top five camel breeding countries are all African, and Saudi Arabia and United Arab Emirates are in the top five camel meat producing countries.² This increased turnover of animals in the Middle East compared with the Canary Islands could also affect the epidemiology of a virus, through more frequent influx of immunologically naive animals.

Targeted studies are needed to confirm our findings and their possible relevance in relation to the human cases of MERS-CoV. Comparative seroprevalence testing of historical and more recent samples from camels for which epidemiological background information is available, as well as virological assessment of specimens from seroconverting animals are needed to identify and characterise this MERS-CoV-related virus. In the meantime we recommend a detailed case history of people with MERS-CoV, with review of any animal exposures including animal products, and targeted, prospective serosurveys to establish whether camels or their products are a potential source of human infections.

Contributors

CBEMR designed and coordinated the study, wrote the report, searched the published work, analysed and interpreted data, and produced the figures. BLH and MAM wrote the report, produced the figures, and analysed and interpreted data. CG collected samples and wrote the report. G-JG, BM, and DM did laboratory testing and analysed, and interpreted data. VSR, LS-DV, VMC, and PR generated and analysed data. J-FD and B-JB did laboratory testing, analysed data, and wrote the

report. SLS did laboratory testing, analysed data, wrote the report, and produced the figures. YEET, KvM, and EH-H collected samples. RDR searched the published work. JvB analysed data and produced the figures. NN collected samples and interpreted data. AO, CD, and MPKG interpreted data and wrote the report. CG-S collected samples, wrote the report, and coordinated the study. MPKG designed the study.

Conflicts of interest

BLH, AO, VSR, and B-JB have a patent pending for a MERS-CoV receptor. The other authors declare that they have no conflicts of interest.

Acknowledgments

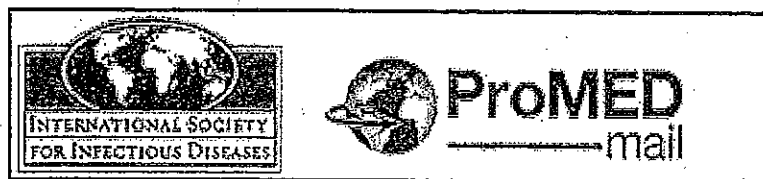
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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2013. 4. 19	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿			公表国 オーストラリア	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社) 新鮮凍結血漿-LR「日赤」J20(日本赤十字社) 新鮮凍結血漿-LR「日赤」J240(日本赤十字社) 新鮮凍結血漿-LR「日赤」J480(日本赤十字社)	研究報告の公表状況	ProMED 20130416.1650747		
研究報告の概要	○バーマフオレストウイルス(BFV) オーストラリア クイーンズランド州保健局によると、2013年1月1日以降メトロサウス保健区のBFV感染症例の報告が急増しており、2012年全体の94症例と比較して、今年は3カ月間で41症例となっている。またレドランド市議会では、BFV感染症例が増加したことで、蚊媒介性疾患を防止するため、庭を清掃し、水溜まりを排除するよう警告している。 BFV感染症の症状は発熱、発疹、関節痛、浮腫、頭痛及び疲労感であり、症例は夏期の多雨とともに増加する。ブリスベン地域では2013年1月以降、平年の約二倍の降雨量が記録された。 BFV:トガウイルス科アルファウイルス属に属する一本鎖RNAウイルスであり、1974年にイエカ(<i>Culex annulirostris</i>)から初めて分離され、ヒト感染症例は1980年代中期に記録された。				
報告企業の意見	オーストラリアのクイーンズランド州でBFV感染症例数が増加しているとの報告である。				
今後の対応	日本赤十字社では、輸血感染症対策として受付時に海外滞在歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き情報の収集に努める。				
	使用上の注意記載状況・ その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分採血 新鮮凍結血漿-LR「日赤」J20 新鮮凍結血漿-LR「日赤」J240 新鮮凍結血漿-LR「日赤」J480 血液を介するウイルス、細菌、原虫等の感染、vCJD等の伝播のリスク				



Published Date: 2013-04-16 19:49:11
Subject: PRO/AH/EDR> Barmah Forest virus - Australia: (QL)
Archive Number: 20130416.1650747

BARMAH FOREST VIRUS - AUSTRALIA: (QUEENSLAND)

A ProMED-mail post

<http://www.promedmail.org>

ProMED-mail is a program of the
International Society for Infectious Diseases

<http://www.isid.org>

Date: Mon 15 Apr 2013

Source: Bayside Bulletin [edited]

<http://www.baysidebulletin.com.au/story/1432346/mossie-warning-after-rise-in-barmah-forest/?cs=213>

Redland City councillor Wendy Boglary is warning residents to clear up stagnant water in gardens to keep mosquito-borne diseases at bay. Mosquito prevention costs Redland City Council AUD 748 000 [USD 776 500] a year.

Redland City Council is urging residents to clean up their back yards in a bid to crack down on mosquitoes and curb the spread of disease after a rise in cases of Barmah Forest virus.

Council Community and Environmental Health portfolio chairman Cr Wendy Boglary issued the warning after Queensland Health statistics showed a spike in the reported cases of Barmah Forest in Metro South Health district since 1 Jan [2013], compared with the same quarter last year. Compared to 94 cases in all of 2012, 41 cases of the mosquito-borne disease were recorded in the district since January [2013].

In Redland, from July 2012 to March 2013, Queensland Health recorded 19 cases of Barmah Forest, a 16 per cent increase in cases over the last 9 months. Council, which keeps records of all notifiable diseases, said the increase in Redland was "not significant" and coincided with a 35 per cent decrease in overall mosquito-transmitted diseases in the financial year to March 2013. It also said there was no evidence that people with the disease were infected within Redland city limits.

The council clean-up warning came a week after an outbreak of the incurable mosquito-borne virus was recorded on the Gold Coast, where 22 people were infected since mid-March 2013, a rate of almost one person a day.

Symptoms of the virus include fever, rash, joint pain, swelling, headaches and feeling tired. The rise in Barmah Forest virus [infections], named after the northern Victoria town, coincided with high rainfall over summer. Since January [2013], more than 702 mm of rain has been recorded in the Brisbane area, compared with the median rainfall for those 3 months of 397 mm. Point Lookout recorded 763 mm since January 2013.

Metro South Public Health senior medical officer Dr Brad McCall warned residents to avoid mossie-prone areas, use insect repellent and cover up to avoid bites. He said protection against mosquito bites would safeguard against the transfer of mosquito-borne viruses such as Ross River and Barmah Forest. "It is worth noting that the increase in Barmah Forest virus in Metro South is from a low base and that rates of infection in Metro South are still amongst the lowest in the state."

Cr Boglary said council had held 6 aerial spraying treatments this financial year [2013] and was vigilant in its control of mosquitoes and was aware of the threat of diseases being spread. "Residents also play an important role and should check they have no containers lying around yards collecting stagnant water," Cr Boglary said.

[Byline: Judith Kerr]

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Communicated by:

ProMED-mail from HealthMap Alerts
<promed@promedmail.org>

[Periods of higher than usual rainfall often bring an increase in mosquito populations, with corresponding increases in transmission of endemic arthropod-borne viruses in those areas. The same situation occurred in 2011 in different localities in Australia. Although not as serious a human health threat as Ross River virus infections, the morbidity rate can be significant, and the disease can be incapacitating.

A Healthmap/ProMED-mail map showing the location of Queensland can be accessed at <http://healthmap.org/r/1kJr>. - Mod.TY]

See Also

2011

 Kunjin/Barmah Forest/Ross River/MVE viruses - Australia [20110305.0721](#)
 Ross River/Barmah Forest viruses - Australia (03): (SA) [20110202.0385](#)
 Ross River/Barmah Forest viruses - Australia (02): (SA) [20110113.0149](#)
 Ross River/Barmah Forest viruses - Australia: (VI) [20110106.0075](#)
sb/ml/ty/msp/ml

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
			2013年7月1日	該当なし。	
一般的名称	別紙のとおり。	研究報告の 公表状況	Emerg Infect Dis: 2013;19:1487-1489	公表国 マラウイ	
販売名(企業名)	別紙のとおり。				
問題点：マラウイで、原因不明の対麻疹患者から新規な cyclovirus が確認された。 マラウイで、2010年から2011年に Queen Elizabeth Central Hospital で治療を受けた原因不明の対麻疹患者の血清及び脳脊髄液 (CSF) サンプルを解析したところ、新規な cyclovirus 種が確認された。検体入手できた12例についてメタゲノム解析を行ったところ、血清及びCSFサンプルの各々15%と10%から同ウイルスのゲノムが検出された。					
研究報告の概要					
報告企業の意見			今後の対応		
別紙のとおり。			今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。		
使用上の注意記載状況・ その他参考事項等 記載なし。					

一般名称	①人血清アルブミン、②人血清アルブミン*、③人血清グロブリン、④人免疫グロブリン、⑤人免疫グロブリン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥スルホ化人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン*、⑫乾燥濃縮人活性化プロテインC、⑬乾燥濃縮人血液凝固第Ⅳ因子、⑭乾燥濃縮人血液凝固第Ⅳ因子、⑮乾燥濃縮人血液凝固第Ⅳ因子、⑯乾燥濃縮人血液凝固第Ⅳ因子、⑰乾燥濃縮人血液凝固第Ⅳ因子、⑱乾燥抗破傷風人免疫グロブリン、⑲乾燥濃縮人免疫グロブリン、⑳抗HBs人免疫グロブリン、㉑抗HBs人免疫グロブリン、㉒トロンビン、㉓フィブリノゲン加第XIII因子*、㉔フィブリノゲン加第XIII因子、㉕乾燥濃縮人アンチトロンビンIII、㉖ヒスタミン加入免疫グロブリン製剤、㉗人血清アルブミン*、㉘人血清アルブミン、㉙乾燥ペプシン処理人免疫グロブリン*、㉚乾燥濃縮人アンチトロンビンIII
販売名(企業名)	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④ガンマーグロブリン筋注450mg/3ml「化血研」、⑤ガンマーグロブリン筋注1500mg/10ml「化血研」、⑥献血グロブリン注射用2500mg「化血研」、⑦献血ベニコロン—I静注用500mg、⑧献血ベニコロン—I静注用1000mg、⑨献血ベニコロン—I静注用2500mg、⑩献血ベニコロン—I静注用5000mg、⑪ベニコロン*、⑫注射用アナクトC2,500単位、⑬コンファクトF注射用250、⑭コンファクトF注射用500、⑮コンファクトF注射用1000、⑯ノバクトM静注用400単位、⑰ノバクトM静注用800単位、⑱ノバクトM静注用1600単位、⑲デタノセラ筋注用250単位、⑳ベパトセラ筋注200単位/ml、㉑ベパトセララ筋注200単位、㉒アンスロピンP500注射用、㉓ヒスタダグロビン皮下注用、㉔アルブミン20%化血研*、㉕アルブミン5%化血研*、㉖静注グロブリン*、㉗アンスロピンP1500注射用
報告企業の意見	cyclo ウイルスは20nm前後の球形で、核酸は一本鎖DNA、エンベロープを持たないウイルスであるが、cyclo ウイルスの人感染については病原性含め不明な点が多い。 今回の報告は、マラウイで、原因不明の対麻疹の患者から新規な cyclo ウイルスが確認された、というものである。 上記製剤の製造工程には、冷エタノール分画工程及びウイルス除去膜ろ過工程といった原理の異なるウイルススクリアランス工程が導入されており、各工程のウイルススクリアランス効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日）」に基づき、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告した新規な cyclo ウイルスのモデルウイルスには、エンベロープの有無、核酸の種類等から、豚バングウイルス（PPV）が該当すると思われるが、上記工程の PPV クリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤による新規な cyclo ウイルスへの感染報告例は無い。 以上の点から、上記製剤は新規な cyclo ウイルスへのウイルス感染に対する安全性を確保していると考えられる。

*：現在製造を行っていない

Novel Cyclovirus in Human Cerebrospinal Fluid, Malawi, 2010–2011

Saskia L. Smits, Ed E. Zijlstra¹,
Jaap J. van Hellemond,
Claudia M.E. Schapendonk, Rogier Bodewes,
Anita C. Schürch, Bart L. Haagmans,
and Albert D.M.E. Osterhaus

To identify unknown human viruses, we analyzed serum and cerebrospinal fluid samples from patients with unexplained paraplegia from Malawi by using viral metagenomics. A novel cyclovirus species was identified and subsequently found in 15% and 10% of serum and cerebrospinal fluid samples, respectively. These data expand our knowledge of cyclovirus diversity and tropism.

The list of diseases caused by viral pathogens is ever changing and growing (1). Breakthroughs in the field of metagenomics had far-reaching effects on the identification of emerging viral pathogens and on the recognition that an increasing number of diseases that were once attributed to unknown causes are actually caused by infectious agents (1). Paraplegia is an impairment of motor or sensory functions of the lower extremities. Although it can be caused by spinal cord injury, nontraumatic paraplegia also should be considered, particularly in a tropical environment; tuberculosis and schistosomiasis may play a role, but in many cases, no firm diagnosis can be made (2). In this study, cerebrospinal fluid (CSF) and serum samples were obtained from 58 patients from Malawi who had paraplegia of unknown etiology and were studied for the presence of known or unknown viruses by using a metagenomics approach.

The Study

During 2010–2011, we enrolled 58 adults who sought care at Queen Elizabeth Central Hospital in Blantyre, Malawi, for unexplained paraplegia in this study. All procedures were performed in compliance with relevant laws and institutional guidelines. The study was approved by

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DOI: <http://dx.doi.org/10.3201/eid1909.130404>

the College of Medicine Research and Ethics Committee (P.99/00/92).

Serum and CSF samples obtained from 12 paraplegia patients were available for virus discovery studies by using random PCR amplification with next-generation sequencing with a 454 GS Junior Instrument (Roche, Indianapolis, IN, USA) (3,4). More than 234,000 trimmed reads were assembled by using de novo assembly in CLC Genomics Workbench 4.5.1 (CLC Bio, Aarhus, Denmark) and analyzed according to nucleotide and translated nucleotide BLAST searches (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Classification of sequences based on the taxonomic origin of the best-hit sequence was performed by using MEGAN 4.40.4 (5), using E-value cut-offs of 0.001 and 10^{-10} for BLASTn and BLASTx searches, respectively.

Most of the sequences were of eukaryotic or bacterial origin or did not have hits to nucleotide or amino acid sequences in GenBank in agreement with previous viral metagenomic studies (6). Serum samples from all patients were positive for viruses from the family *Anelloviridae* in serum. Anellovirus infections are acquired during early childhood, when the virus establishes a chronic productive infection with long-lasting detectable viremia (7). Two of these patients were positive for anelloviruses in the CSF, which has been described (7). In 2 patients, hepatitis B virus sequences were detected. Hepatitis B virus infection occurs worldwide but is most prevalent in Southeast Asia and sub-Saharan Africa, with reported prevalence rates of 3%–26% (8). CSF from 2 other patients showed evidence of HIV infection in CSF samples (Table). None of these viral infections seem to explain the paraplegia.

A cyclovirus genome was obtained by 454-sequencing from serum of patient VS5700009. Cycloviruses (family *Circoviridae*, genus *Cyclovirus*) have been detected in human and chimpanzee feces and tissues of farm animals, bats, and dragonflies (9–12). They are nonenveloped viruses with a single-stranded circular DNA genome of ≈ 2 kb (13). The genome contains 2 major inversely arranged open reading frames (ORFs) encoding the putative replication-associated protein (Rep) and capsid protein (Cap). A potential stem-loop structure with a conserved nonanucleotide motif located between the 5'-ends of these 2 ORFs is required to initiate the replication of the viral genome (13).

The genome organization of human cyclovirus VS5700009 (GenBank accession no. KC771281) resembles that of human cycloviruses TN18 and TN25 (11). The Rep ORF of human cyclovirus VS5700009 was interrupted by a 96-bp intron with a splice donor-site (GT)

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Table. Viruses detected in serum and CSF samples from patients with paraplegia in a study of novel human cyclovirus, Malawi, 2010–2011*

Patient no.	Next-generation sequence		Cyclovirus	
	Serum	CSF	Serum	CSF
VS5700001	Anelloviridae			
VS5700002	Anelloviridae			+
VS5700003	Anelloviridae			
VS5700004	Anelloviridae, HBV			
VS5700005	Anelloviridae			
VS5700006	Anelloviridae	HIV-1		
VS5700007	Anelloviridae			
VS5700008	Anelloviridae, HBV	HIV-1, Anelloviridae		
VS5700009	Anelloviridae, Cyclovirus		+	+
VS5700010	Anelloviridae	Anelloviridae	+	
VS5700011	Anelloviridae			
VS5700012	Anelloviridae			
VS5700021	NA	NA	+	
VS5700022	NA	NA	NA	+
VS5700025	NA	NA	+	
VS5700029	NA	NA	+	
VS5700031	NA	NA	+	
VS5700040	NA	NA	+	NA
VS5700042	NA	NA	+	NA
VS5700044	NA	NA		+

*CSF, cerebrospinal fluid; +, positive; HBV, hepatitis B virus; NA, not available. Blank cells indicate negative results.

and splice acceptor site (AG). The 3' intergenic region between the Rep and Cap ORFs was only 7 bp long. The cyclovirus stem-loop structure with the conserved nonamer sequence (5'-TAATACTAT-3') in the 5' intergenic region was observed, as were 3 other potential ORFs (Figure, panel A, Appendix, wwwnc.cdc.gov/EID/article/19/9/13-0404-F1.htm). These latter ORFs are not conserved among cycloviruses and show low partial homology to bacterial enzymes, NAD-dependent DNA ligase of *Psychrobacter* (ORF3), transketolase of *Sinorhizobium* (ORF4), and dTDP-D-glucose 4,6-dehydratase of *Actinomyces* (ORF5). The complete Rep protein of human cyclovirus VS5700009 and representative strains of cycloviruses were used for phylogenetic analysis (Figure, panel B, Appendix). Multiple alignments were created by using ClustalX (2.0.10) (14). Phylogenetic analyses were conducted with MEGA5 (15). The human cyclovirus VS5700009 Rep protein was most closely related to the Rep proteins of human cycloviruses TN18 and TN25 (~75% aa identity) (11). Similar relations, but with much lower amino acid identities, were observed in Cap proteins (~37% identity; data not shown). Cycloviruses belong to the same species when sharing >85% aa identity in the Rep region (11). Thus, human cyclovirus VS5700009 represents a new cyclovirus species. Most of the closest relatives of human cyclovirus VS5700009 (which include TN18 and TN25) detected in feces from children with nonpolio acute flaccid paralysis (11), a condition related to paraplegia, which may indicate that TN25/VS5700009-

like viruses may be more pathogenic than other cycloviruses (Figure, panel B, Appendix).

To determine the prevalence of human cyclovirus VS5700009 in the serum and CSF samples of the 58 patients, a VS5700009-specific PCR was performed. Total nucleic acid was extracted from an aliquot (~100 µl) of serum and CSF samples by using the MagNAPure LC total nucleic acid isolation kit and the MagNAPure LC isolation station (Roche). A genomic area, corresponding to nt 373–752 in human cyclovirus VS5700009, was amplified by nested PCR, with primers VS711 (5'-CGAGCGCA-CATTGAGAAAG-3') and VS712 (5'-CCATCCCAC-CATTCTCCTC-3') by using Amplitaq gold DNA polymerase (Roche). Negative water controls were taken along. Eight (15%) of 54 serum samples and 4 (10%) of 40 CSF samples from paraplegia patients were human cyclovirus positive (Table). For several amplicons, the cyclovirus nature was confirmed by Sanger sequencing, and the amplicons showed 75%–99% identity to human cyclovirus VS5700009 (Figure, panel C, Appendix). Only in patient VS5700009 were both CSF and serum samples positive for human cyclovirus.

Conclusions

Our results indicate that cycloviruses are commonly found in serum and CSF of paraplegia patients from Malawi. Diverse cycloviruses have been discovered in human and chimpanzee fecal samples and in muscle tissue of farm animals, such as cows, sheep, goats, and chickens (11). Cycloviruses have been suggested to cause human enteric infections and were not derived from consumed food because the human and animal cycloviruses showed limited genetic overlap (11). Our data indicate that cycloviruses may cause systemic infections and are present in multiple organ compartments in humans. Whether cycloviruses play a role in development of paraplegia remains to be determined; this study lacks a control group of healthy persons, and the relatively high virus prevalence in persons with paraplegia may also reflect high overall prevalence in healthy persons. In addition, the apparent interleaved evolution of human and animal cycloviruses suggests the potential for frequent cross-species exposure and zoonotic transmission.

Our observations expand the knowledge of cycloviruses in humans and show how epidemiologic baseline information on virus host range and tropism in animals (11) may indicate the presence of similar viruses in different organ systems of humans. To clarify the epidemiology and pathogenicity of cycloviruses in humans, additional surveillance should be conducted, especially because the prevalence and diversity of human cycloviruses is relatively high (11; this study), cross-species transmission of cycloviruses seems plausible (10), and closely related cyclovirus species may be pathogenic.

This work was partially funded by the European Community's Seventh Framework Program 8 (FP7/2007–2013) under the project "European Management Platform for Emerging and Reemerging Infectious Disease Entities" European Commission agreement no. 223498; and by Virgo Consortium.

Dr Smits is a senior scientist at the Virology Department, Erasmus Medical Center and at Viroclinics Biosciences B.V. in Rotterdam. Her primary research interest is virus discovery.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2013年7月3日	新医薬品等の区分 該当なし。	総合機構処理欄	
一般的名称	別紙のとおり。	研究報告の 公表状況	mBio. 2013;4:Issue 3, 1-10	公表国 ベトナム		
販売名(企業名)	別紙のとおり。					
研究報告の概要		問題点：ベトナムで、病原因子不明の急性中枢神経系感染症の患者から新規な cyclovirus が特定された。 急性中枢神経系感染症は高い発症率や死亡率を伴い、多くの症例で病原因子が不明である。ベトナムで、病原因子不明の同感染症を生じたベトナム人の患者2例の脳脊髄液から新規な cyclovirus (暫定的に cyclovirus-Vietnam (CyCV-VN) と命名) が特定された。CyC-VN は、原因不明の同感染症を生じたベトナム人患者の CSF (脳脊髄液) 標本 642 の4%で検出され、非感染性神経障害患者 CSF122 標本からは検出されなかった。患者の住む地域のニワトリやブタの糞便調査の結果から、それらの約半数が同ウイルスを保持していることが判明し、これらの動物が感染源となっていることも示唆している。				使用上の注意記載状況・ その他参考事項等 記載なし。
報告企業の意見		今後の対応 今後とも関連情報の収集に努め、本剤の安全性の確保を図っていききたい。				
別紙のとおり。						

*: 現在製造を行っていない



Identification of a New Cyclovirus in Cerebrospinal Fluid of Patients with Acute Central Nervous System Infections

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RESEARCH ARTICLE

Identification of a New Cyclovirus in Cerebrospinal Fluid of Patients with Acute Central Nervous System Infections

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ABSTRACT Acute central nervous system (CNS) infections cause substantial morbidity and mortality, but the etiology remains unknown in a large proportion of cases. We identified and characterized the full genome of a novel cyclovirus (tentatively named cyclovirus-Vietnam [CyCV-VN]) in cerebrospinal fluid (CSF) specimens of two Vietnamese patients with CNS infections of unknown etiology. CyCV-VN was subsequently detected in 4% of 642 CSF specimens from Vietnamese patients with suspected CNS infections and none of 122 CSFs from patients with noninfectious neurological disorders. Detection rates were similar in patients with CNS infections of unknown etiology and those in whom other pathogens were detected. A similar detection rate in feces from healthy children suggested food-borne or orofecal transmission routes, while high detection rates in feces from pigs and poultry (average, 58%) suggested the existence of animal reservoirs for such transmission. Further research is needed to address the epidemiology and pathogenicity of this novel, potentially zoonotic virus.

IMPORTANCE Acute central nervous system (CNS) infections cause substantial morbidity and mortality, but the etiology frequently remains unknown, which hampers development of therapeutic or preventive strategies. Hence, identification of novel pathogens is essential and is facilitated by current next-generation sequencing-based methods. Using such technology, we identified and characterized the full genome of a novel cyclovirus in cerebrospinal fluid (CSF) specimens from two Vietnamese patients with CNS infections of unknown etiology, which was subsequently detected in none of 122 CSF specimens from patients with noninfectious neurological disorders but 4% of 642 CSF specimens from Vietnamese patients with suspected or confirmed CNS infections. Similar detection rates in feces from healthy children suggested food-borne or orofecal transmission routes, while frequent detection in feces from Vietnamese pigs and poultry (average, 58%) suggested the existence of animal reservoirs for such transmission. Further studies are needed to address the epidemiology and pathogenicity of this novel, potentially zoonotic virus.

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Acute central nervous system (CNS) infections cause substantial morbidity and mortality in children and adults, especially in tropical regions. While the causative microorganisms include a wide variety of bacterial, parasitic, fungal, and particularly viral pathogens, the majority of cases remain undiagnosed, despite extensive diagnostic efforts (1). The identification of previously unknown pathogens in patients with acute CNS infections remains essential to improve prevention and clinical management of this frequently devastating syndrome. The discovery of such novel

pathogens is facilitated by the availability of sensitive sequence-independent molecular methods.

Using a combination of an amplified fragment length polymorphism (AFLP)-based virus discovery assay (VIDISCA) and next-generation sequencing (2), we set out to look for nucleic acid sequences of potential novel pathogens in cerebrospinal fluid (CSF) specimens from Vietnamese adults and children with acute CNS infections of unknown etiology. Here we report the identification and full genome characterization of a novel virus belonging

TABLE 1 Oligonucleotide sequences of primers and a probe used in this study

Primers	Oligonucleotide sequence (5'–3')	Source
CyCV31_53F	GAGCGCACATTGAAAGAGCTAAA	Newly designed
CyCV178-153R	TCTCCTCCTCAATGACAGAAACAAC	Newly designed
CyCV65-96Probe*	FAM-CGADAATAAGGMATACTGCTCTAAAGSTGGCG-BHQ1	Newly designed
CVF1	GGIAYICCCAYYTICARGG	3
CVR1	AWCCAICCRTARAARTCRTC	3
CVF2	GGIAYICCI CAYYTICARGGIT	3
CVR2	TGYTGYTCRTAICCRTCCACCA	3
CyCV31_53F-rc	TTTAGCTCTTTCAATGTGCGCTC	Newly designed
CyCV178-153R-rc	GTTGTTTCTGTCATTGAAGGAGGAGA	Newly designed
CyCV_FL1	ACTTATTTCTAATTCATATTGCCGGGTA	Newly designed
CyCV_FL2	TCAGCGTCCAGCAGAATCTAC	Newly designed
CyCV_FL3	AAGTCATCGTATTGATCCATCC	Newly designed
CyCV_FL4	CCTCACTGAGCTATGTAAATTGCT	Newly designed

to the recently proposed genus *Cyclovirus* in the family *Circoviridae*. In addition, we report the results of initial studies to explore the pathogenic potential of this virus in humans as well as the existence of possible animal reservoirs for zoonotic transmission.

RESULTS

Identification of a cyclovirus-like sequence in cerebrospinal fluid. The analysis of the (pooled) CSF specimens generated 321,000 reads, which were reduced to 161,000 sequence reads for further analysis after excluding *Escherichia coli*-like sequences suspected to be derived from VIDISCA-454 (454: Roche 454 Genome Sequencer FLX System) reagents (data not shown). Of these, a 190-bp sequence was identified in a pool consisting of 5 CSF samples that showed 82% identity to a replication-associated gene (*Rep*) of cycloviruses (CyCV). To confirm this VIDISCA-454 read, a specific PCR (Table 1) targeting the sequence was employed and successfully detected a fragment of the expected size in 2 out of 5 original CSF samples (Fig. 1A). The fragment was sequenced, which confirmed the CyCV-like sequence that was obtained from the VIDISCA-454 analysis (data not shown). These results indicated that a CyCV-like virus was present in CSF of two patients (one child and one adult) (Table 2) with acute CNS infections of unknown etiology.

Genome characterization and phylogenetic analysis of a new cyclovirus. To further characterize the CyCV-like virus, an initial attempt was made to amplify the partial *Rep* gene (primers CVFs

and CVRs) (Table 1), but this was unsuccessful. Therefore, inverse PCR (iPCR) was used for direct amplification of the viral genome from clinical specimens. An amplified product of less than 2 kb was successfully obtained from only one sample (Fig. 1B) and was then subjected to direct sequencing with use of iPCR primers and primers FL1 to FL4 (Table 1) to generate the full viral genome. The cyclovirus species of the completed genome was tentatively named cyclovirus-Vietnam (CyCV-VN). The single-stranded circular DNA genome consisted of 1,856 bp and revealed a genomic structure characteristic of the genera *Circovirus* and *Cyclovirus* in the family *Circoviridae* with the typical feature of two open reading frames (ORFs) encoding two major proteins, the replication-associated (*Rep*) and capsid (*Cap*) proteins, arranged in opposite directions (Fig. 2A). These two deduced proteins are 292 amino acids (aa) and 222 aa long, respectively. The *Rep* gene of CyCV-VN was predicted to be interrupted by a 166-bp intron with a typical splice donor (GT) and splice acceptor site (AG), as in other CyCVs (3, 4). Amino acid sequencing of the *Rep* protein showed that the conserved motifs (WWDGY, DDFYGW, DRYP, FTLNN, TPHLQG, CSK, and G-GSK) that are commonly observed among circoviruses, including CyCVs, were also found in CyCV-VN (data not shown) (3). As in other CyCVs, the intergenic region between the 3' ends of the two ORFs was absent (3–5), and a highly conserved stem-loop structure was present at the 5' intergenic region of the viral genome (Fig. 2B). The latter structure

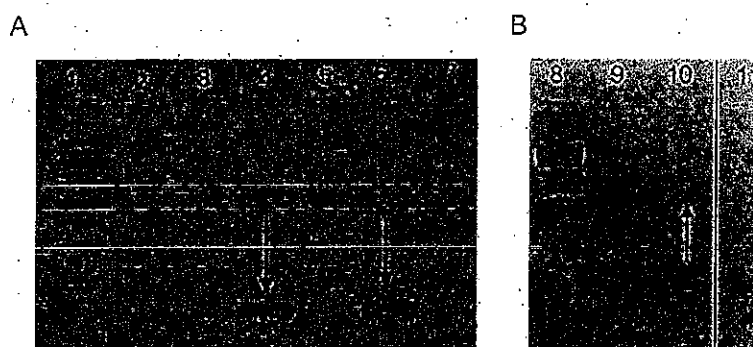


FIG 1 Gel electrophoresis of PCR products. (A) Cyclovirus-like sequence PCR; (B) inverse PCR of cyclovirus. Lanes 1 and 8, 100-bp and 1-kb ladders, respectively; lanes 2 to 6, 9, and 10, patient samples; lanes 7 and 11, negative controls. Arrows indicate products of expected sizes. The thin black line indicates the spliced margin where the two unrelated lanes between lanes 10 and 11 were removed from the original gel picture.

TABLE 2 Characteristics and clinical outcome of patients with CNS infections and positive for CyCV-VN by PCR

Group and patient (n) ^a	Other confirmed diagnosis ^b	Sample date	No. (%) of males in group or sex of patient ^c	Age (yr) ^d	Length of hospital stay (days) ^d	Illness day	Fever	Convulsions	Neck stiffness	Limb weakness	Headache	Vomiting	GCS score ^e	CSF white blood cell count	% of lymphocytes in CSF	CSF/blood glucose ratio	Outcome ^f	Sequelae at discharge
Group 1 (12)			9 (75)	19.5 (<1–50)	8 (2–41)	5	11 (92)	4 (33)	7 (58)	2 (18)	6 (86)	8 (89)		40 (1–464)				
1	None	Jan. 5, 2004	F	<1	7	5	Y	N	N	Y	NA	NA	2 (BCS)	1		0.88	1	None
2	None	Jun. 8, 2004	M	<1	8	7	Y	N	N	N	NA	NA	3 (BCS)	3		0.53	1	None
3	None	Feb. 7, 2004	M	12	8	3	Y	N	N	N	NA	NA	6	26	90	0.96	1	None
4	None	Oct. 18, 1999	M	27	5	7	Y	N	Y	NA	NA	Y	15	54	68	0.55	1	None
5	None	Jun. 12, 2008	M	31	2	2	Y	Y	N	N	Y	Y	15	10		0.54	3	Unknown
6	None	Nov. 25, 2009	F	<2	9	2	Y	Y	N	N	NA	Y	5 (BCS)	10	60	0.56	1	Unknown
7	None	Nov. 29, 2008	M	20	41	20	Y	Y	Y	Y	Y	Y	7	160	30	0.73	1	Unknown
8	None	Sep. 4, 2009	M	15	15	9	Y	N	Y	N	Y	Y	15	78	20	0.62	1	Unknown
9	None	Sep. 7, 2009	F	50	15	7	Y	N	Y	N	Y	Y	15	105	22	0.44	1	Unknown
10	None	Nov. 18, 2009	M	19	5	5	Y	Y	Y	N	Y	N	11	14	36	0.55	1	Unknown
11	None	Mar. 31, 2008	M	43	3	5	N	N	Y	N	Y	Y	9	78	20	0.57	3	Unknown
12	None	Apr. 16, 2008	M	24	17	4	Y	N	Y	N	N	Y	15	464	20	0.51	1	Unknown
Group 2 (4)			3 (75)	14 (2–19)	14 (4–38)	5.5 (2–7)	2 (50)	3 (100)	2 (50)	1 (25)	2 (100)	2 (100)		50 (0–110)				
1	JEV	Jan. 30, 2004	M	2	4	2	N	N	N	N	NA	NA	13	0		0.98	1	None
2	JEV	Aug. 12, 2004	M	11	5	5	Y	N	Y	N	NA	NA	11	22	65	0.81	1	None
3	DENV	Oct. 24, 2008	M	19	38	7	N	NA	Y	Y	Y	Y	9	79	21	0.41	1	Unknown
4	JEV	May 05, 2008	F	17	23	6	Y	N	N	N	Y	Y	15	110	25	0.71	1	Unknown
Group 3 (7)			6 (85)	5 (<1–61)	19 (1–34)	2 (1–3)	6 (85)	2 (28)	5 (71)	1 (14)	4 (100)	4 (57)		1,439 (2–9,160)				
1	<i>S. suis</i>	Aug. 31, 2008	M	29	21	1	N	N	Y	N	Y	Y	15	3,500		0.04	1	Unknown
2	<i>S. pneumoniae</i>	Nov. 17, 2008	M	25	16	2	Y	N	Y	N	Y	Y	15	5,513	20	0.23	1	Unknown
3	<i>H. influenzae</i>	Jan. 20, 2009	M	<1	19	1	Y	N	Y	N	NA	N	5 (BCS)	9,160	9	0.36	1	Unknown
4	<i>S. pneumoniae</i>	Jan. 27, 2009	M	5	1	1	Y	Y	N	Y	Y	Y	8	30	11	0.18	2	Unknown
5	<i>S. suis</i>	Mar. 16, 2009	F	61	34	3	Y	N	Y	N	NA	N	11	1,439	28	0.19	1	Unknown
6	<i>S. pneumoniae</i>	Apr. 17, 2009	M	2	4	2	Y	Y	N	N	NA	N	0 (BCS)	2		0.65	1	Unknown
7	<i>S. pneumoniae</i>	Dec. 23, 2009	M	3	20	2	Y	N	Y	N	Y	Y	11	80	33	0.95	1	Unknown
Group 4 (5)			1 (20)	32 (29–68)	4 (2–10)	5 (3–7)	4 (80)	0	4 (80)	0	NA	NA		296 (100–950)				
1187	<i>M. tuberculosis</i>	Aug. 4, 2009	F	30	4	7	Y	N	Y	N	NA	NA	13	296	NA	0.18	3	Unknown
1847	None	Feb. 16, 2009	M	32	10	3	Y	N	Y	N	NA	NA	15	100	20	0.13	1	Unknown
1854	None	Jul. 24, 2009	F	29	2	3	Y	N	Y	N	NA	NA	15	603	20	0.34	1	Unknown
2117	<i>M. tuberculosis</i>	Feb. 9, 2010	F	55	NA	7	N	Unknown	Y	N	NA	NA	7	950	60	NA	3	Unknown
2460	None	Nov. 13, 2008	F	68	NA	5	Y	N	Y	N	NA	NA	15	296	NA	0.72	3	Unknown

^a Group 1, patients with CNS infections of unknown etiology; group 2, patients with laboratory-confirmed CNS infections; group 3, bacterial meningitis patients; group 4, PCR confirmed/clinically suspected tuberculous meningitis patients. Names of provinces from which the patients were originated are intentionally not shown; patients 5 and 6 were the index cases in whose samples the CyCV-like sequence was detected by VIDISCA-454.

^b For *M. tuberculosis*, denominators may vary: JEV, Japanese encephalitis virus; DENV, dengue virus.

^c M, male; F, female.

^d Data are numbers (percentages) of patients; continuous variables are presented as medians (ranges). NA, not available; Y, yes; N, no.

^e Scores are on the Glasgow coma scale (GCS) unless the Blantyre coma scale (BCS) is indicated.

^f 1, recovery; 2, death; 3, unknown.

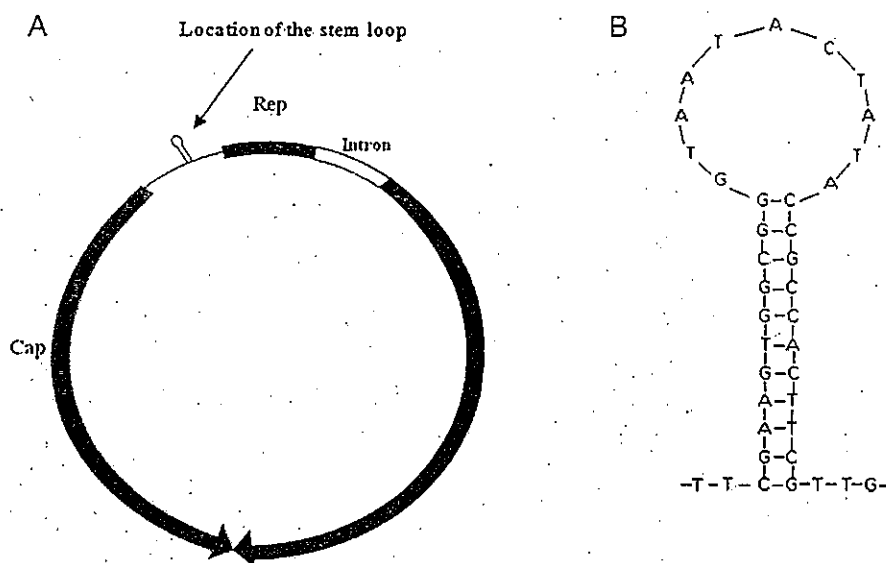


FIG 2. (A) Predicted genome organization of CyCV-VN; (B) stem-loop structure of CyCV-VN, with nonamer sequence in red.

is thought to initiate the rolling-cycle replication of the virus (6). The nonamer sequence of CyCV-VN (TAATACTAT) was identical to that of other CyCVs (Table 3), and the sequence of the loop was 11 bp long, which is within the 9- to 13-bp range of other CyCV loops (3–5) (Table 3).

There are no formally defined criteria for classifying a new viral species within the recently proposed genus *Cyclovirus*. However, criteria for a new species within the genus *Circovirus* proposed by the International Committee on Taxonomy of Viruses are whole-genome sequence identities of less than 75% at the nucleotide level and amino acid sequence identities of the Cap protein of less than 70% (6). Li and colleagues, who first described the genus *Cyclovirus*, proposed to use these species demarcation criteria of the genus *Circovirus* to classify cycloviral species (3). To determine whether CyCV-VN represents a new cyclovirus species, its com-

plete genome sequence was compared to 15 representative full-length genomes of 15 different cyclovirus species. The degrees of similarity ranged from 44 to 70% at the nucleotide level, with highest similarity being observed with CyCV-TN18, a cyclovirus detected in the stool of a Tunisian patient with acute flaccid paralysis (Table 4). Phylogenetic analysis confirmed that CyCV-VN was genetically distinct from known cycloviruses, albeit most closely related to CyCV-TN18 and CyCV-TN25, both derived from human stool samples from Tunisia and belonging to the same species as proposed by Li et al. (Fig. 3A) (3). Analysis of amino acid sequences of the Cap proteins from the same viruses yielded results similar to those of whole-genome analysis (Table 4).

Twenty-nine cyclovirus species have been proposed thus far (3–5, 7). While complete genomes of only 15 species are available, partial sequences of the Rep gene are available for the remaining 13 species. It has recently been proposed that separate CyCV species should have less than 85% similarity at the amino acid level in the partial Rep protein (3, 4). To further determine the relationships between CyCV-VN and reported CyCVs for which complete genomes were not available, pairwise comparison and phylogenetic analysis were performed on nonrepetitive amino acid sequences of Rep proteins of 28 cyclovirus species. Results of these analyses confirmed the findings of our analyses of whole genome sequences and Cap proteins (Fig. 3B; Table 4).

Prevalence of CyCV-VN DNA detection in CSF of patients with central nervous system disorders of suspected infectious and noninfectious etiology. To study the prevalence of CyCV-VN in CSF, we first determined the frequency of viral DNA detection by CyCV-VN-specific real-time PCR in 642 randomly selected CSF samples from patients with suspected acute CNS infections enrolled in three descriptive studies in southern and central Vietnam from which the acute-infection CSF samples were selected for our virus detection experiments. We detected CyCV-VN in 26 of 642 (4%) acute-infection CSF specimens, including 10 of 273 (3.7%) CSF specimens from patients with CNS

TABLE 3 Nonamer sequences of CyCV-VN and 15 different CyCV species and lengths of the stems

CyCV species	Nonamer sequence ^a	Loop length
VN	TAATACTAT	11
PK 5006	TAATACTAT	13
PK 5034	TAATACTAT	13
PK 5222	TAATACTAT	13
PK 5510	TAATACTAT	12
Chimp 11	TAATACTAT	13
NG12	TAATACTAT	13
NG 14	TAATACTAT	11
TN 18	TAATACTAT	12
NG chicken 8	TAATACTAA	12
PK goat 11	TAATACTAT	12
PK goat 21	TAATACTAG	13
TB bat USA	TAATACTAT	12
Bat GF-4c	TAATACTAT	11
NG 13	TAGTATTAC	9
DFCyCV-A1	TAATACTAT	13
FWCasCyV-GS140	TAGTATTAC	11

^a Underlined nucleotides are those that differ from the consensus sequence.

TABLE 4 Degree of sequence identities between CyCV-VN and other cycloviruses^a

CyCV species	% identity of:			Host
	Complete genome (nt level)	Cap protein (aa level)	Rep protein (aa level)	
USAbat-TB/2009	46	22	51	Bats
BatGF-4c	45	22	50	Bats
Chimp12	43	24	46	Chimpanzees
NG12	44	26	51	Humans
NG13	40	14	43	Humans
NG14	44	22	50	Humans
NGchicken15/2009	45	23	49	Chickens
PK5006	46	22	48	Humans
PK5034	44	23	51	Humans
PK5222	44	30	49	Humans
PK5510	46	28	51	Humans
PKgoat11/2009	44	25	49	Sheep
PKgoat21/2009	67	50	70	Sheep
TN18	70	48	74	Humans
DfCyCV-A1	50	30	47	Dragonflies
FWCasCyV-GS140	45	23	33	Cockroaches
NG23	NA	NA	50	Humans
Chimp13	NA	NA	51	Chimpanzees
PK5192	NA	NA	40	Humans
PKgoat24	NA	NA	30	Sheep
Pkbeef25	NA	NA	76	Bovines
TN12	NA	NA	46	Humans
TN9	NA	NA	46	Humans
TN26	NA	NA	49	Humans
NG6	NA	NA	46	Humans
NG15	NA	NA	46	Humans
Chimp73	NA	NA	45	Chimpanzees
TN6	NA	NA	46	Humans
Chimp53	NA	NA	48	Chimpanzees

^a NA, not available. Sequence accession numbers are shown in Fig. 3.

infections of unknown etiology and 16 of 369 (4.3%) from patients in whom laboratory-confirmed CNS infection with other pathogens was established. The latter included 4/140 (2.9%) patients with confirmed viral encephalitis (Japanese encephalitis virus [JEV] and Dengue virus [DENV]), 7/108 (6.5%) patients with bacterial meningitis (*Streptococcus pneumoniae*, *Haemophilus influenzae*, or *Streptococcus suis* serotype 2) and 5/121 (4.1%) patients with laboratory-confirmed or clinically suspected tuberculous meningitis (Table 2). The viral burden as estimated by cycle threshold (C_T) values was not different between specimens of unknown etiology and those diagnosed with other pathogens (data not shown). Details on characteristics and clinical outcome of CyCV-VN patients are presented in Table 2 and are compatible with that of other patients with CNS infections, although the mortality observed (4%) was lower than the overall mortalities of patients in cohorts from the PVE (a pediatric viral encephalitis study), BMD (an adult viral encephalitis study), and OISS (a study of children and adults with suspected CNS infections) studies (29%, 10% and 12%, respectively). CyCV-DNA could not be detected in 122 CSF specimens collected from Vietnamese patients with noninfectious neurological disorders.

CyCV-VN detection in human specimens other than CSF. We tested the presence of CyCV-VN DNA in available specimens other than CSF that were collected in parallel from patients whose CSF was positive and found CyCV-VN DNA in 1 of 5 rectal swabs, 1 of 4 throat swabs, and 0 of 3 available sera obtained at the time of lumbar puncture.

No viral DNA was detected in 90 residual blood specimens obtained from routine hospital biochemistry laboratories in Vietnam. However, CyCV-VN DNA could be detected in 8/188 (4.2%) fecal specimens from healthy children.

CyCV-VN detection in animal samples. Previous reports have shown the detection of diverse cycloviruses in farm animal meats (3). The existence of possible animal reservoirs as potential sources for human exposure was investigated using CyCV-VN-specific real-time PCR. We tested fecal material from poultry and pigs from the home province (Dong Thap) of the "index" patient from whom the complete genome of CyCV-VN was obtained. Viral DNA was detected in 38/65 (58%) animal specimens, including 12/20 (60%) pigs (5/10 boot swabs and 7/10 individual fecal droppings), 12/12 (100%) chickens (3/3 boot swabs and 9/9 individual fecal droppings), and 14/33 (42%) ducks (6/13 boot swabs and 8/20 individual fecal droppings).

Genetic diversity of CyCV-VN. In order to explore the genetic relationship between the cycloviruses detected in human and animal specimens, we obtained additional 6 full-genome sequences (4 from human CSF and 2 from chicken and pig feces), 1 nearly full-genome sequence from a duck fecal sample, and 19 partial Rep gene sequences (10 from CSF, 4 from human stool samples, and 5 from animal fecal samples). Pairwise comparison of the obtained sequences revealed that the obtained human and animal cyclovirus sequences belong to the same CyCV-VN species, as reflected by a degree of sequence similarity of >97% between the detected strains (data not shown). This low intraspecies sequence

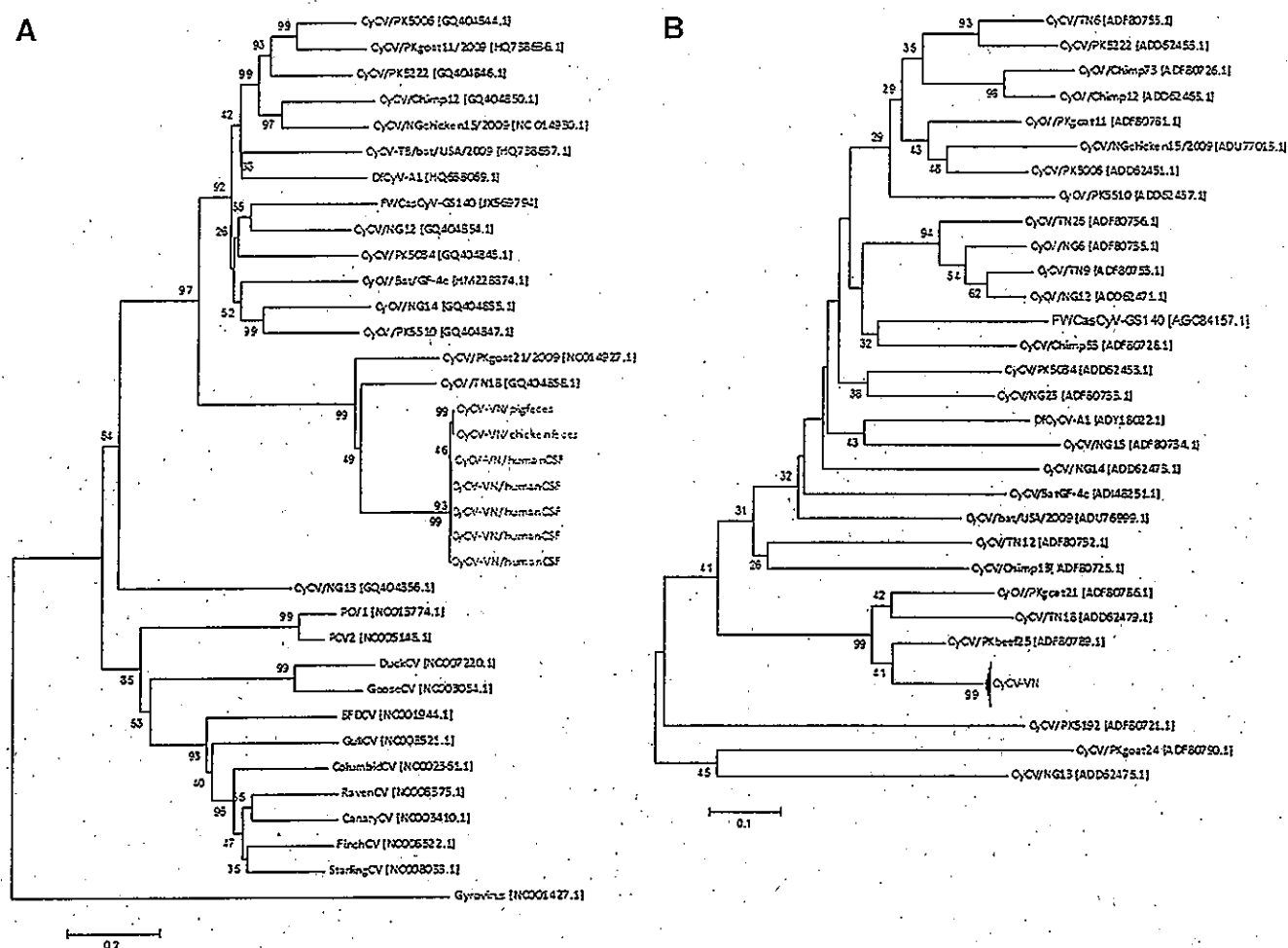


FIG 3 Reconstructed phylogeny trees of cycloviruses. (A) Tree based on complete genome sequences of 7 strains of CyCV-VN (red), 16 cycloviruses, 12 circoviruses, and a gyrovirus as an outlier. (B) Tree based on partial amino acid sequences of Rep proteins of 28 reported CyCV species and 27 CyCV-VN strains, indicated by the compressed branch. Trees were built by means of neighbor joining (MEGA 4.1); bootstrap tests of the reconstructed trees were done with 1,000 replicates. Sequence accession numbers are in brackets. CV, circovirus.

divergence of the CyCV-VN is consistent with that of other cyclovirus species (3–5). The results of phylogenetic analyses are presented in Fig. 3.

DISCUSSION

The availability of highly sensitive molecular methods such as PCR and sequence-independent techniques based on next-generation sequencing technology allows enhanced detection of (novel and unknown) pathogens in clinical specimens, which is essential to guide prevention strategies, aid clinical management, and identify the sources of outbreaks (8). Using a sequence-independent virus discovery assay, VIDISCA-454, we identified a cycloviral sequence in the CSF of patients with acute encephalitis with unknown etiology. Whole-genome characterization indicated that the sequence represented a novel species within the genus *Cyclovirus* of the family *Circoviridae* that we tentatively named CyCV-VN.

Circoviridae is a family of nonenveloped viruses containing a circular single-stranded DNA genome and is subdivided into two

genera, *Circovirus* and *Gyrovirus*, both of which have been associated only with disease in animals, including birds and pigs. Avian circoviruses have been associated with beak abnormalities, lethargy, and anorexia in pigeons and production losses and mortality in geese (9), and porcine circovirus 2 causes respiratory and enteric diseases, dermatitis, and reproductive problems in pigs, resulting in economic losses in the pork industry (10–12). Recently, novel viruses belonging to the family *Circoviridae* have been identified which have been proposed to represent a novel genus, *Cyclovirus* (3). In addition to being found in farm animals (e.g., sheep and bovines), chimpanzees, bats, cockroaches, and dragonflies (3, 5, 7, 13), cycloviruses have been detected in the blood of patients with febrile illness, stool samples of children with acute flaccid paralysis, and skin swabs and feces of healthy adults and children, respectively (3, 14, 15). However, a causal link between cycloviruses and human or animal disease has not been proven (3).

After initial discovery and characterization, we detected CyCV-VN by PCR in a total of 12 CSFs from 273 patients with acute encephalitis of unknown etiology. We also tested the CSF

TABLE 5 Samples for PCR screening of CyCV-VN

Sample ^a	Disease/syndrome	No.	Collection period	Hospital ^b	Hospital location
CSF ^c	CNS	642	1999-2009	— ^d	Southern and central Vietnam
CSF ^d	Noninfectious condition	122	1997-2011	Hospital for Tropical Diseases and Cho Ray Hospital	Ho Chi Minh City, southern Vietnam
Blood ^e	Unknown	90	2009-2010	Hue Central Hospital	Hue City, central Vietnam
Human feces	Healthy children	188	2011	NA	Southern Vietnam
Animal feces ^f	NA	65	2011	NA	Dong Thap Province, southern Vietnam

^a Prior to PCR screening, nucleic acid was isolated from clinical samples with use of the easyMAG (bioMérieux, Marcy l'Étoile, France) or the MagNA pure96 system (Roche), following the manufacturer's instructions.

^b The hospitals from which the noninfectious CSF and blood samples were collected are referral hospitals for southern and central provinces in Vietnam. NA, not applicable.

^c From children and adult patients enrolled in the BMD, PVE, or OISS study (see Materials and Methods for study details).

^d Indications for lumbar puncture included headache/migraine ($n = 17$), trauma ($n = 16$), postoperative CSF leakage ($n = 2$), tumors ($n = 13$), benign intracranial hypertension ($n = 1$), epilepsy/convulsion ($n = 15$), encephalopathy ($n = 5$), external ventricular draining ($n = 4$), hemorrhage/stroke ($n = 29$), Guillain-Barré syndrome ($n = 3$), cranial nerve palsies ($n = 6$), and others ($n = 11$). The patients included both children and adults [age median (range): 36 (1 to 91) years; 67% male], and they were from 26 provinces in central or southern Vietnam.

^e Anonymized residual blood samples from an influenza sero-surveillance study.

^f Including samples collected from individual animals and boot swabs—from pigs ($n = 20$), chickens ($n = 12$), and ducks ($n = 33$).

from patients with other laboratory-confirmed CNS infections, and found a detection rate similar to that in patients with unknown etiology (16/369; 4.3%). In contrast, CyCV-VN DNA was not detected in 122 CSF specimens from patients with noninfectious CNS conditions ($P = 0.015$). However, it should be noted that 88% of 122 CSF samples from patients without CNS infections were collected over a short period of time (August to October 2011), possibly introducing bias.

The detection of CyCV-VN in CSF of patients with CNS infections, both with known and with unknown etiology, but not in CSF from patients with noninfectious CNS disorders may imply a pathogenic role of CyCV-VN, either as a single agent or as a coinfecting agent, e.g., by enhancing severity of disease or facilitating entry of other pathogens into the CNS compartment. While the mortality of CyCV-VN-infected patients seemed relatively low, comparison of clinical severities and outcomes between CyCV-VN patients with and without coinfection with other pathogens could not be done reliably because of incomplete data collection at discharge and the small numbers of patients (Table 2).

CyCV-VN was detected in patients of all ages, from less than 1 year to over 60 years, from 7 different provinces in southern and central Vietnam over a 10-year period from 1999 to 2009. This suggests that this virus can affect persons of all ages, has a broad geographic distribution in Vietnam, and did not emerge very recently.

No CyCV-VN DNA was found in 3 available parallel sera of CSF-positive patients, suggesting that the detection of CyCV-VN DNA in CSF does not reflect ongoing viremia with spillover into the CSF or blood contamination of CSF after possible traumatic lumbar puncture. However, the possibility that past viremia was responsible for CNS entry cannot be excluded. The fact that CyCV-VN was not detected in 90 randomly selected blood from Vietnamese subjects renders it unlikely that cyclovirus is a ubiquitous commensal virus, like torque teno virus (TTV), which is asymptotically present in blood of 7 to 78% of people, with occasional detection in CSF (16–21).

CyCV-VN DNA was also detected in throat and rectal swabs of CSF-positive patients as well as in fecal specimens of healthy children. This suggests a possible food-borne or fecal-oral transmission route for infection, although detection in feces of healthy children may also merely reflect consumption of CyCV-VN-infected food rather than true infection.

To explore the existence of possible animal reservoirs of CyCV-VN for such transmission, we tested fecal samples from animals in Dong Thap Province, where the index patient lived, and found remarkably high detection rates in fecal samples collected from pigs, ducks, and chickens, ranging from 42 to 100%. Sequence identities between CyCV-VN strains in humans and animals were high (around 98%) (Fig. 3), which may suggest interspecies transmission between animals and possibly from animals to humans.

It should be noted that the PCR screening method was designed for specific detection of only CyCV-VN and closely related viruses and that complete or partial genomic sequences were not obtained in all CyCV-VN PCR-positive samples; hence, the possibility exists that genetically diverse, more distantly related CyCV species are cocirculating in Vietnam. Future research is needed to investigate this possibility and to determine whether human infections are restricted to CyCV-VN species. While the discovery of CyCV-VN and its frequent detection in the usually sterile CSF compartment are remarkable in themselves, the results of the present study should be interpreted with caution, because the mere detection of a novel virus in clinical specimens of patients with disease does not prove a causative role (22, 23). This is illustrated by the recent discovery and subsequent "dediscovery" of xenotropic murine leukemia virus-related virus in patients with prostate cancer and patients with chronic fatigue syndrome (24, 25), which attracted much attention among scientists, the media, and patient groups and required stringent follow-up studies to show the absence of a link between the virus and the disease or syndrome (26, 27). According to criteria recently proposed by Lipkin, our findings only point to a possible causal relationship at present (22, 23). In order to detect a more definitive causal link between infection with CyCV-VN and CNS disease, studies to determine whether CyCV-VN fulfills Koch's postulates or adapted versions of these are necessary (22, 23, 28–30). For these, efforts to isolate the virus in cell culture or animal models and the detection of a specific immune response to CyCV-VN are imperative and ongoing.

MATERIALS AND METHODS

Patients and samples. Clinical data and CSF specimens analyzed in this study were derived from three prospective clinical studies designed to establish the etiology of CNS infections in Vietnam. These included a previously published study (PVE) on children with presumed viral en-

cephalitis admitted to Children's Hospital Number One in Ho Chi Minh City in 2004 ($n = 194$) (31); a study (BMD) on adults with clinically suspected viral encephalitis admitted to the Hospital for Tropical Diseases, Ho Chi Minh City, between 1997 and 2008 ($n = 291$) (unpublished data), and a study (OISS) of children and adults with suspected CNS infections admitted to 13 provincial hospitals in central and southern Vietnam between 2008 and 2010 ($n = 1,241$) (32).

Acute-infection CSF specimens were collected, divided into aliquots, and stored at -80°C until analysis. In addition, acute-infection blood specimens and rectal and throat swabs were collected from children participating in the PVE study. Detailed demographic and clinical data, including routine blood and CSF hematology and chemistry laboratory investigations, were collected on case record forms at enrollment and during follow-up.

In all three studies, initial diagnostic efforts included CSF Gram stains, CSF culture, and specific PCR-based methods and serology targeting herpes simplex viruses, enteroviruses, JEV, dengue virus, *Streptococcus pneumoniae*, *Streptococcus suis* serotype 2, *Haemophilus influenzae*, and *Neisseria meningitidis*. Additional diagnostic tests for flaviviruses (generic), influenza A virus, and cytomegalovirus were performed in the PVE and BMD studies. Parechoviruses and Semliki Forest virus were also tested for in the PVE study (33). Adult patients in the BMD study were further tested for varicella zoster virus, influenza B virus, Epstein-Barr virus, mumps virus, and Nipah virus by PCR and for *Mycobacterium tuberculosis* by CSF culture and microscopy. A subset of 121 patients in the OISS cohort with clinically suspected tuberculosis meningitis was also tested by *M. tuberculosis* PCR (32).

From these case series, CSF specimens with unknown etiology were selected for VIDISCA-454 analysis, for which the following selection criteria were used to increase the probability of detecting a novel viral pathogen causing relevant disease: (i) fever or a history of fever ($\geq 37.5^{\circ}\text{C}$), (ii) illness onset of ≤ 5 days, (iii) CSF white cell count between 6 and 1,000 cells/ μL , (iv) normal CSF glucose (CSF glucose/blood glucose ratio ≥ 0.5), and (v) absence of an etiological diagnosis by the methods described above. In total, 125 CSF specimens from patients with CNS infections with unknown etiology (PVE, $n = 6$; BMD, $n = 45$; and OISS, $n = 74$) were selected.

After the discovery of the novel cyclovirus in the initial clinical series, we investigated its clinical relevance and potential animal reservoirs by further testing of CSF specimens from patients with infectious and non-infectious neurological illness, residual blood specimens, stool samples from healthy children, and fecal samples from poultry and pigs in Vietnam (Table 5).

VIDISCA-454 analysis. The selected CSF specimens were analyzed by VIDISCA-454 either as single samples ($n = 27$, for samples with CSF cell counts of ≥ 152 cells/ μL) or as pools of ≤ 5 specimens ($n = 23$, for the remaining 98 samples with CSF cell counts of ≤ 151 cells/ μL).

The VIDISCA-454 procedure was done as previously described (2). In brief, prior to nucleic acid (NA) isolation, samples were centrifuged to eliminate cell debris and mitochondria, followed by DNA digestion by Turbo DNase I (Ambion, Austin, TX). NAs were then extracted by a guanidinium thiocyanate-based procedure (34) and recovered in 50 μL of sterile water containing a 10 μM concentration of rRNA-blocking oligonucleotides (2 μM for each oligonucleotide). Blocking oligonucleotides were used to avoid reverse transcription and amplification of abundant human rRNA, thereby increasing the sensitivity of the VIDISCA-454 (2). The sequences of these oligonucleotides were designed based on human 18S and 28S rRNA sequences but contain a 3' dideoxy-C-6 modification which cannot be extended. Reverse transcription of viral RNA was done using nonribosomal hexamer primers (35) and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA), followed by double-stranded-DNA (dsDNA) synthesis using DNA polymerase I Klenow fragment (Westburg, Leusden, the Netherlands) and RNase H (Amersham, Uppsala; Sweden). The resulting dsDNA was subjected to restriction enzyme digestion with MseI (New England Biolabs, Ipswich, MA), MseI-adaptor

ligation with DNA ligase (Invitrogen), and PCR amplification with primers containing a restriction site adaptor and a multiplex identifier (MID; Roche Diagnostics GmbH, Mannheim, Germany). Finally, the amplified product was subjected to emulsion PCR (GS emPCR kit II; Roche) followed by DNA sequencing on a FLX genome sequencer (454 Life Science, Roche Applied Science, Roche), following the supplier's instructions.

Conventional and real-time PCR. To confirm the presence of the cyclovirus-like sequence generated by high-throughput sequencing, specific PCR primers (CyCV31-53F and CyCV178-53R) (Table 1) were designed based on the obtained sequence and were used to test the 5 individual original CSF specimens of the initial pool in which the sequence was detected. Sequence alignment indicated that these primers could detect CyCV-VN and a closely related CyCV species—CyCV-20—classified by Li et al. (3) (see Fig. S1 in the supplemental material). The PCR was done in a final 25- μL volume reaction mixture containing 1.25 U of Hot-StarTaq DNA polymerase (Qiagen GmbH, Hilden, Germany), an 800 μM concentration of each primer, a 400 μM concentration of each deoxynucleoside triphosphate (dNTP), 5 mM MgCl_2 (provided with the polymerase), 1 $\times$ PCR buffer (provided with the polymerase), and 3 μL of extracted NAs. PCR was performed in a Mastercycler (Eppendorf, Hamburg, Germany) with an initial polymerase activation step at 95°C for 14 min 30 s, followed by 45 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. Confirmation of amplified product was done by sequencing, using BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems, Carlsbad, CA) in an ABI377 automatic sequencer (Applied Biosystems), following the manufacturer's instructions.

For high-throughput screening of clinical specimens, we designed a real-time PCR using the same primers and a 6-carboxyfluorescein (FAM)-labeled probe (CyCV65-96) (Table 1) at a final concentration of 100 μM . The real-time PCR was performed on a Chromo4 platform (MJ Research, Bio-Rad, Hercules, CA) or a LightCycler480 real-time PCR system (Roche) using the above-described thermal cycling program.

Inverse PCR and (full-length) genome sequencing. For whole-genome sequencing, iPCR was employed and performed in a final reaction volume of 50 μL containing 400 μM of the primers CyCV31-53F-rc and CyCV178-53R-rc (Table 1), 250 μM of each dNTPs (Roche Diagnostics), 1 $\times$ PFU ultra buffer (provided with the polymerase), 1 μL of PfuUltra II fusion HS DNA polymerase (Stratagene, Santa Clara, CA), and 5 μL of extracted NAs. PCR was performed in a Mastercycler (Eppendorf, Germany) with an initial polymerase activation step at 95°C for 2 min, followed by 50 cycles of 95°C for 20 s, 55°C for 20 s, and 72°C for 1 min.

To obtain a partial replication associated protein-coding gene sequence, primers CyCV-FL3 and CyCV-FL4 (Table 1) were employed, and the PCR was carried out with PfuUltra II fusion HS DNA polymerase under the conditions used for iPCR.

The product was directly sequenced in duplicate using the same PCR primers and walking primers (CyCV-FL1 to -FL4) (Table 1), whose design was based on the sequences obtained with the iPCR primers. All sequencing reactions were done using a BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems), following the manufacturer's instructions.

All the PCR experiments were performed in molecular diagnostic facilities that consist of three physically separated laboratories for reagent preparation, extraction, and amplification, and these used a unidirectional workflow.

Sequence analysis. Sequence reads were imported into CodonCode Aligner v. 3.7.1 (CodonCode, Dedham, MA) to remove adaptor sequences and DNA fragments of *Escherichia coli* genome. The remaining reads were then subjected to a sequence similarity search by BLASTn. Pairwise comparisons and phylogenetic analyses were done using AlignX (Vector NTI Advance 11; Invitrogen) and neighbor-joining in MEGA version 4 (36), respectively. Prediction of ORF of the virus genome was done using Vector NTI advance 11 (Invitrogen).

Ethics statement. All clinical studies were reviewed and approved by the Institutional Review Boards of collaborating hospitals/authorities and

the Oxford Tropical Research Ethics Committee (OxTREC), University of Oxford, United Kingdom.

Nucleotide sequence accession number. The GenBank accession numbers for the CyCV-VN sequences are KF031465–KF031492.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00231-13/-/DCSupplemental>.

Figure S1, TIF file, 1.2 MB.

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一般的名称	新鮮凍結人血漿		公表国	
販売名(企業名)	新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分血(日本赤十字社) 新鮮凍結血漿-LR「日赤」J120(日本赤十字社) 新鮮凍結血漿-LR「日赤」J240(日本赤十字社) 新鮮凍結血漿-LR「日赤」J480(日本赤十字社)	研究報告の公表状況	中国	
研究報告の概要	○大規模シークエンシングにより発見された血清反応陰性を呈する中国人肝炎患者におけるハイブリッドDNAウイルスの詳細な解明は不十分であるが、重篤な合併症と関連している血清反応陰性肝炎(非A、B、C、D、E型肝炎)が報告されている。1999年から2007年に、中国重慶市で非A-E型肝炎患者92人の血清検体を10プールとし、Solexa大規模シークエンシング法を用いてスクリーニングした。その結果、全てのプールで、パルボウイルスに対してBLASTx Eスコア7e-05~0.008である3,780bpのコンティグが存在した。<i>In silico</i>で組み立てられた3,780bpのコンティグの完全配列が、重複領域の遺伝子増幅により確認され、ウイルスは暫定的にNIH-CQVと命名された。更なる分析により、コンティグが2つの主要なORFから構成されることが明らかとなった。タンパク質BLASTでは、ORF1とORF2によってコードされるアミノ酸配列はそれぞれコウモリサーコウイルスの複製関連タンパク質とブタパルボウイルスのカプシドタンパク質に比較的高い相同性を示し、系統発生解析では、NIH-CQVはパルボウイルス科とサーコウイルス科の境界に位置することを示した。定量PCRを用いてNIH-CQV感染率を調べた結果、血清反応陰性肝炎患者90人のうち63人(70%)が陽性であったが、45人の健常対照群は全員陰性であった。患者検体の平均ウイルス力価は1.05×10^4 コピー/μLであった。免疫プロットを用いてNIH-CQV特異抗体を調べた結果、患者の84%がIgG陽性、31%がIgM陽性であり、一方健常対照群では78%がIgG陽性、IgM陽性は0%であった。ヒトの疾患へのNIH-CQVの病因的役割については更なる研究が必要であるが、これらのデータは、非A-E型肝炎患者集団でパルボウイルス様ウイルスの保有率が高いことを示している。			
報告企業の意見	今後の対応			
中国の非A-E型肝炎患者の血清検体から、パルボウイルス様ウイルスが検出されたとの報告である。	今後も引き続き情報の収集に努める。			
	使用上の注意記載状況・その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分血 新鮮凍結血漿-LR「日赤」J120 新鮮凍結血漿-LR「日赤」J240 新鮮凍結血漿-LR「日赤」J480 血液を介するウイルス、細菌、原虫等の感染、vCJD等の伝播のリスク			

Hybrid DNA virus in Chinese patients with seronegative hepatitis discovered by deep sequencing

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Seronegative hepatitis—non-A, non-B, non-C, non-D, non-E hepatitis—is poorly characterized but strongly associated with serious complications. We collected 92 sera specimens from patients with non-A–E hepatitis in Chongqing, China between 1999 and 2007. Ten sera pools were screened by Solexa deep sequencing. We discovered a 3,780-bp contig present in all 10 pools that yielded BLASTx E scores of 7e-05–0.008 against parvoviruses. The complete sequence of the *in silico*-assembled 3,780-bp contig was confirmed by gene amplification of overlapping regions over almost the entire genome, and the virus was provisionally designated NIH-CQV. Further analysis revealed that the contig was composed of two major ORFs. By protein BLAST, ORF1 and ORF2 were most homologous to the replication-associated protein of bat circovirus and the capsid protein of porcine parvovirus, respectively. Phylogenetic analysis indicated that NIH-CQV is located at the interface of *Parvoviridae* and *Circoviridae*. Prevalence of NIH-CQV in patients was determined by quantitative PCR. Sixty-three of 90 patient samples (70%) were positive, but all those from 45 healthy controls were negative. Average virus titer in the patient specimens was 1.05 e4 copies/μL. Specific antibodies against NIH-CQV were sought by immunoblotting. Eighty-four percent of patients were positive for IgG, and 31% were positive for IgM; in contrast, 78% of healthy controls were positive for IgG, but all were negative for IgM. Although more work is needed to determine the etiologic role of NIH-CQV in human disease, our data indicate that a parvovirus-like virus is highly prevalent in a cohort of patients with non-A–E hepatitis.

hepatitis-associated aplastic anemia | liver failure | Solexa sequencing | virological diagnosis

Most viral hepatitis is secondary to infection by known hepatotropic viruses: hepatitis A virus (HAV) (1), hepatitis B virus (HBV) (2), hepatitis C virus (HCV) (3), hepatitis delta virus (HDV) (4), and hepatitis E virus (HEV) (5). Other hepatitis-associated viruses, including CMV (6), Epstein–Barr virus (EBV) (7), herpes simplex virus 1 and 2 (8), varicella-zoster virus, human herpesvirus 6 (9), human parvovirus B19V (B19V) (10), and adenoviruses (11), may cause liver injury, ranging from mild and transient elevation of aminotransferases to acute hepatitis and occasionally acute liver failure. Despite the number of hepatitis viruses and hepatitis-associated viruses, etiology cannot be determined in 10–20% of the cases of acute hepatitis (12), in 30% of cases of cryptogenic chronic liver diseases (13), in hepatitis-associated aplastic anemia (14), and in a large proportion of cases of acute liver failure (15). Patients with acute seronegative hepatitis have fewer parenteral risk factors, more severe bilirubin and transaminase elevations, and worse hepatocellular synthetic function than do patients with hepatitis C (16). Moreover, seronegative hepatitis is more strongly associated with serious complications, especially bone marrow failure (14) and childhood fulminant hepatitis (17).

Next-generation sequencing (NGS) technology has had an increasing impact on biological research and clinical diagnosis, because it provides high-resolution genome-scale data rapidly and reliably. The first application of NGS for pathogen discovery in

a patient who died of a febrile illness after solid-organ transplantation led to the identification of an arenavirus (18). NGS-based platforms, such as 454 and Solexa deep sequencing, have been applied successfully to the investigation of emerging human pathogens, resulting in major discoveries of new human viruses (19–21).

In the present study, we established an experimental and analytic procedure for identifying virus identification in human specimens based on Solexa deep sequencing. We applied the method to screen for potential viral infection in human sera specimens and discovered a human virus in Chinese patients who had seronegative hepatitis. From its genome organization and phylogeny, the virus is unusual, because it is evolutionarily at the interface of the parvovirus and circovirus families, perhaps having emerged from interfamilial recombination. The virus was highly prevalent in a patient population, and active infection in seronegative hepatitis cases was suggested by the presence of viral DNA in the blood and serology. However, more work is needed to establish a firm disease association.

Results

Detection of a Virus in Patients with Seronegative Hepatitis. Ten pools were made of sera from 92 patients with non-A–E hepatitis and were screened for potential pathogens by Solexa deep sequencing. After filter sterilization, the samples were digested with DNase and RNase to eliminate host nucleotide contamination, and the remaining nucleic acids were extracted using carrier RNA [synthetic poly(A)]. cDNA was synthesized from extracted viral nucleic acids with non-poly(A) random hexamers, which were designed specifically to block reverse transcription of the carrier RNA. With non-poly(A) random hexamers, redundant sequence tags derived from the carrier RNA were reduced to a level of <0.001%. High capacity enabled multiplex sequencing of more than 70 individual samples in a single Solexa run. Short DNA sequences were analyzed and filtered at the nucleotide level to exclude potential DNA contamination from human cells, mouse cells, and known bacteria. For detection of known viruses, non-redundant reads were mapped against virus genomes from the National Center for Biotechnology Information (NCBI) Reference

Author contributions: B.X., N.Z., K.Z., Q.M., and N.S.Y. designed research; B.X., N.Z., G.H., Z.W., X.Z., X.L., S.W., and S.K. performed research; B.X., N.Z., G.H., and X.Z. analyzed data; Z.W. and Q.M. collected the clinical specimens; and B.X., N.Z., and N.S.Y. wrote the paper.

The authors declare no conflict of interest.

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Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. KC617868).

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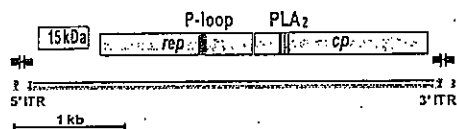


Fig. 1. Schematic diagram of the NIH-CQV genome. The putative ORFs are diagrammed in boxes, and the arrows indicate the orientation of the ORFs. The conserved P-loop is shown as a shaded box in rep, and the conserved PLA₂-like motifs are shown as shaded boxes with the Ca²⁺ binding loop in black and the catalytic residues in gray.

Sequence (RefSeq) database using the short sequence alignment tool Bowtie. HAV and HCV sequences were detected in samples derived from sera pools 2 and 4, respectively. Individual samples from these two pools then were sequenced to clarify the source of the hepatitis viruses, and high copy numbers of HAV or HCV genomes were detected in one patient in each pool (these two samples were excluded from further analysis of seronegative hepatitis cases). Furthermore, sequences from *Adenoviridae*, *Herpesviridae*, *Parvoviridae*, and *Anelloviridae* were also commonly detected. For virus discovery, filtered short sequences were subjected to de novo assembly. Contigs from different samples were clustered based on nucleotide-level similarities. The resulting contigs were further compared with the NCBI nonredundant (NR) protein database using tBLASTx. A contig was predicted as viral origin if the best tBLASTx hit was from the kingdom of viruses, and a cluster was defined as a candidate if it contained a contig with viral origin. Contigs for each cluster were subjected to multiple alignments to define a consensus contig for later data analysis and experimental validation. By applying this pipeline to the 10 pooled samples, we identified a 3,780-bp contig that yielded BLASTx E scores of 7e-05–0.008 against parvoviruses (GenBank accession no.: KC617868).

Characterization of a Human Virus Genome. The 3,780-bp contig was composed of three major ORFs (Fig. 1 and Fig. S1). Protein Blast (BLASTp) searches showed that ORF1 encoded a 45-kDa protein, which contained a domain (GxxxGK[T/S]) (22) for a phosphate-binding loop (P-loop) that is conserved among different DNA viruses (Table S1); ORF1 was homologous to the replication-associated protein (Rep) of bat circovirus with E values of 7e-04. ORF2 encoded a 55-kDa protein which was homologous to Parvovirus coat protein 1 (VP1) of porcine parvovirus (PPV) and goose parvovirus, with E scores of 2e-05. Because of low homology, only the first 87 amino acid residues encoded by ORF2 could be aligned reliably to the first 100 amino acids of VP1 of PPV. The first 100 amino acids of the N-terminal region of the PPV VP1 region constituted an active phospholipase A₂ (PLA₂), which is highly conserved among the members of the *Parvoviridae* and is essential for parvovirus infection (23). The putative capsid protein (CP) encoded by ORF2 included a PLA₂-like motif that is highly conserved among the members of the *Parvoviridae* (Table S2). ORF3 was located on the left side of the genome on the viral negative strand and encoded a 15-kDa protein. There was no homology at the nucleic acid level between the 3,780-bp contig and known viruses in GenBank.

Sequence analysis also revealed inverted terminal repeats (ITR) at both ends of the genome (Fig. S1). The ITR was 156 nucleotides long. The first 75 nucleotides complemented nucleotides 82–156, allowing the formation of a stem of a hairpin, whereas nucleotides 76–81 were mismatched and could form a loop. By sequence analysis of the intergenic region between ORF1 and ORF3, we found multiple direct repeats and TATA boxes (Fig. S1). *In silico* promoter prediction suggested that consensus sequences for a bidirectional eukaryote promoter were present in this region (www.fruitfly.org/seq_tools/promoter.html) and that the NIH-CQV might have an ambisense genome.

To confirm the sequence of the de novo-assembled 3,780-bp contig, eight pairs of PCR primers (Table S3) were designed, based on the sequence predicted and were used to amplify overlapping DNA fragments from the pooled patient samples. Eight amplified DNA fragments were of the expected lengths (Fig. S2), and their sequences could be assembled into a 3,629-bp contig. Sequencing analysis revealed that the 3,629-bp contig, composed of the entire coding region plus about half of the ITRs from both 5' and 3' termini, aligned exactly with that of the de novo-assembled 3,780-bp contig, indicating that the contig contained the nearly complete virus genome, was indeed present in the patient samples, and was not an artifact generated by mis-assembly. The virus was provisionally designated NIH-CQ virus (NIH-CQV), because the samples were collected in a hospital in Chongqing and the laboratory experiments were conducted at the National Institutes of Health (NIH).

Because ORF1 of NIH-CQV encodes a putative Rep protein homologous to circovirus Rep, we speculated that the virus might have a circular form of its genome. To test this hypothesis, we performed inverse PCR with a primer pair (Table S3) oriented outwardly with respect to each other. Amplification with the inverted-primer pair generated an amplicon of 116 bp from two of three patient samples tested. Sequencing and alignment of the inverse PCR product showed a junction region between the 5' and 3' termini in a head-to-tail orientation. In comparison with the linear genome of NIH-CQV, a 419-bp region composed of both 5' and 3' ITRs present in the linear virus genome was absent in the inverse PCR product (Fig. S3), indicating a closed circular form of the virus genome of 3,361 bp present in the patient samples. Circularization of the viral genome resulted in an extension of the ORF3 at the left end of the genome, from 369 bp to 435 bp, with predicted encoding of a 17-kDa protein. The circular form of NIH-CQV was designated NIH-CQV-Co.

Phylogenetic and Evolutionary Analyses of NIH-CQV. Because ORF1 and ORF2 of NIH-CQV were homologous to different viral families, phylogenetic analysis of the two ORFs was performed separately by comparison with members of *Circoviridae* and *Parvoviridae*. Although BLASTp searches showed that the amino acid sequences encoded by ORF1 were homologous to Rep of bat circovirus, by gene tree analysis NIH-CQV was not closely related to any known circoviruses (Fig. 2A). For ORF2, the amino acid similarity between NIH-CQV and known parvoviruses was below 20%. Because of low homology, only the first 87 amino acid

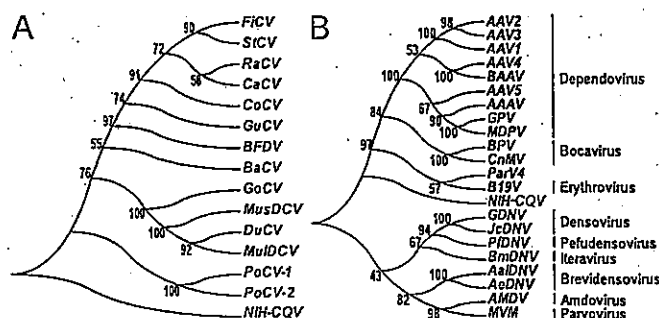


Fig. 2. Phylogenetic analysis of Rep and CP of NIH-CQV. (A) Phylogenetic relationship of the Rep of NIH-CQV and other related circoviruses based on an alignment of amino acid sequences. (B) Phylogenetic relationship of the CP of NIH-CQV and other related parvoviruses based on an alignment of amino acid sequences. The phylogenetic trees were constructed by neighbor joining (NEIGHBOR program from PHYLIP) based on alignments generated with CLUSTAL V, and 1,000 bootstrap replications were performed. All sequences were downloaded from GenBank and SwissProt with a BioPerl script. Detailed information is available in Dataset S1A.

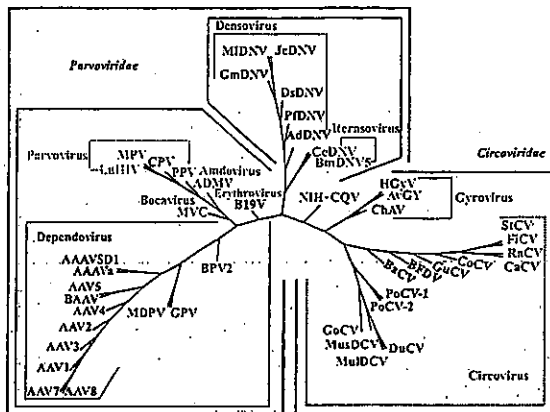


Fig. 3. Whole-proteome tree of NIH-CQV and members of *Parvoviridae* and *Circoviridae*. A total of 18 circoviruses and 28 parvoviruses are included in the analysis. The tree was constructed by neighbor joining based on protein sequences using dynamic language method for $K = 4$. The different viral genera are color-coded to the branches of the tree based on the taxonomic partitions. Detailed information regarding abbreviations and accession numbers are available in Dataset S18.

residues encoded by ORF2 could be aligned reliably with the VP1 of porcine parvovirus and goose parvovirus, and in this region amino acid identity was ~31%. NIH-CQV was not closely related to any known parvoviruses and thus appeared to be deeply rooted by lineage between human and animal parvoviruses and parvoviruses identified in arthropods (Fig. 2B). To confirm further the phylogenetic location of NIH-CQV, whole-proteome phylogeny analysis of NIH-CQV with 14 circoviruses and 21 prototypes of parvoviruses was conducted using a dynamic language model (24), which correctly divided the 35 selected viruses into two families, *Parvoviridae* and *Circoviridae*, and placed NIH-CQV at the interface of *Parvoviridae* and *Circoviridae* (Fig. 3).

Deep-sequencing data showed that NIH-CQV exhibited remarkable intrapatient genetic heterogeneity, suggesting the presence of closely related variants or quasispecies in the patients infected with NIH-CQV. To assess the dynamics of circulating quasispecies, we measured the ratios of nonsynonymous (K_a) versus synonymous (K_s) substitutions across the entire *rep* and *cp* of NIH-CQV from patients with acute ($n = 13$) or chronic ($n = 9$) non-A-E hepatitis. A higher value of K_a/K_s reflects selection favoring amino acid mutation (positive selection), whereas lower values are consistent with selection against nonsynonymous variants (negative selection). Positive selection results in the dominance of virus variants with a survival advantage, whereas negative selection acts to eliminate variants with a diminished capacity to complete the virus-replication cycle. The average K_a/K_s ratio of the *cp* of the virus was greater in patients with chronic hepatitis than in patients with acute hepatitis (0.98 ± 0.27 versus 0.71 ± 0.17 ; $P < 0.01$) (Fig. 4A). There were three regions in *cp*, ranging from nucleotides 2,360–2,448, 2,562–2,732, and 2,969–3,036 of the NIH-CQV genome, in which K_a/K_s ratios were greater than 1 and were significantly higher than in other regions ($P < 0.05$) (Fig. 4B), suggesting positive selection acting on specific regions of the *cp* gene. In contrast, although the average K_a/K_s ratio of the *rep* from patients with chronic hepatitis was greater than that for patients with acute hepatitis [0.47 ± 0.08 versus 0.39 ± 0.08 ($P < 0.05$)] (Fig. 4C), the overall K_a/K_s ratio was much lower (0.42), and there was no evidence for positive selection of this gene (Fig. 4D).

Prevalence of the NIH-CQ Virus in Clinical Specimens. We designed a quantitative PCR (qPCR) assay for the *rep* region of NIH-CQV and generated a standard curve using a synthetic DNA *rep* fragment. Sensitivity of detection was <10 copies. We conducted

screening of the 90 sera specimens from the patients with seronegative hepatitis and 45 sera specimens from healthy blood donors. Seventy percent (63/90) of the patient samples were positive, but all the samples from 45 healthy controls were negative. The average virus titer in the patient specimens was $1.05 \text{ e4 copies}/\mu\text{L}$, and the highest was $3.1 \text{ e4 copies}/\mu\text{L}$ (Fig. 5). No significant difference in NIH-CQV DNA levels was observed between patients with acute or chronic hepatitis (Table S4). The two patients with cryptic HAV and HCV infection were negative for NIH-CQV by qPCR.

Detection of Specific Antibody Against NIH-CQV in Patients with Non-A-E Hepatitis. To investigate the immune response against NIH-CQV, a synthetic DNA fragment encoding a 184-amino acid polypeptide of the C-terminal portion of CP was cloned into an expression vector, and the affinity-purified recombinant CP (rCP) was used for serological studies. Cross-reactivities between NIH-CQV and other human parvoviruses, including B19V, human parvovirus 4 (Parv4), human bocavirus (HBov), and adeno-associated virus 2 (AAV2), were tested by immunoblotting. No cross-reactivity was seen between CP of NIH-CQV and other major human parvoviruses (Fig. 6A). Immunoreactivity of rCP with a total of 135 human sera specimens was examined. By immunoblotting, 84% (76/90) of the patients with seronegative hepatitis were positive for NIH-CQV IgG, and 31% (28/90) were positive for IgM, suggestive of recent infection. In contrast, 78% (35/45) of healthy controls were positive for IgG, but all were negative for IgM, consistent with past NIH-CQV exposure (Fig. 6B, Fig. S4 A and B, and Table 1). There was a significant difference in NIH-CQV-specific IgM, but not in IgG, between the patients with acute and chronic hepatitis (Table S4).

Discussion

We report the identification of a virus, NIH-CQV, in patients with non-A-E hepatitis. Phylogenetically NIH-CQV appears to lie at the interface of parvoviruses and circoviruses. The genome of

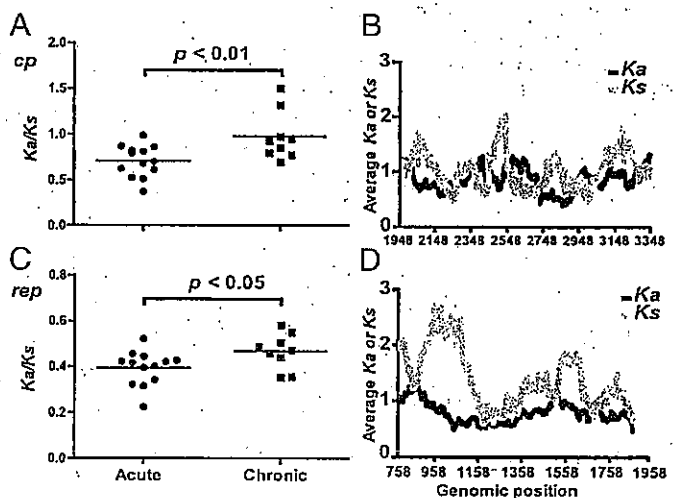


Fig. 4. Quasispecies of NIH-CQV in patients with acute and chronic hepatitis. (A and C) Scatterplot presentations of K_a/K_s ratios of *cap* and *rep* genes of NIH-CQV from patients with acute or chronic hepatitis. The average values of K_a/K_s ratios of *cap* or *rep* of NIH-CQV from 22 individual patients (acute: $n = 13$; chronic: $n = 9$) are indicated. (B and D) Distribution of K_a/K_s ratios throughout the *cp* and *rep*. The y-axis is the averaged values of K_a or K_s within a sliding window of 100 amino acids. The average values of K_a/K_s across the whole gene region of NIH-CQV from the 22 patients are indicated. Genomic regions with significant high K_a/K_s ($P < 0.05$; binomial test) are shown in gray.

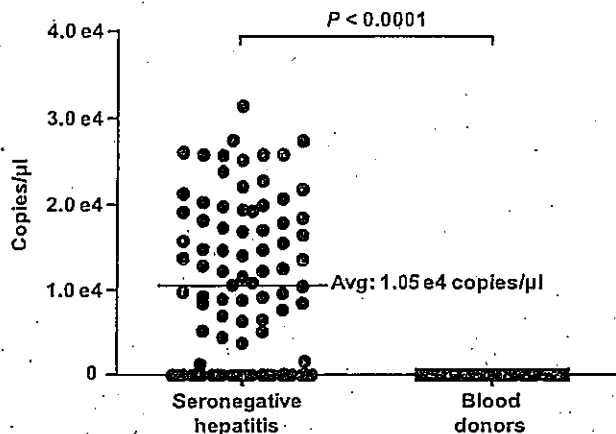


Fig. 5. Detection of NIH-CQV DNA by qPCR. Scatterplot showing virus load per microliter of sera specimens obtained from patients ($n = 90$) and healthy controls ($n = 45$). Each dot represents one specimen. Bars show the average copy numbers of virus genome.

NIH-CQV has no homology to any known viruses in GenBank. Although comparative analysis revealed that the Rep and CP proteins of NIH-CQV have limited homologies with circoviruses and parvoviruses, respectively, the overall genome organization of NIH-CQV has the basic characteristics of viruses in *Parvoviridae*.

NIH-CQV has a small, compact linear DNA genome with two tandemly arranged major ORFs encoding Rep and CP proteins, respectively, and a pair of ITRs at the left and right ends of the genome. *In silico* promoter prediction suggested that transcription of the *rep* and *cp* was regulated by a single promoter upstream of the *rep*, because there was no apparent promoter sequence immediately upstream of *cp*. A pre-mRNA encoded by a single

promoter that is processed by alternative splicing and alternative polyadenylation to generate multiple mRNAs had been reported for other parvoviruses, such as B19V (25) and Aleutian mink disease virus (26). By amino acid sequence analysis, CP protein of NIH-CQV is minimally homologous to porcine and goose parvovirus VP1 (27). The homologous region is mainly in the N terminus of CP and contains the conserved PLA₂-like motif, which is present in the N-terminal extension of the VP1 unique region of members of *Parvoviridae* (23). The phylogenetic tree constructed using amino acid sequences of CP of NIH-CQV and VP1 of other representative viruses in *Parvoviridae* showed that NIH-CQV represents a deeply rooted lineage between two groups: (i) human and animal parvoviruses and (ii) insect parvoviruses. Thus, NIH-CQV appears to be a parvovirus-like virus. However, NIH-CQV also has features shared by members of the *Circoviridae* family (28). First, a BLASTp search revealed that the Rep of NIH-CQV is more related to circoviral Rep than to those of other viruses. Second, NIH-CQV appears to have an ambisense genome because there are bidirectional putative promoters in the intergenic region between the *rep* and *15-kDa protein* and because *rep* and *15-kDa protein* arranged head-to-head flank the intergenic region, which is a feature of circoviruses. Finally, sequencing and alignment of the inverse PCR products indicated the existence of the circular genome of NIH-CQV in the clinical samples. The circular form of NIH-CQV genome might represent a subviral DNA or an intermediate structure of viral replication, as reported for other parvoviruses (29). Therefore, NIH-CQV appears to be a "hybrid" virus, perhaps formed by interfamilial recombination between a parvovirus and a circovirus. This idea was supported further by whole-proteome phylogenetic analysis, which showed NIH-CQV located between *Parvoviridae* and *Circoviridae*.

Both recombination and a high mutation rate are often cited as key in evolutionary innovation. For many viruses, recombination

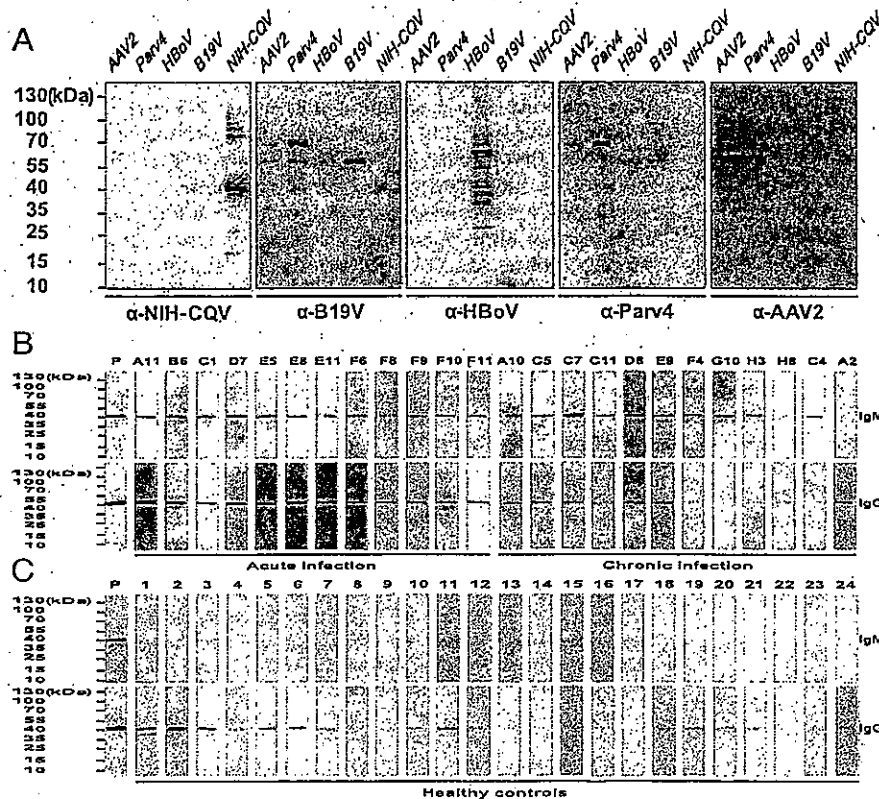


Fig. 6. Immunoblotting of sera from patients with non-A-E hepatitis and from healthy controls using rCP of NIH-CQV. (A) Specificity test for the rCP of NIH-CQV. The cell lysates derived from the cells transfected with the plasmids that expressed capsid proteins of AAV2, Parv4, HBov, and B19V and purified rCP were subjected to SDS/PAGE and then were transferred to a nitrocellulose membrane. After blocking with 5% (wt/vol) nonfat milk for 2 h, the membrane was incubated with respective antisera at 1:1,000 dilution. (B and C) Detection of specific antibodies against the rCP of NIH-CQV. Samples subjected to SDS/PAGE consisted of 25 ng of affinity-purified rCP. These proteins were transferred to a nitrocellulose membrane and incubated with a 1:1,000 dilution of patient sera. The numbers at the top are patient identification numbers. Only representative result are shown; complete data are shown in Fig. S4 A and B. Mouse monoclonal antibody against the polyhistidine tag was used as a positive control. The numbers on the left indicate molecular masses in kilodaltons based on the PageRuler Prestained Protein Ladder (Fermentas).

Table 1. Results of real-time PCR and immunoblot analysis

qPCR	Seronegative hepatitis patients				Healthy controls			
	IgM		IgG		IgM		IgG	
	+	-	+	-	+	-	+	-
+	27.8% (25/90)	42.2% (38/90)	70.0% (63/90)	0 (0/90)	0 (0/45)	0 (0/45)	0 (0/45)	0 (0/45)
-	3.3% (3/90)	26.7% (24/90)	14.4% (13/90)	15.6% (14/90)	0 (0/45)	100% (45/45)	77.8% (35/45)	22.2% (10/45)
Total	31.1% (28/90)	68.9% (62/90)	84.4% (76/90)	15.6% (14/90)	0 (0/45)	100% (45/45)	77.8% (35/45)	22.2% (10/45)

allows single-step acquisition of multiple genetic changes, and a high mutation rate enhances adaptation to new hosts (30). These processes are critical driving forces in viral evolution and lead to viral emergence, host-switching, and adaptation, which often result in disease outbreaks (31, 32). Recent studies had demonstrated that small eukaryotic ssDNA viruses have high rates of nucleotide substitutions and genetic recombination, which may surpass rates seen in RNA viruses (33, 34). These observations may explain the extensive diversity seen in ssDNA viruses. Parvoviruses (family *Parvoviridae*) and circoviruses (family *Circoviridae*) are both ssDNA viruses that infect a wide variety of animal species, including mammals, birds, and insects. Although horizontal gene transfer and high mutation rates have been documented in these viruses, cross-species viral transfers resulting from interfamilial recombination and leading to a infection in the new host species are not common.

A high rate of viral evolution has been reported for emerging parvoviruses (35). Canine parvovirus (CPV) is one well-characterized example (35). CPV emerged from feline panleukopenia parvovirus or a closely related virus, differing at several amino acid residues. Sequences of the viruses collected at time points before and after host-switching suggest that the emergence of CPV was dependent on a high mutation rate and positive selection of the major capsid gene. Our deep-sequencing data showed that NIH-CQV has a high substitution mutation rate throughout its genome. However, the impact of selective pressures, as measured by the K_a/K_s ratio, appeared to vary depending on location. The *cp* gene contained three regions in which the K_a/K_s ratio exceeded 1, suggesting positive selection. These substitutions would be presumed to enhance fitness. In contrast, selective pressure on the *rep* of NIH-CQV appeared to be dominated by purifying selection, because the average K_a/K_s ratio for the *rep* from 14 patients was only 0.42. The K_a/K_s ratios for the regions encoding the PLA₂ motif in the CP protein or P-loop nucleoside triphosphatase (NTPase) in the Rep proteins were lower than in other regions, as is consistent with strong purifying selection and functional importance of this region in viral replication.

NIH-CQV exhibited remarkable genetic heterogeneity within patients, indicating the presence of closely related variants or quasispecies in NIH-CQV-infected individuals. The K_a/K_s ratios of both viral *cp* and *rep* were greater in patients classified as having chronic hepatitis than in those with acute hepatitis, suggesting greater variation or more diversity of quasispecies of NIH-CQV over time. For hepatitis viruses C and E, diversification of quasispecies during infection is driven by the interaction between the viruses and host immune response and may be associated with different clinical outcomes (36, 37). With a limited number of serial samples from a single individual, we were unable to conclude whether quasispecies dynamics of NIH-CQV in patients correlated with disease.

Despite technological advances in molecular biology, an etiology cannot be determined in as many as 20% of hepatitis cases. As of yet, no causative agent may be defined in a significant proportion of patients with chronic liver disease and cirrhosis, whose disease therefore is characterized as "cryptogenic." Additional hepatitis agents have been suspected. Over several decades, several human

viruses, such as GB virus C (38), Torque teno virus (39), and SEN virus (40), have been putatively claimed to be hepatitis viruses, but subsequent investigation revealed no conclusive association between these viruses and liver disease. Therefore, the etiology of non-A-E hepatitis remains unresolved. In this study, we applied NGS methodology to virus discovery and identified a parvovirus-like virus in the sera samples collected from a cohort of patients with non-A-E hepatitis. Our results showed that 28% of patients were positive for both NIH-CQV-specific IgM and viral DNA, suggesting recent infection in patients. Most patients and healthy controls were positive for virus-specific IgG, suggesting that exposure was common in the population studied. The patients who yielded positive specimens suffered from symptoms of hepatitis with a wide range of liver dysfunction, including acute and chronic hepatitis. There was no difference between patients diagnosed with acute versus or chronic hepatitis with respect to detection of NIH-CQV DNA, but viral DNA was not detected in healthy controls. Although these findings are suggestive of an etiologic relationship, our study has limitations. By electron microscopy, we observed virus-like particles in NIH-CQV qPCR-positive sera which cosedimented with viral DNA, but we were unable to confirm their identity by immunogold labeling with our antisera. In addition, because of the limited availability of samples, we had insufficient material for viral propagation and isolation in cell culture, and efforts are in progress to detect viral proteins in a few liver biopsy tissue blocks. Additionally, with the limited number of samples available, we cannot exclude the possibility of reactivation of a latent virus in the course of liver inflammation.

In general, parvoviruses cause systemic infection, but clinical manifestations of the infection are varied and may depend on specific tissue tropisms as well as on host immunologic competency. For two human pathogenic parvoviruses, B19V (10) and HBoV (41), clinical symptoms range from none to acute systemic manifestations involving skin and joints to subtle persistence of infection; in immunocompromised populations, B19V chronic infection causes pure red cell aplasia. In addition, coinfection of parvoviruses with other viruses, especially related DNA viruses, can lead to synergy and more severe clinical symptoms in the affected host. Because of the complexity of disease associations, more comprehensive epidemiology and in vitro studies are needed to establish a pathogenic role of NIH-CQV in hepatitis.

Materials and Methods

Study Subjects. We studied a total of 92 sera samples from patients with non-A-E hepatitis who were admitted to the Institute of Infectious Disease of Southwest Hospital, Third Military Medical University, China, between 1999 and 2007. According to the criteria of the Chinese Society of Infectious Disease and Parasitology, and the Chinese Society of Hepatology (42), 33 patients were diagnosed with acute hepatitis by clinical and biochemical parameters. Fifty-nine patients had chronic aggressive hepatitis confirmed by biopsy, and 10 of them had cirrhosis; eight outpatients with chronic persistent hepatitis did not have liver biopsies performed. The patients had a median age of 42 y (range: 12–73 y); among of them, 55 males had a median age of 41 y (range: 12–73 y), and 37 females had median age of 43 y (range: 15–70 y). On admission, mean liver function tests were as follows: total bilirubin 174 ± 130 μ mol/L (range: 14–735 μ mol/L), alanine aminotransferase 421 ± 623 IU/L (range: 5–3,150 IU/L). Serological tests for anti-HAV IgM (HAV IgM, ELISA Kit; Wantai Biological Pharmacy,

HBV markers (HBsAg and HBeAg; Roche Diagnostic Systems) anti-HCV antibodies (HCV ELISA Kit; Wantai Biological Pharmacy), anti-HDV IgM (HDV IgM ELISA Kit; Wantai Biological Pharmacy), anti-HEV IgM (HAV IgM ELISA Kit; Wantai Biological Pharmacy), HCV RNA tests (HCV Quantitative RT-PCR Kit; Kehua Bio-engineering), anti-HIV-1 and 2 antibodies (HIV ELISA Kit; Wantai Biological Pharmacy), HIV viral load test (HIV Quantitative RT-PCR Kit; Roche Diagnostic Systems), and serological tests for anti-CMV IgM and (CMV IgM ELISA Kit; Wantai Biological Pharmacy) and anti-EBV IgM (EBV IgM Kit; YHLO Biotech) were all negative. Additional tests for antinuclear antibody, rheumatoid factor, anti-mitochondrial antibody (Microplate ELISA Test Systems for Autoimmunity; EUROIMMUN), as well as blood cultures, urine cultures, and throat swabs for bacteria were negative. The research protocol was approved by the Human Bioethics Committee of the Third Military Medical University, and all participants provided written informed consent.

Solexa Deep Sequencing and Phylogenetic and Evolutionary Analysis. Details of deep sequencing and phylogenetic and evolutionary analysis are provided in *SI Material and Methods*.

Overlapping PCR and Reverse PCR. To verify the sequence of the viral genome assembled from the Solexa data, eight sets of overlapping primer pairs were designed (Table S3). Viral DNA was extracted from sera as described in the Solexa deep sequencing. Extracted DNA (5 μ L) was used as template for the PCR. The 50- μ L reaction mix consisted of 1x Platinum Taq PCR Buffer (Invitrogen), 1.5 mM MgCl₂, each dNTP at 0.2 mM, and 25 pmol each of the primers. After 2 min at 94 °C, 35 cycles of amplification (94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min) were performed. To detect circularized viral DNA, inverse PCR with a primer pair (Table S3) that oriented outwardly with respect to each other was used for amplification. PCR was performed as

described above, and amplified products were visualized on an agarose gel. All PCR products were sequenced.

Diagnostic qPCR. For quantitative PCR, DNA was extracted by the QIAamp MinElute Virus Kit (Qiagen), and 5 μ L of the resulting DNA was used for analysis with the NIH-CQV rep primers set (NIH-CQV_Rep-1295F and NIH-CQV_Rep-1470R), and NIH-CQV_Rep probe was used for qPCR (Table S3). All reactions were performed using the Chromo4 real-time detector (Bio-Rad). The reaction started with activation of the polymerase at 95 °C for 15 min, followed by 45 cycles of 15 s at 94 °C and 1 min at 60 °C. The quantitation of amplicon was performed by interpolation with the standard curve to the synthesized rep gene (ORF1) with serial dilutions.

Immunoblotting. To investigate the immune response against NIH-CQV, a synthetic DNA fragment encoding a 184-amino acid polypeptide of the C-terminal portion of CP was cloned into an expression vector, and the affinity-purified rCP was used for immunoblotting. Cross-reactivities between NIH-CQV and other human parvoviruses, including B19V, Parv4, HBoV, and AAV2, were tested by immunoblotting. Details are provided in *SI Material and Methods*.

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日		第一報入手日		新医薬品等の区分		総合機構処理欄	
一般的名称		研究報告の公表状況		http://www.promedmail.org/direct.php?id=20130602.1750302		公表国 中国		使用上の注意記載状況・ その他参考事項等 曹頭部 本剤は、貴重なヒト血液を原料として製剤化したものである。原料となった血液を採取する際には、問診、感染症関連の検査を実施するとともに、製造工程において一定の不活化・除去処理などを実施し、感染症に対する安全対策を講じているが、ヒト血液を原料としていていることが、感染症伝播のリスクを完全に排除することはできないため、疾病の治療上の必要性を十分に検討の上、必要最小限の使用にとどめること。	
販売名(企業名)		—		—		—			
研究報告の概要		我々は中国の重慶で1999～2007年に非ABCD E型肝炎の患者から92の血清標本を収集し、Solexa deep sequencingによってスクリーニングしたところ、パルボウイルスに関連づけられた10の血清プールにおいて、3780bpの連結断片(contig)を発見し、それを含むハイブリッドDNA ウイルスを暫定的にNIH-CQVと命名した。この連結断片はコウモリサージコウイルスとブタパルボウイルスに類似した2つの主要な配列により構成されていた。 ポリメラゼ連鎖反応法では、90の患者サンプル中63(70%)が陽性であり、一方、45の健康コントロールは全て陰性であった。また、NIH-CQVに対する特定免疫抗体を免疫ブロット法で調査したところ、患者の84%はIgG陽性、31%がIgM陽性であったのに対して、健康コントロールでは78%がIgG陽性であり、IgMは全ての患者で陰性であった。 我々のデータはこのパルボウイルス様のウイルスが非ABCD E型肝炎の患者で広がっていることを示しているが、人の病気におけるNIH-CQVの病因学上の役割については更なる研究が必要である。							
報告企業の意見		今後の対応							
情報で述べられているように人に対する病因学上の役割ははっきりしていないことから、今後の情報に注目していきたい。		今後とも未知の病原体に関する情報に留意していく。							



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HEPATITIS NON-A/B/C/D/E: PARVOVIRUS-LIKE HYBRID

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[ref: Xu B, Zhi N, Hu G, et al: Hybrid DNA virus in Chinese patients with seronegative A-E hepatitis discovered by deep sequencing. Proc Natl Acad Sci USA. 2013; Epub ahead of print]

Abstract

Seronegative hepatitis -- non-A, non-B, non-C, non-D, non-E hepatitis -- is poorly characterized but strongly associated with serious complications. We collected 92 sera specimens from patients with non-A-E hepatitis in Chongqing, China between 1999 and 2007. 10 sera pools were screened by Solexa deep sequencing. We discovered a 3780-bp contig present in all 10 pools that yielded BLASTx E [basic local alignment search tool] scores of 7e-05-0.008 against parvoviruses. The complete sequence of the in silico-assembled 3780-bp contig was confirmed by gene amplification of overlapping regions over almost the entire genome, and the virus was provisionally designated NIH-CQV. Further analysis revealed that the contig was composed of 2 major ORFs [open reading frames]. By protein BLAST, ORF1 and ORF2 were most homologous to the replication-associated protein of bat circovirus and the capsid protein of porcine parvovirus, respectively. Phylogenetic analysis indicated that NIH-CQV is located at the interface of *Parvoviridae* and *Circoviridae*. Prevalence of NIH-CQV in patients was determined by quantitative PCR. 63 of 90 patient samples (70 per cent) were positive, but all those from 45 healthy controls were negative. Average virus titer in the patient specimens was 1.05 e4 copies/microL. Specific antibodies against NIH-CQV were sought by immunoblotting. 84 per cent of patients were positive for IgG, and 31 per cent were positive for IgM; in contrast, 78 per cent of healthy controls were positive for IgG, but all were negative for IgM. Although more work is needed to determine the etiologic role of NIH-CQV in human disease, our data indicate that a parvovirus-like virus is highly prevalent in a cohort of patients with non-A-E hepatitis.

Most viral hepatitis are secondary to infection by known hepatotropic viruses: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis delta virus (HDV), and hepatitis E virus (HEV). Other hepatitis-associated viruses, including CMV [cytomegalovirus], Epstein-Barr virus, herpes simplex virus 1 and 2, varicella-zoster virus, human herpesvirus 6, human parvovirus B19V (B19V), and adenoviruses, may cause liver injury, ranging from mild and transient elevation of aminotransferases to acute hepatitis and occasionally acute liver failure. Despite the number of hepatitis viruses and hepatitis-associated viruses, etiology cannot be determined in 10-20 per cent of the cases of acute hepatitis, in 30 per cent of cases of cryptogenic chronic liver diseases, in hepatitis-associated aplastic anemia, and in a large proportion of cases of acute liver failure. Patients with acute seronegative hepatitis have fewer parenteral risk factors, more severe bilirubin and transaminase elevations, and worse hepatocellular synthetic function than patients with hepatitis C. Moreover, seronegative hepatitis is more strongly associated with serious complications, especially bone marrow failure and childhood fulminant hepatitis.

Next-generation sequencing (NGS) technology has had an increasing impact on biological research and clinical diagnosis, because it provides high-resolution genome-scale data rapidly and reliably. The 1st application of NGS for pathogen discovery in a patient who died of a febrile illness after solid-organ transplantation led to the identification of an arenavirus. NGS based platforms, such as 454 and Solexa deep sequencing, have been applied successfully to the investigation of emerging human pathogens, resulting in major discoveries of new human viruses.

In the present study, we established an experimental and analytic procedure for identifying virus identification in human specimens based on Solexa deep sequencing. We applied the method to screen for potential viral infection in human sera specimens and discovered a human virus in Chinese patients who had seronegative hepatitis. From its genome organization and phylogeny, the virus is unusual, because it is evolutionarily at the interface of the parvovirus and circovirus families, perhaps having emerged from interfamilial recombination. The virus was highly prevalent in a patient population, and active infection in seronegative hepatitis cases was suggested by the presence of viral DNA in the blood and serology. However, more work is needed to establish a firm disease association.

[Interested readers should access the original text at the source URL above to view the data, figures, and references. Only the Discussion is reproduced here.- Mod.CP]

Discussion

We report the identification of a virus, NIH-CQV, in patients with non-A-E hepatitis. Phylogenetically NIH-CQV appears to lie at the interface of parvoviruses and circoviruses. The genome of NIH-CQV has no homology to any known viruses in GenBank.

Although comparative analysis revealed that the Rep and CP proteins of NIH-CQV have limited homologies with circoviruses and parvoviruses, respectively, the overall genome organization of NIH-CQV has the basic characteristics of viruses in *Parvoviridae*.

NIH-CQV has a small, compact linear DNA genome with 2 tandemly arranged major ORFs encoding Rep and CP proteins, respectively, and a pair of ITRs [inverted terminal repeats] at the left and right ends of the genome. In silico promoter prediction suggested that transcription of the rep and cp was regulated by a single promoter upstream of the rep, because there was no apparent promoter sequence immediately upstream of cp. A pre-mRNA encoded by a single promoter that is processed by alternative splicing and alternative polyadenylation to generate multiple mRNAs had been reported for other parvoviruses, such as B19V and Aleutian mink disease virus. By amino acid sequence analysis, CP protein of NIH-CQV is minimally homologous to porcine and goose parvovirus VP1. The homologous region is mainly in the N terminus of CP and contains the conserved PLA2-like motif, which is present in the N-terminal extension of the VP1 unique region of members of *Parvoviridae*. The phylogenetic tree constructed using amino acid sequences of CP of NIH-CQV and VP1 of other representative viruses in *Parvoviridae* showed that NIH-CQV represents a deeply rooted lineage between 2 groups: (i) human and animal parvoviruses and (ii) insect parvoviruses. Thus, NIH-CQV appears to be a parvovirus-like virus.

However, NIH-CQV also has features shared by members of the *Circoviridae* family. First, a BLASTp search revealed that the Rep of NIH-CQV is more related to circoviral Rep than to those of other viruses. Second, NIH-CQV appears to have an ambisense genome because there are bidirectional putative promoters in the intergenic region between the rep and 15-kDa protein and because rep and 15-kDa protein arranged head-to-head flank the intergenic region, which is a feature of circoviruses. Finally, sequencing and alignment of the inverse PCR products indicated the existence of the circular genome of NIH-CQV in the clinical samples. The circular form of NIH-CQV genome might represent a subviral DNA or an intermediate structure of viral replication, as reported for other parvoviruses.

Therefore, NIH-CQV appears to be a "hybrid" virus, perhaps formed by interfamilial recombination between a parvovirus and a circovirus. This idea was supported further by whole-proteome phylogenetic analysis, which showed NIH-CQV located between *Parvoviridae* and *Circoviridae*.

Both recombination and a high mutation rate are often cited as key in evolutionary innovation. For many viruses, recombination allows single-step acquisition of multiple genetic changes, and a high mutation rate enhances adaptation to new hosts. These processes are critical driving forces in viral evolution and lead to viral emergence, host-switching, and adaptation, which often result in disease outbreaks. Recent studies had demonstrated that small eukaryotic ssDNA [single stranded DNA] viruses have high rates of nucleotide substitutions and genetic recombination, which may surpass rates seen in RNA viruses. These observations may explain the extensive diversity seen in ssDNA viruses. Parvoviruses (family *Parvoviridae*) and circoviruses (family *Circoviridae*) are both ssDNA viruses that infect a wide variety of animal species, including mammals, birds, and insects. Although horizontal gene transfer and high mutation rates have been documented in these viruses, cross-species viral transfers resulting from interfamilial recombination and leading to a infection in the new host species are not common.

A high rate of viral evolution has been reported for emerging parvoviruses. Canine parvovirus (CPV) is one well-characterized example. CPV emerged from feline panleukopenia parvovirus or a closely related virus, differing at several amino acid residues. Sequences of the viruses collected at time points before and after host-switching suggest that the emergence of CPV was dependent on a high mutation rate and positive selection of the major capsid gene. Our deep-sequencing data showed that NIH-CQV has a high substitution mutation rate throughout its genome. However, the impact of selective pressures, as measured by the Ka/Ks ratio, appeared to vary depending on location. The cp gene contained three regions in which the Ka/Ks ratio exceeded 1, suggesting positive selection. These substitutions would be presumed to enhance fitness. In contrast, selective pressure on the rep of NIH-CQV appeared to be dominated by purifying selection, because the average Ka/Ks ratio for the rep from 14 patients was only 0.42. The Ka/Ks ratios for the regions encoding the PLA2 motif in the CP protein or P-loop nucleoside triphosphatase (NTPase) in the Rep proteins were lower than in other regions, as is consistent with strong purifying selection and functional importance of this region in viral replication.

NIH-CQV exhibited remarkable genetic heterogeneity within patients, indicating the presence of closely related variants or quasispecies in NIH-CQV-infected individuals. The Ka/Ks ratios of both viral cp and rep were greater in patients classified as having chronic hepatitis than in those with acute hepatitis, suggesting greater variation or more diversity of quasispecies of NIH-CQV over time. For hepatitis viruses C and E, diversification of quasispecies during infection is driven by the interaction between the viruses and host immune response and may be associated with different clinical outcomes. With a limited number of serial samples from a single individual, we were unable to conclude whether quasispecies dynamics of NIH-CQV in patients correlated with disease.

Despite technological advances in molecular biology, an etiology cannot be determined in as many as 20 per cent of hepatitis cases. As of yet, no causative agent may be defined in a significant proportion of patients with chronic liver disease and cirrhosis, whose disease therefore is characterized as "cryptogenic". Additional hepatitis agents have been suspected. Over several decades, several human viruses, such as GB virus C, Torque teno virus, and

SEN virus, have been putatively claimed to be hepatitis viruses, but subsequent investigation revealed no conclusive association between these viruses and liver disease. Therefore, the etiology of non-A-E hepatitis remains unresolved. In this study, we applied NGS methodology to virus discovery and identified a parvovirus-like virus in the sera samples collected from a cohort of patients with non-A-E hepatitis. Our results showed that 28 per cent of patients were positive for both NIH-CQV-specific IgM and viral DNA, suggesting recent infection in patients. Most patients and healthy controls were positive for virus-specific IgG, suggesting that exposure was common in the population studied. The patients who yielded positive specimens suffered from symptoms of hepatitis with a wide range of liver dysfunction, including acute and chronic hepatitis. There was no difference between patients diagnosed with acute versus or chronic hepatitis with respect to detection of NIH-CQV DNA, but viral DNA was not detected in healthy controls.

Although these findings are suggestive of an etiologic relationship, our study has limitations. By electron microscopy, we observed virus-like particles in NIH-CQV qPCR [quantitative PCR]-positive sera which cosedimented with viral DNA, but we were unable to confirm their identity by immunogold labeling with our antisera. In addition, because of the limited availability of samples, we had insufficient material for viral propagation and isolation in cell culture, and efforts are in progress to detect viral proteins in a few liver biopsy tissue blocks. Additionally, with the limited number of samples available, we cannot exclude the possibility of reactivation of a latent virus in the course of liver inflammation.

In general, parvoviruses cause systemic infection, but clinical manifestations of the infection are varied and may depend on specific tissue tropisms as well as on host immunologic competency. For 2 human pathogenic parvoviruses, B19V and HBoV, clinical symptoms range from none to acute systemic manifestations involving skin and joints to subtle persistence of infection; in immunocompromised populations, B19V chronic infection causes pure red cell aplasia. In addition, coinfection of parvoviruses with other viruses, especially related DNA viruses, can lead to synergy and more severe clinical symptoms in the affected host. Because of the complexity of disease associations, more comprehensive epidemiology and in vitro studies are needed to establish a pathogenic role of NIH-CQV in hepatitis.

communicated by:
ProMED-mail rapporteur Kunihiro Iizuka.

[The authors report the identification of a virus, designated NIH-CQV, in patients with non-A/B/C/D/E hepatitis. Phylogenetically this virus appears to lie at the interface of parvoviruses and circoviruses. The genome of NIH-CQV has no homology to any known hepatitis viruses. NIH-CQV has a small, compact linear DNA genome with 2 tandemly arranged major ORFs encoding Rep and CP proteins, respectively, and a pair of ITRs at the left and right ends of the genome. NIH-CQV represents a deeply rooted lineage between 2 groups: the human and animal parvoviruses and the insect parvoviruses. Thus, NIH-CQV appears to be a parvovirus-like virus.

However, NIH-CQV also has features shared by members of the Circoviridae family. NIH-CQV exhibits ambisense encoding of sequences a feature shared with members of the Circoviridae family. Therefore, NIH-CQV appears to be a "hybrid" virus, perhaps formed by interfamilial recombination between a parvovirus and a circovirus. This idea is supported further by whole-proteome phylogenetic analysis, which showed NIH-CQV located between the families Parvoviridae and Circoviridae. Parvoviruses (family Parvoviridae) and circoviruses (family Circoviridae) are both ssDNA viruses that infect a wide variety of animal species, including mammals, birds, and insects. However, although horizontal gene transfer and high mutation rates have been documented in these viruses, cross-species viral transfers resulting from interfamilial recombination and leading to a infection in the new host species are not common.

Furthermore NIH-CQV exhibited remarkable genetic heterogeneity within patients, indicating the presence of closely related variants or quasispecies in NIH-CQV-infected individuals. For hepatitis viruses C and E, diversification of quasispecies during infection is driven by the interaction between the viruses and host immune response and may be associated with different clinical outcomes. With a limited number of serial samples from a single individual, the authors were unable to conclude whether quasispecies dynamics of NIH-CQV in patients correlated with disease.

The authors are aware that over the years several human viruses, such as GB virus C, Torque teno virus, and SEN virus, have been claimed to be hepatitis viruses, but subsequent investigation revealed no conclusive association between these viruses and liver disease. Therefore, the etiology of non-A/B/C/D/E hepatitis remains unsolved. In this study, the authors applied NGS methodology to virus discovery and identified a parvovirus-like virus in sera samples collected from a cohort of patients with non-A-E hepatitis. Nonetheless, the authors recognise that because of the complexity of disease associations, more comprehensive epidemiology and in vitro studies will be required to confirm NIH-CQV virus as a 6th hepatitis virus. - Mod.CP]

.....cp/mj/sh

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医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一 般 的 名 称	別紙のとおり。	研究報告の 公表状況	2013年9月4日	該当なし。	公表国 日本
販売名（企業名）	別紙のとおり。				
問題点：日本で初めて、ボレリア属菌ボレリア・ミヤモトイ (<i>Borrelia miyamotoi</i>) の国内感染による回帰熱症例が確認された。 四類感染症に指定されている回帰熱について、感染症の予防及び感染症の患者に対する医療に関する法律施行以降これまでに国内での感染例は報告されていないが、厚生労働科学研究班が実施した、過去にライム病が疑われた患者血清約800検体を用いた後向き疫学調査の結果、ボレリア属菌ボレリア・ミヤモトイ (<i>Borrelia miyamotoi</i>) の国内感染による回帰熱2症例が国内で初めて確認された。2例とも抗生剤の投与により回復している。 厚生労働省は、国内における回帰熱又はライム病を疑う症例について、検査依頼があった場合の検査協力を求めている。					
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 記載なし。			
報告企業の意見		今後の対応			
別紙のとおり。	今後とも関連情報の収集に努め、本剤の安全性の確保を図っていききたい。				

※：現在製造を行っていない

健感発 0903 第 1 号

平成 25 年 9 月 3 日

各 { 都 道 府 県
保健所設置市
特 別 区 } 衛生主管部（局）長 殿

厚生労働省健康局結核感染症課長

（ 公 印 省 略 ）

ボレリア・ミヤモトイによる回帰熱の国内症例の確認及び
ライム病を含むボレリア感染症の病原体診断検査について
（情報提供及び協力依頼）

感染症の予防及び感染症の患者に対する医療に関する法律（平成10年法律第114号。以下「感染症法」という。）において四類感染症に指定されている回帰熱については、感染症法施行以降、国内での感染例は報告されていませんでしたが、今般、別添1のとおり、ボレリア属菌ボレリア・ミヤモトイ (*Borrelia miyamotoi*) による回帰熱の症例が、厚生労働科学研究班が実施した遡り調査の結果、国内で確認されました。

これを受けて、別添2・3のとおり本感染症に関するQ&Aやライム病との比較表など、資料を取りまとめました。つきましては、本件について関係者への周知方をお願いします。

なお、今回、回帰熱であると確認された2症例については、当初、ライム病と診断されていましたが、実際には回帰熱であったか、又は、ライム病と回帰熱に同時に感染した可能性が示唆されています。これは、現在、ライム病の診断で行われている血清中の抗ボレリア抗体の検出検査のみでは、ライム病と回帰熱を鑑別することが困難なためです。

よって、国内における回帰熱とライム病の発生動向を正確に把握するためにも、今後、貴管内医療機関から、回帰熱又はライム病を疑う症例について、病原体診断のための検査依頼があった場合は、回帰熱・ライム病両方の検査を実施するよう、御協力よろしくお願いいたします。なお、検査については、国立感染症研究所細菌第一部において実施することが可能です。

参考資料

別添 1 : 病原微生物検出情報 (IASR) 速報 国内感染が確認された回帰熱の 2 例

別添 2 : マダニ媒介性の回帰熱に関する Q&A

別添 3 : ライム病と回帰熱の比較

本疾患に関する技術的な問い合わせ : 国立感染症研究所細菌第一部第四室

電 話 : 03-5285-1111 (内線 2224)

病原微生物検出情報 (IASR) 速報

国内感染が確認された回帰熱の2例

回帰熱はスピロヘータ科ボレリア属細菌感染による一病態であり、その病原体ボレリアはヒメダニやシラミによって媒介される。これに加えて、2011年、ロシアでマダニが媒介する *Borrelia miyamotoi* による回帰熱が報告され^{1,2)}、また2013年には米国の疫学調査により、ライム病流行地では本ボレリア感染による回帰熱症例が存在することが報告された^{3,4)}。

この回帰熱の病原体である *B. miyamotoi* は、北海道で1995年に発見されたボレリアで、*Ixodes* 属ダニによって伝播される⁵⁾。北海道やロシアでは *Ixodes persulcatus* (シュルツェマダニ) によって保菌されている。米国や欧州では *I. ricinus*、*I. scapularis*、*I. pacificus* から本菌のDNAが検出されている。また、シュルツェマダニはライム病ボレリアも伝播することが知られている。

一方で本ボレリアは培養が困難なため、これまでに適切な実験室診断法が確立されていなかったこと、また *B. miyamotoi* を媒介するマダニはライム病ボレリアも保菌している場合もあり、このボレリアとの重複感染がしばしば起るため、臨床診断が極めて難しいことから、その実態はほとんど把握されていなかった。

国立感染症研究所では、過去にライム病が疑われた患者血清約800検体を用いた後ろ向き疫学調査を実施し、このうち発症後の有熱期に採血された2検体から *B. miyamotoi* DNAを検出した。またこの内の1検体では *B. miyamotoi* HT31株由来の組換えGlpQ抗原を用いた *B. miyamotoi* 特異的な抗体検査により、回復期ペア血清で抗体上昇が確認された。これら2検体は北海道在住の患者より採取されたものであり、いずれもライム病血清診断でも抗体陽性と判定されている。これら2症例は国内でのマダニ刺咬により感染したものと考えられている。いずれの症例もミノサイクリンもしくはセフトリアキソン投与により回復している。

シュルツェマダニの主な生息地域は北海道であり、その活動期は春～秋である。また本マダニは、長野県など本州中部の高山帯(標高約1200m以上)等でも生息が確認されている。本マダニ刺咬後におこる原因不明の発熱性疾患等を呈した患者では、ライム病に加えて本疾患を鑑別対象として加えることが必要^{1,3,6,7)}である。

<回帰熱の検査について>

回帰熱の検査は国立感染症研究所・細菌第一部で実施可能です。検査検体は、マダニ刺咬後に発熱、頭痛、倦怠感等を示した患者の、1)発熱期の全血もしくは血清、2)髄膜炎を呈した場合には髄液です。また抗体検査を依頼される場合には、回復期血清による確認が重要です。これら検査をご依頼される場合には、最寄りの保健所などへお問い合わせください。

<回帰熱に関する問合せ先>

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マダニ媒介性の回帰熱に関するQ&A

(平成 25 年 9 月 3 日作成)

回帰熱には、シラミによって媒介されるものと、マダニによって媒介されるものの 2 つのタイプがあります。シラミ媒介性の回帰熱は、主に、戦争や飢饉等によって、衛生環境が悪化した際に見られる感染症で、世界でも限られた地域でのみ流行しています。一方、マダニ媒介性の回帰熱は、世界の多くの地域で発生が見られます。ここでは、我が国でも発生が確認されたマダニ媒介性の回帰熱について解説します。

<一般の方向け>

問1 回帰熱とはどのような病気ですか？

答 我が国では、ボレリア属の細菌「ボレリア・ミヤモトイ (*Borrelia miyamotoi*)」を保有するマダニ類に咬まれることにより、細菌が体内に侵入し、感染します。ただし、ヒトからヒトには感染しません。また、動物から直接感染することはありません。感染すると、主に、発熱や頭痛、筋肉痛など風邪のような症状が出ます。

問2 ボレリア・ミヤモトイ (*Borrelia miyamotoi*) とは、どのような細菌ですか？

答 ボレリア・ミヤモトイ (*Borrelia miyamotoi*) は、1995 年に北海道で発見された新種のボレリア菌です。発見当時は、この菌が病気を引き起こすかどうかについては不明でしたが、その後、2011 年にロシアで、さらに 2013 年に米国で、ボレリア・ミヤモトイを原因とする回帰熱の報告がなされ、その病原性が明らかとなりました。

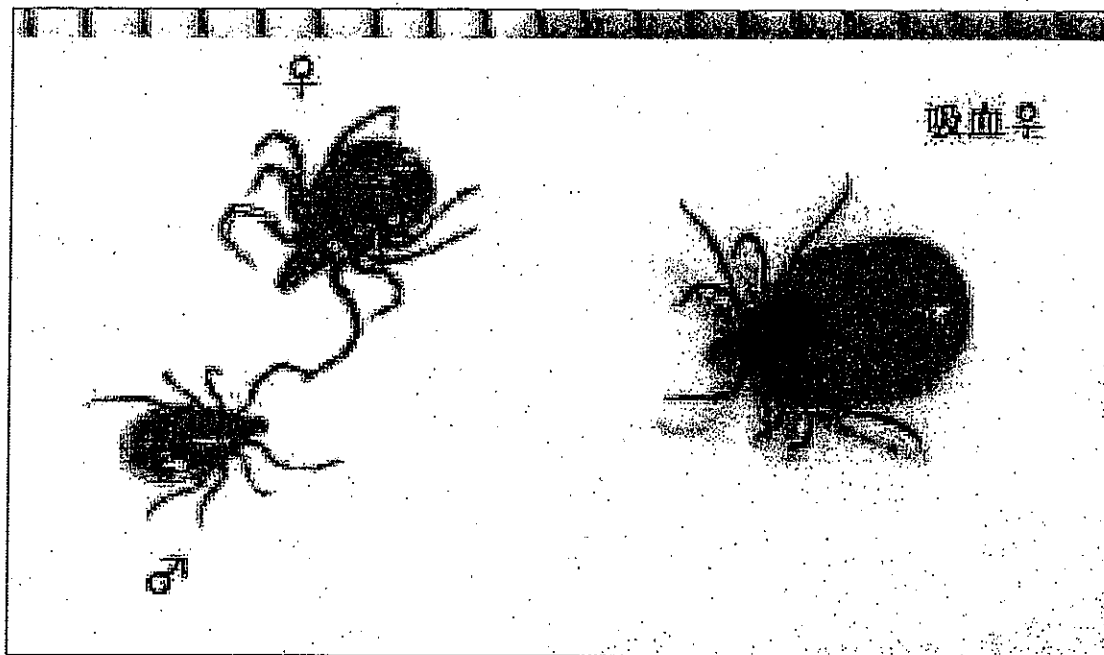
問3 回帰熱は、我が国ではどのくらい発生していますか？

答 海外で感染し、帰国後に発症した数例を除き、過去数十年間、日本国内で回帰熱の患者の報告はありませんでしたが、近年の遡り調査の結果、2011 年以降に、北海道でボレリア・ミヤモトイ感染による回帰熱の患者 2 名が発生していたことが明らかになりました。

問4 どのような種類のマダニがボレリア・ミヤモトイを保有しているのですか？

答 我が国では、主にシュルツェマダニ (*Ixodes persulcatus*) からボレリア・ミヤモトイが見つかっています。このマダニは、主として、本州中部以北の山間部（標

高・1200m以上)や寒冷地に生息しており、北海道では平地でも見られます。そのほか、パブロブスキーマダニ (*Ixodes pavlovski*) もこの菌を保有することが分かっています。これらマダニは春から秋にかけて活動的になります。



■シュルツェマダニ

成ダニの体長は、吸血前で 3mm、吸血後で 5-10mm 程度。左は吸血前の成ダニ(♂、♀)。右は吸血した成ダニ(♀)。一目盛りは 1mm を示す。

問5 全てのマダニが病原体を保有しているのですか？

答 いいえ、全てのマダニが病原体を保有しているわけではありません。ロシアの調査では、数%から 16%のマダニがボレリア・ミヤモトイを保有していることが報告されています。我が国では、調査の結果、シュルツェマダニとパブロブスキーマダニがそれぞれ 1-5%程度、ボレリア・ミヤモトイを保有していることが明らかになっています。

問6 回帰熱にかかっても回復するのですか？

答 回帰熱の治療には、テトラサイクリン系の抗菌薬(ミノサイクリン、ドキシサイクリンなど)の投与が有効です。これまでに、ボレリア・ミヤモトイに感染したことによる回帰熱患者で、死亡例は報告されていません。

ただし、海外の報告によれば、ボレリア・ミヤモトイ以外のボレリア属菌による回帰熱全般で見た場合、適切な治療が行われなかった時の致死率は 5%未満とされています。

問7 回帰熱にかかりやすい、または、重症化しやすい年齢はあるのですか？

答 ロシアの報告では、患者の多くは50歳代以上です。我が国での状況を明らかにするためには今後の調査が必要です。

問8 回帰熱にかからないためには、どのように予防すればよいですか？

答 ダニに咬まれないようにすることが重要です。ダニに咬まれないことで予防できる疾患は、回帰熱だけではなく、つつが虫病、日本紅斑熱等のリケッチア感染症、ライム病、重症熱性血小板減少症候群（SFTS）、アナプラズマ症などがあります。マダニは春から秋にかけて、またツツガムシは全国的に通年で活動が見られます。草むらや藪など、マダニが多く生息する場所に入る場合には、長袖・長ズボン（シャツの裾はズボンの中に、ズボンの裾は靴下や長靴の中に入れる、または登山用スパッツを着用する）、足を完全に覆う靴（サンダル等は避ける）、帽子、手袋を着用し、首にタオルを巻く等、肌の露出を少なくすることが大事です。服は、明るい色のもの（マダニを目視で確認しやすい）がおすすめです。また、^{ディート}DEETという成分を含む虫除け剤の中には服の上から用いるタイプがあり、補助的な効果があると言われています。屋外活動後は入浴し、マダニに刺されていないか確認してください。特に、わきの下、足の付け根、手首、膝の裏、胸の下、頭部（髪の毛の中）などがポイントです。

問9 マダニが体に付いているのを見つけました。どうすればよいですか？

答 マダニの多くは、ヒトや動物に取り付くと、皮膚にしっかりと口器を突き刺し、長時間（数日から、長いものは10日間以上）吸血しますが、咬まれたことに気がつかない場合も多いと言われています。吸血中のマダニが体に付いているのを見つけた場合、無理に引き抜こうとするとマダニの一部が皮膚内に残って化膿したり、マダニの体液を逆流させてしまったりする恐れがあるので、医療機関（皮膚科）で処置（マダニの除去・洗浄など）をしてもらってください。また、マダニに咬まれた後、数週間程度は体調の変化に注意し、発熱等の症状が認められた場合は医療機関で診察を受けてください。

問10 厚生労働省ではどのような対応を行っていますか？

答 回帰熱は、感染症の予防及び感染症の患者に対する医療に関する法律（感染症法）において、診断した医師による届出が必要な四類感染症に指定されています。今般、我が国においてもボレリア・ミヤモトイによる回帰熱が確

認められたことから、全国の地方自治体及び医療機関に対して情報提供と注意喚起を行っています。

また、厚生労働科学研究事業において、マダニのボレリア菌の保有状況、迅速診断法の開発、抗菌薬の効果等、回帰熱対策に関する総合的な研究が行われています。

<参考：ダニが媒介するその他の感染症>

- ・[重症熱性血小板減少症候群に関する Q&A](#)
- ・[ダニ媒介脳炎に関する Q&A](#)
- ・[つつが虫病（感染研ホームページ）](#)
- ・[日本紅斑熱（感染研ホームページ）](#)
- ・[ライム病とは（感染研ホームページ）](#)
- ・[ライム病検査について（感染研ホームページ）](#)

<医療従事者の方向け>

問1 回帰熱の病原体は何ですか？

答 スピロヘータ科ボレリア (*Borrelia*) 属の微好気性らせん菌です。マダニ媒介性回帰熱の病原体ボレリアとしては、世界的には *B. duttoni* など十数種類が知られています。北海道の2症例では、*B. miyamotoi* が検出されています。

ボレリア属菌は、酸や熱に弱く、一般的な消毒剤（消毒用アルコールなど）や台所用洗剤、紫外線照射等で死滅します。

問2 潜伏期間はどのくらいですか？

答 マダニに刺された後、12～16日程度（平均15日）です。

問3 どのような症状が出ますか？

答 発熱、頭痛、悪寒、筋肉痛、関節痛、全身の倦怠感などの症状が主で、時に、神経症状（意識障害、けいれん、昏睡）、リンパ節腫脹、呼吸不全、出血症状（歯肉出血、紫斑、下血）が出現します。

問4 検査所見の特徴はどのようなものですか？

答 限られた情報しかありませんが、肝機能異常を示す AST、ALT の上昇が報告されています。また米国の2症例では、血小板減少、好中球数減少と核の左方移動が報告されています。

問5 鑑別を要する疾患は何ですか？

答 回帰熱と同様の症状を呈しうる疾患には様々なものが考えられますが、ダニ媒介性の疾患としては、つつが虫病、日本紅斑熱を含むリケッチア感染症、ライム病、アナプラズマ症、エーリキア症などが挙げられます。そのほか、レプトスピラ症、ブルセラ症（波状熱）、鼠咬症、野兔病や、海外渡航歴がある場合は、さらに、マラリア、デング熱などと鑑別が必要です。

問6 どのようにして診断すればよいですか？

答 マダニによる咬傷後、上記問3・4にあるような症状・検査所見が認められた場合は、回帰熱を疑います。ただし、全ての症状や検査所見が認められるわけではありませぬ。また、患者がマダニに咬まれたことに気がついていなかったり、刺し口が見つからなかったりする場合があります。そのため確定診断には、発熱期の血液検体（全血、血清、血液培養ボトル等）を用いて、以下のような方法により、病原体診断を行います。

- ・ボレリア菌の分離・同定
- ・暗視野顕微鏡下鏡検による病原体の検出
- ・蛍光抗体法等による末梢血スメアの観察による病原体の抗原の検出
- ・PCR法による病原体の遺伝子の検出

なお、同じくボレリア属菌によるライム病については、これまで実施されてきた検査では、回帰熱との鑑別ができないことから、過去にライム病と診断された症例についても、実際には回帰熱であったか、もしくは、回帰熱との共感染であった可能性があります。よって、回帰熱もしくはライム病を疑い、病原体診断を行う場合は、回帰熱・ライム病両方の検査を依頼する必要があります。これら確定診断のための検査は、国立感染症研究所で実施することが可能ですので、まずは最寄りの保健所にご相談ください。

問7 治療方法はありますか？

答 ミノサイクリンやドキシサイクリンなどのテトラサイクリン系抗菌薬の投与が有効です。ペニシリン系の抗菌薬も有効ですが、殺菌的な抗菌薬を使用した場合、希にJarisch-Herxheimer反応（ヤーリッシュ-ヘルクスハイマー反応）*を起こす場合があります、注意が必要です。

*発熱、悪寒、頭痛、筋肉痛、頻脈などを主徴とする急性全身症状。死菌から放出されたエンドトキシン（菌体内毒素）等により引き起こされると考えられている。

問8 検査で回帰熱であることが確定した場合、どう対応したらよいですか？

答 回帰熱は感染症法において四類感染症に位置付けられていますので、患者を回帰熱と診断した場合には、直ちに最寄りの保健所長を通じて届け出てください。

→届出様式

問9 患者や患者検体（サンプル）を取り扱う上で、特に注意することはありますか？

答 回帰熱は、ヒトからヒトには感染しませんが、発熱期患者の血液には回帰熱の病原体が存在するので、標準予防策を遵守してください。

問10 検査方法等、技術的な内容の相談窓口を教えてください。

答 国立感染症研究所 info@niid.go.jp にお問い合わせください。

<参考文献>

回帰熱 診断マニュアル

http://www.nih.go.jp/niid/images/lab-manual/relapsing_fever_120608.pdf

ライム病と回帰熱の比較

疾病名	ライム病 (四類感染症)	回帰熱 (四類感染症)
国内で確認されている病原体	スピロヘータ科ボレリア属菌 <i>Borrelia garinii</i> , <i>B. afzelii</i> など	スピロヘータ科ボレリア属菌 現在のところ、 <i>Borrelia miyamotoi</i> のみ(※)
感染経路	主としてマダニによる咬傷 (回帰熱については、シラミ媒介性のものである)	
主な媒介マダニ	シュルツェマダニ (病原体保有率：20～30%程度)	シュルツェマダニ (病原体保有率：1～5%程度)
国内発生届出数	年間 10 件程度 (死亡例は 2007 年に 1 件報告あり)	感染症法施行後の届出無し (輸入症例 2 例を除く)
国内患者発生地域	シュルツェマダニの生息地域に一致 (主に中部以北の高地、寒冷地)。北海道、長野県など。	国立感染症研究所が保存していた血清約 800 検体を検査したところ、北海道の 2 検体から <i>B. miyamotoi</i> を検出。
潜伏期間	(マダニ咬傷から) 3～32 日 (平均 7～10 日)	(マダニ咬傷から) 12～16 日 (平均 15 日)
主症状	- マダニ刺咬部を中心とした遊走性紅斑が特徴的 - 発熱 (回帰熱ほどではない)、頭痛、関節痛、筋肉痛 - などインフルエンザ様症状を伴うこともある	- 発熱 (39℃以上)、頭痛、筋肉痛、関節痛、全身の倦怠感など - 回帰熱 (3～6 日の発熱期と 7～10 日の無熱期の反復) <i>B. miyamotoi</i> による死亡例の報告は無い
確定診断検査	- 血清中の抗ボレリア抗体の検出：可 - 血液検体を用いた PCR：不適 - 皮膚生検による病原体の分離・同定：可	- 血清中の抗ボレリア抗体の検出：可 - 有症期の血液検体を用いた PCR：可
治療・予防	*抗ボレリア抗体の検出検査のみでは、ライム病と回帰熱の鑑別不能 テトラサイクリン系の抗菌薬 (ミノサイクリン、ドキシサイクリンなど) の投与が有効	

※*Borrelia miyamotoi* は、1995 年に我が国で新種として発見された。当時、その病原性は不明であったが、2011 年にロシア、また、2013 年に米国において、回帰熱の病原体であることが確認されている。

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日 2013. 7. 29	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	新鮮凍結人血漿	研究報告の公表状況	Gradoni L. Euro Surveill. 2013 Jul 25;18(30). pii: 20539.	公表国 イタリア	使用上の注意記載状況・ その他参考事項等 新鮮凍結血漿-LR「日赤」 新鮮凍結血漿-LR「日赤」成分 採血 新鮮凍結血漿-LR「日赤」120 新鮮凍結血漿-LR「日赤」240 新鮮凍結血漿-LR「日赤」480 血液を介するウイルス、 細菌、原虫等の感染 vCJD等の伝播のリスク
販売名（企業名）	新鮮凍結血漿-LR「日赤」（日本赤十字社） 新鮮凍結血漿-LR「日赤」成分採血（日本赤十字社） 新鮮凍結血漿-LR「日赤」120（日本赤十字社） 新鮮凍結血漿-LR「日赤」240（日本赤十字社） 新鮮凍結血漿-LR「日赤」480（日本赤十字社）	○欧州連合（EU）におけるリーシェンマニア症の疫学的サーベイランス：運用と研究課題 リーシェンマニア症は媒介動物であるサシチョウバエの刺咬を通じてリーシェンマニア属の寄生原虫に感染し引き起こされる、病型が多様な疾患であり、熱帯、亜熱帯、温帯地域の101カ国で発生している。重症となる内臓リーシェンマニア症（VL）は年間20～40万件発生し、軽症の皮膚リーシェンマニア症（CL）は年間70～120万人が罹患している。 多くの医療関係者がリーシェンマニア症はまだまだ熱帯地域の疾患であると考えているが、既に南欧でも風土病となっている。VLはEUの9カ国で、2003～2008年に毎年410～620症例が発生したと推定された。人獣共通CLはVL流行地域で一般的に発生するが、<i>Leishmania infantum</i> による皮膚症は通常軽症であるため過小報告されているとみられる。無症候性原虫キャリアの存在は、EUにおける公衆衛生上の問題であり、南欧から北欧への輸入感染も報告されている。診断の遅れや誤診の発生は、医師が欧州におけるリーシェンマニア症の流行を認識しなくてはならないことを示している。リーシェンマニア症は欧州の一部では稀であるが、既に流行している地域では更なる拡大の可能性もあるため、サーベイランスを慎重に行い、国内外における届出システムを統一させなくてはならない。			
研究報告の概要	報告企業の意見 これまで熱帯地域の疾患であると認識されていたリーシェンマニア症が既に南欧で風土病化しており、更なる拡大の可能性もあるため注意が必要であるとの報告である。 今後の対応 日本赤十字社では、輸血感染症対策として問診時に海外滞在歴の有無を確認し、帰国（入国）後4週間は献血不適としている。また、問診時にリーシェンマニア症の既往歴を確認し、該当する場合は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。				

EDITORIALS

Epidemiological surveillance of leishmaniasis in the European Union: operational and research challenges

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Leishmaniasis is complex of vector-borne diseases caused by protozoan parasites of the genus *Leishmania* transmitted by the bite of phlebotomine sandflies. A dozen nosogeographical entities – characterised by different parasite, vector and reservoir host species, geographical distribution and clinical features in humans – affect 101 countries in tropical, subtropical and temperate zones of the world [1,2]. More than 90% of 200,000–400,000 global cases of visceral leishmaniasis (VL), the most severe form, are estimated to occur annually in India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil. A less severe form, cutaneous leishmaniasis (CL), is more widely distributed, accounting for 0.7–1.2 million cases each year in countries of Latin America, Mediterranean basin, Middle East and Central Asia.

Even though many physicians and public health experts still consider leishmaniasis a tropical disease, two entities associated with several *Phlebotomus* species are endemic in southern Europe: (i) zoonotic VL and CL caused by *L. infantum* throughout the region, having dogs as reservoir host; and (ii) anthroponotic CL caused by *L. tropica*, which occurs sporadically in Greece. More recently, a third parasite species (*L. donovani*, assumed to be anthroponotic) has been recorded in Cyprus, where it causes both VL and CL [3].

VL is endemic in nine countries of the European Union (EU). The World Health Organization's Department for the Control of Neglected Tropical Diseases has estimated a total VL incidence of approximately 410–620 cases each year during 2003 to 2008 in these endemic countries, adjusted to take into account a 'mild' 1.2–1.8-fold under-reporting [2]. Recent experiences from six of the nine countries – Bulgaria, Greece, Croatia, Italy, France and Spain – are presented in this special issue.

Zoonotic CL usually occurs in the same areas endemic for VL, but there are probably many more cases than those registered (2.8–4.6-fold under-reporting has been estimated for the EU region [2]). As pointed out by Lachaud et al. for France [4], but also applicable to

other EU countries endemic for CL, cutaneous lesions due to *L. infantum* are often benign and patients are seen by general practitioners or dermatologists who generally do not report these cases or notify them even when mandatory.

Despite provoking a limited number of overt clinical cases – in comparison with global leishmaniasis figures – *L. infantum* represents a latent public health threat in the EU because studies performed in several endemic foci have disclosed a high prevalence of asymptomatic parasite carriers [5]. A recent example is provided for Croatia by Šiško-Kraljević et al. [6]. Hence, immunosuppressive conditions, either due to co-morbidities (e.g. human immunodeficiency virus (HIV) infection) or therapies (e.g. organ transplantation or treatment of immunological disorders [7]) may result in the reactivation of latent infections. In this regard, it should be emphasised that dermatropic *L. infantum* genotypes – the usual agents of benign CL – may disseminate to cause severe VL in immunosuppressed individuals [8]. Such elevated prevalence of human infections could have been predicted from two strands of evidence: humans are frequently bitten by sandflies and *L. infantum* infections are widespread in dogs, a highly susceptible host [9]. In large parts of countries of southern EU, canine seroprevalence rates are estimated to be in the range of 5–30%, which means that infection rates may reach values of 40–80% [10].

Some European countries at the north of regions with natural transmission of leishmaniasis have reported large series of VL and CL imported cases, many of which have acquired the parasitic infection during holidays in southern Europe [11–14]. In several instances, a definitive diagnosis of VL proved difficult and for one case, the period before symptom onset and specific treatment was longer than a year. Delay in diagnosis or misdiagnosis can also occur in southern European countries endemic for VL, but in parts where cases occur rarely, as has been reported from a northern Italian region [15]. These observations suggest that awareness about leishmaniasis endemicity in Europe should be greatly increased among general practitioners and clinicians.

TABLE

Operational and research challenges concerning epidemiological surveillance of leishmaniasis in the European Union

Topic	Challenges
Surveillance by passive notification systems	Notification of leishmaniasis is not compulsory everywhere in Europe. Some endemic countries have national notification systems centralised at the Ministry of Health; others have compulsory or voluntary surveillance systems in endemic regions but not in non-endemic ones. Non-endemic countries of northern Europe rely on single (or a network of) reference centres that collect information on a voluntary basis.
	There is limited harmonisation of the existing notification systems as regards case definition, clinical presentation and patient information.
	In countries with compulsory notifiable systems, under-reporting of visceral leishmaniasis is estimated to be 1.2–1.8-fold, that of cutaneous leishmaniasis 2.8–4.6-fold [2].
Transnational information	Travellers to endemic countries are not provided with adequate information on leishmaniasis risk and physicians often do not include leishmaniasis in differential diagnosis of travel-related diseases.
	There is a lack of feedback from non-endemic countries registering leishmaniasis cases in travellers to the endemic countries visited by patients, which can hamper early identification of new or re-emerging foci.
Disease vs infection	Increasingly, there is evidence that clinical cases of leishmaniasis represent the tip of an 'infection iceberg', whose size (i.e. prevalence) is unknown in most of the endemic countries
	Determinants for human clinical susceptibility are largely unknown, apart from some co-morbidities (e.g. human immunodeficiency virus (HIV) infection) or immunocompromising conditions, i.e. through immunosuppressive therapies.
Parasite identification	Multilocus enzyme electrophoresis (MLEE), the gold standard for <i>Leishmania</i> identification, is available at reference centres of three European countries (France, Italy and Spain) [1]; however, there is risk that MLEE typing activities will be ended soon because they are expensive and laborious.
	Different levels of accuracy may be required (e.g. species level at clinical centres, genotype level for epidemiological investigations); however, common protocols for molecular <i>Leishmania</i> identification are not available yet.
Domestic vs wild reservoir hosts	Updated geographical distribution of canine leishmaniasis, representing the most efficient sentinel for leishmaniasis transmission in a territory, is not available for all endemic countries
	The epidemiological role of domestic hosts other than dogs (e.g. cats) is still unclear
	The potential role of wild mammals (rodents, lagomorphs, carnivores) as reservoir hosts of <i>Leishmania</i> requires investigation because it can change with man-made environmental changes such as witnessed by the recent outbreak in Madrid, Spain [21].
Phlebotomine vectors	Taxonomy and biology investigations on European phlebotomine species rely on a limited group of experts. Updated information on vector distribution is therefore lacking in some endemic countries and in neighbouring non-endemic ones.
	Competence of permissive sandfly species needs to be elucidated as regards potential transmission of exotic <i>Leishmania</i> species imported into Europe.
	The vectorial role of continental European species of sandflies (e.g. <i>Phlebotomus mascitti</i>) is still to be ascertained.
Control measures	The primary control measure is avoiding deaths from the most severe form of leishmaniasis (visceral). General public and health professional awareness of the disease (both leading to early diagnosis) and appropriate therapy should be the mainstay for both endemic and non-endemic countries.
	Vaccination combined with topical insecticides with sandfly anti-feeding properties should be recommended for dogs living in endemic areas or temporarily travelling from non-endemic to endemic areas.

As an endemic country comprises known areas or foci of endemicity, it is interestingly to note that in some instances, travellers became infected after visiting an area that was not considered as endemic by the health authorities of the country visited [16]. This should encourage the development of systems for appropriate transnational information following leishmaniasis diagnosis in travellers.

Deaths due to VL, although possible, are rare. The disease has a slow chronic course, so that fatal cases may be patients with individual risk factors such as severe co-morbidities or, in case of young children, malnutrition associated with late diagnosis. On the other hand, deaths due to inappropriate use of VL drugs can be even more frequent. In some European countries, antimonial drugs are still in use for some categories of patients because of the high cost of liposomal amphotericin B [17] and it is well known that overdose of pentavalent antimony in adults can cause severe cardiac failures in addition to pancreatitis.

This special issue of *Eurosurveillance*, published in two parts, is a useful instrument to review diverse aspects of leishmaniasis in Europe related to topics such as the information and surveillance systems in place in countries within the EU, the current epidemiological situation and novel aspects related to parasite identification [18,19], domestic and wild reservoir hosts [20] and vectors [9]. The main challenges associated with these topics are summarised in the Table.

In conclusion, leishmaniasis, a neglected disease, is rare in some countries of Europe, but endemic in others, having a great impact on individuals and the potential to spread further. The disease should be monitored carefully and systems for its notification should be harmonised at both national and transnational levels.

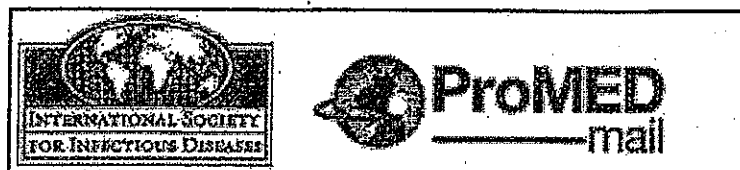
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醫藥部外品 化粧品

識別番号・報告回数	報告日 年 月 日	第一報入手日 2013 年 8 月 19 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	回	研究報告の公表状況	公表国 イギリス	
販売名（企業名）				
研究報告の概要	イギリスにおいて、食肉処理業者がウシ結核に感染し死亡した。この症例はイギリスで最初の死亡症例であった。イギリス中西部の町 Uttoxeter において、53 歳の食肉処理業者がウシ結核に感染し、2012 年 5 月 28 日に Burton's Queen's Hospital で死亡した。この症例はイギリスで最初の死亡症例であった。イギリスの Health and Safety Executive は、患者が働いていた食肉処理場に対して調査を実施している。調査会において、Queen's Medical Center の顧問病理医師は、患者の胸部において詳細な検死解剖を実施し、死因確認のための検査試料を Birmingham に送付したと報告している。この病理医師は、感染ウシの血液や尿などのエアロゾルを介して感染した可能性を指摘している。患者は、全身に結核が蔓延していた。結核に感染していた期間を推定することは困難であるが、恐らく、比較的最近に感染し、急速に蔓延した可能性があると述べている。患者は、肉牛の生育農家、および、食肉処理場で働いていた。なお、問題の食肉処理場は、結核に感染したウシの食肉処理施設として登録されていた。Health and Safety Executive の検査官は、結核に感染した牛が屠殺されているにもかかわらず、屠体から発生するエアロゾルを阻止するための処置がされていないことに関し懸念していると述べている。検査官は、感染が食肉処理場の業務が原因であるとは断定できず、患者が生育農場で暴露された可能性も考えられる、としている。Health and Safety Executive はリスク監視のために極めて詳細な調査を実施しており、現在、リスク評価の結果が出るのを待っているところであると述べている。一方、多くの発展途上国においてウシ結核が蔓延している。これらの中では、ウシ結核のヒトへの感染が認められている。一方、多くの先進国では、診断検査が複雑、高価なので実施には限度があることから、十分な検査が実施されていない可能性がある。			
報告企業の意見		今後の対応		
ウシ結核の感染による死亡例の報告である。先進国では、感染ウシの殺処分、汚染された乳および乳製品に対する殺菌の普及などにより、ヒトへの感染は減少した。しかし、感染ウシの排泄物や屠体のエアロゾルを介した感染があることに注意が必要である。コージネイト FS の製造工程においてアフィニティークロマトグラフィーを用いているが、このリガンドであるマウス IgG モノクロナール抗体産生細胞の培養液にウシインスリンが添加されている。このウシインスリンの一連の製造・精製工程は細菌やウイルスを高率に除去できていることが確認されている。なお、2007 年 4 月以降、コージネイト FS の販売は行っていない。				現時点で新たな安全対策上の措置を講じる必要はないと考える。今後も、新規人畜共通感染症や新たなウイルス感染症に関する情報収集に努める。
使用上の注意記載状況・その他参考事項等 BYL-2013-420 ProMED mail: Bovine Tuberculosis, Human-UK http://www.promedmail.org/direct.php?id=20130809.1871322				

MedDRAバージョン 16.0



Published Date: 2013-08-09 10:00:28
Subject: PRO/AH/EDR> Bovine tuberculosis, human - UK: (England)
Archive Number: 20130809.1871322

BOVINE TUBERCULOSIS, HUMAN - UK: (ENGLAND)

A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Mon 1 Jul 2013 [accessed 8 Aug 2013]
Source: Uttoxeter Advertiser/Post & Times [edited]
<http://www.utttoxeter-news.co.uk/News/Investigation-is-still-ongoing-at-Bramshall-abattoir-20130701122754.htm>

Investigations are still ongoing at a Uttoxeter abattoir [slaughterhouse] after a slaughterman became the only meat worker in the country to die after contracting bovine tuberculosis (TB).

[The patient], of Stramshall, died at Burton's Queen's Hospital on 28 May 2012 after contracting the disease, which is usually a major killer of cattle and wildlife.

Now the Health and Safety Executive has revealed a full investigation into TS Sargeant Abattoir, in Roycroft Grange, Bramshall, where [the patient] worked, is ongoing.

Dr Ian Scott, a consultant pathologist at Queen's Medical Centre, in Nottingham, told the inquest, held at Burton Town Hall, that the limited post mortem concentrated on his chest and samples were sent to Birmingham, which confirmed the unusual cause of death.

He said the bovine TB is contracted by contact with an infected animal carcass through "aerosol" spread such as blood and urine.

Dr Scott said it was difficult to know how long the 53-year-old had the infection as it had spread throughout his body. However, he had suspicions it was a probably a more recent infection that had spread quickly.

[The patient] was always with beef cattle as he would rear them and then work as a slaughterman for the abattoir, which is registered to slaughter cattle infected with bovine TB.

Lynne Spooner, an inspector for the HSE [Health and Safety Executive], said an investigation had originally been carried out to ensure there was not an epidemic as the abattoir was registered to take infected cattle from surrounding farms as well as from [the patient]'s own farm.

Her investigation did raise concerns as she found the cattle infected with bovine TB were being slaughtered but there were no controls in place to stop the aerosols from the carcasses.

Miss Spooner found there was a presence of bovine TB and the workers were being exposed to it through the aerosols.

She added: "What we don't know is the exact point when he contracted bovine TB as we cannot categorically say [it was] because of his work at the abattoir. There is also the potential he was exposed to it on his own farm as well. The HSE has done extensive work to monitor the risk and this has been looked into in great detail. We are currently awaiting the results of a risk assessment."

Miss Spooner told the inquest that they had seen a significant increase in cattle infected with the disease in the last 10 years and samples sent off for testing from TS Sargeant found 50 per cent were returned positive.

She said the transmission of bovine TB to humans in the general population had decreased but it is difficult to say from occupational exposure as workers may develop it later in life when their immune system has weakened.

South Staffordshire coroner Andrew Haigh said [the patient] died from multiple organ failure, pulmonary (bovine) tuberculosis, and ulcerative colitis.

He ruled his death was due to industrial disease.

Mr Haigh said: "Where and when he became infected is unclear but maybe it was a more recent rather than historic infection. The colitis he was being treated for may have made the infection develop quicker. The likelihood is he caught the infection from his work at the slaughterhouse as opposed to the farm but we cannot be sure about this."

He added that this was the only case they are aware of in the country.

TS Sargeant Abattoir said it had no comment on the investigation.

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[Uttoxeter is a town in Staffordshire, in the West Midlands region of England.]

Mycobacterium bovis has been identified in humans in most countries where isolates of mycobacteria from human patients have been fully characterised. The incidence of pulmonary tuberculosis caused by M. bovis is higher in farm and slaughterhouse workers than in urban inhabitants. The transmission of M. bovis to humans via milk and its products is eliminated by the pasteurisation of milk. One of the results of bovine tuberculosis eradication programmes has been a reduction in disease and death caused by bovine tuberculosis in the human population.

It is necessary to distinguish M. bovis from the other members of the "tuberculosis complex," namely M. tuberculosis (the primary cause of tuberculosis in humans), M. africanum (occupies an intermediate phenotypic position between M. tuberculosis and M. bovis), M. microti (the "vole bacillus," a rarely encountered organism), M. pinnipedii, and M. caprae. For the prescribed laboratory techniques, please refer to "Chapter 2.4.7. Bovine Tuberculosis" in OIE's Terrestrial Manual (2009 version) at http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.04.07_BOVINE_TB.pdf. - Mod.AS]

[Conventional microbiologic diagnosis of human tuberculosis (TB) relies on methods that allow only identification of "Mycobacterium tuberculosis complex". "M. tuberculosis complex" is composed of Mycobacterium tuberculosis (Mtb), as well as Mycobacterium bovis (Mb) and several other genetically closely related species: the BCG bacillus, M. africanum, M. caprae, M. microti, and M. canetti. Mb is inherently resistant to pyrazinamide (PZA), a drug that is an essential component of the standard multidrug short-course regimen to treat human tuberculosis due to drug-susceptible Mtb.

Differentiation of Mb from Mtb is therefore important clinically as well as epidemiologically. To distinguish Mb from these other species, special tests have to be done, such as spoligotyping (spacer oligonucleotide typing), which is a polymerase chain reaction-based amplification of a region of the mycobacterial genome, or growth in selective media and specific biochemical reactions. If these special tests are not done, the diagnosis of Mb infection might be missed and the patient treated inappropriately.

The pathology of Mb infection in cattle is similar to that of TB in humans, with cavitary pulmonary tuberculosis leading to chronic debilitation and coughing, and potential hematogenous spread to other organs. Infectious aerosols can be generated when an animal with lung cavities, which contain high numbers of mycobacteria, coughs or sneezes. Mb can then spread from cow-to-cow by inhalation of the aerosolized organisms. One to 2 per cent of MB-infected cows develop mycobacterial mastitis with shedding of the organism in milk and possible spread of infection in their calves.

Mb can be transmitted to humans by either ingestion of unpasteurized milk or milk products or

inhalation of infectious aerosols from infected animals. Although rare, person-to-person transmission of Mb does occur. Following ingestion of Mb-contaminated food, Mb most commonly localizes in cervical lymph nodes (scrofula) or the gastrointestinal tract and related lymph nodes. These forms of Mb infection occur commonly in children and were common in people in developed countries during the pre-pasteurization era. Following inhalation of infectious aerosols of Mb, primary pulmonary Mb infection develops with subsequent hematogenous dissemination to the lungs and other organs. Reactivation of latent Mb infection can occur in later life and especially in those individuals with underlying immunosuppressive disorders such as HIV infection or who are receiving corticosteroid therapy, in whom the disease can be rapidly fatal.

No mention is made in the report above of the patient ever having scrofula, so we can assume this patient likely inhaled aerosolized respiratory secretions from an infected animal on his own farm or aerosols generated in the slaughterhouse. The fact that he had ulcerative colitis suggests that he may have received immunosuppressive medication for this diagnosis, which would have a detrimental effect on his immune system's ability to control TB. In fact, all patients with TB should be tested for HIV infection and there is no mention of this patient's HIV status in the news report above.

Since his farm and the slaughterhouse where he worked were known to have Mb-infected animals, there should be environmental control measures in the workplace to prevent or reduce generation of infectious aerosols, and thus reduce exposure of employees to Mb. Also, employees suspected or known to have developed Mb TB should be rapidly diagnosed and treated to prevent further spread of infection. Workers in such facilities should be wearing face masks that filter infectious aerosols and they should be screened by periodic tuberculin skin testing or interferon-gamma release assay to assess for latent TB and they should have periodic medical exams.

This patient certainly should have been evaluated for latent TB if he were started on immunosuppression for ulcerative colitis and at the time relieved him from work in this potentially life-threatening environment. To assess for spread of Mb from slaughtered cattle or this infected employee, other employees should be tuberculin skin tested or interferon-gamma release tested and an attempt should be made to assess if the patient's Mb genotype matches that of Mb isolated from recently slaughtered cattle or his farm animals.

Human Mb TB still occurs in many developing countries, where Mb TB remains endemic, and may be under-appreciated in many developed countries because of the limited use of complex and expensive diagnostic laboratory testing. The same is also likely true in much of the developing world, where bacteriologic diagnostic testing is generally limited to acid-fast stained sputum smear examination. - Mod.ML

A HealthMap/ProMED-mail map can be accessed at: <http://healthmap.org/r/7XiC.>

See Also

2012

Bovine tuberculosis, human - UK (02): (England) ex alpaca [20120510.1128707](#)
Bovine tuberculosis, human - UK: (England) ex alpaca [20120427.1116358](#)

2009

Bovine tuberculosis, human - UK: (England) [20090808.2815](#)
2008

Bovine tuberculosis, human, canine - UK: (England) (02) [20080927.3054](#)
Bovine tuberculosis, human, canine - UK: (England) [20080903.2751](#)
2006

Bovine tuberculosis, human - UK (England) (02) [20061015.2967](#)
Bovine tuberculosis, human - UK (England) [20061009.2896](#)
2005

Tuberculosis, bovine, human ex cervid - USA (MI) [20050112.0102](#)
2004

Bovine tuberculosis, human - UK (Gloucestershire) (02) [20040716.1930](#)
Bovine tuberculosis, human - UK (Gloucestershire) [20040714.1890](#)
.....arn/je/ml/mj/mpp

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