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Unexpectedly High Incidence of Indigenous Acute Hepatitis E Within South Hampshire: Time for Routine Testing?

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Hepatitis E indigenous to developed countries (hepatitis E^{IDC}) is a form of hepatitis E in persons with no travel history to highly endemic areas. It has been recognized recently as an emerging clinical entity in a significant number of economically developed countries including UK. However, it is still perceived as a rare disease and routine laboratory testing for hepatitis E is not performed. A series of 13 cases of hepatitis E^{IDC}, diagnosed in a 13-month period from June 2005 within a single center in South Hampshire, UK, is presented. These patients were identified after implementing a novel-screening algorithm that introduced routine hepatitis E serological investigations. Patients were middle aged or elderly and males were affected more commonly. Four patients (31%) required hospital admission. All reverse transcriptase-polymerase chain reaction (RT-PCR) confirmed cases carried hepatitis E virus (HEV) genotype-3, which bore close sequence homology to HEV circulating in UK pigs. None of these patients recalled eating undercooked pork products or close contact with pigs during the 2 months preceding the onset of acute hepatitis. In comparison, during the same period, only two cases of hepatitis A and five cases of acute hepatitis B were diagnosed. These data illustrate the importance of introducing routine hepatitis E testing in all patients with unexplained acute liver disease and absence of relevant travel history. Routine testing can clarify hepatitis E epidemiology whilst improving the clinical management of patients with acute liver disease. ***J. Med. Virol. 80:283–288, 2008.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: non-travel associated hepatitis E; serology; RT-PCR

INTRODUCTION

Hepatitis E virus (HEV) is a small non-enveloped virus, with a single-stranded RNA genome of positive polarity. First documented as the cause of non-A, non-B enterically transmitted hepatitis in the eighties [Gandhi et al., 1982; Balayan et al., 1983; Bradley and Maynard, 1986], HEV was cloned and sequenced in the early nineties [Reyes et al., 1990; Tam et al., 1991] and classified as the sole member of the genus *Hepevirus*, family *Hepeviridae*, in 2004 [Emerson et al., 2004]. In developing countries, where sanitation is poor, HEV can cause epidemics of acute hepatitis E when the water supply is fecally contaminated [Tsega et al., 1991; Naik et al., 1992; Rab et al., 1997]. In this setting hepatitis E is generally a mild disease of young adults; however, pregnant women may suffer significant morbidity and mortality [Hussaini et al., 1997; Kumar et al., 2004; Boccia et al., 2006]. In developed countries, by contrast, hepatitis E is a sporadic disease identified predominantly in travelers returning from developing countries. More recently, a form of hepatitis E with no travel history to highly endemic areas has been identified and referred to as "hepatitis E indigenous to developed countries" or "hepatitis E^{IDC}" [Teo, 2006]. This form appears to affect predominantly elderly males [Sainokami et al., 2004; Ijaz et al., 2005].

Genotyping of HEV has given insights into the epidemiology of this infection. There are four main genotypes of HEV. Hepatitis E in developing countries is

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caused by genotypes 1 and 2. However, hepatitis E^{IDC} is caused by genotype 3 in most countries and by genotypes 3 and 4 in Japan [Lu et al., 2006; Okamoto, 2007]. Genotype 4 causes hepatitis E in China where it appears to be increasingly common compared to genotype 1 [Li et al., 2006]. Critically, HEV genotypes 3 and 4 are known to infect a range of animals [Wang et al., 2002; Michitaka et al., 2007], particularly pigs [Banks et al., 2004; Zheng et al., 2006; Herremans et al., 2007], suggesting that exposure to animals or animal products may be the source of infection in humans. Indeed acquisition of hepatitis E^{IDC} by dietary consumption of wild boar, deer, and pig meat or viscera, contaminated with HEV, has been documented in Japan [Tei et al., 2003; Yazaki et al., 2003; Takahashi et al., 2004; Masuda et al., 2005].

Hepatitis E^{IDC} was first reported as a clinical entity in the United Kingdom (UK) 7 years ago, following a report of four cases [McCrudden et al., 2000]. Subsequent investigations of stored serum samples, together with enhanced prospective surveillance in several UK centers, have shown that hepatitis E^{IDC} is indeed an under-diagnosed disease in UK [Ijaz et al., 2005; Lewis et al., 2006; Dalton et al., 2007]. The relatively low sensitivity [Zhang et al., 2002; Mansuy et al., 2004; Myint et al., 2006] and high costs of currently available diagnostic tests have meant that they are not used routinely in the diagnosis of unexplained abnormal liver function tests. Therefore, to date, systematic testing for hepatitis E is not routinely performed in UK diagnostic laboratories, and the true incidence and the clinical impact of this disease remain to be fully clarified.

In order to address this, a novel diagnostic algorithm, introducing routine testing for antibodies to HEV, was defined and implemented. The experience of a single diagnostic center in Hampshire, UK, is presented.

METHODS

Patients and Samples

This work was performed at Southampton University Hospital NHS Trust. Between May 2005 and June 2006, 139 (70 females, 69 males) serum samples received at the Health Protection Agency (HPA) South East Regional laboratory of Southampton, which were negative for markers of acute infection by hepatitis viruses A, B, C, Epstein–Barr virus (EBV), and cytomegalovirus (CMV), and with an ALT level greater than 300 IU/L (normal range of 10–40 IU/L), were tested for HEV IgM and IgG.

All patients with laboratory data consistent with acute hepatitis E were investigated for travel history to highly endemic areas in the 2 months preceding symptoms onset. Whenever travel history was negative, patients were asked to complete a questionnaire, which assessed contacts with animals, including pigs, dietary habits, and exposure to other jaundiced individuals. The questionnaire, developed by the Center for Infections, HPA, London (www.hpa.org.uk), after the initial cases of hepatitis E^{IDC} had been detected in UK in 1999 [Mc Crudden et al., 2000], is part of an enhanced surveillance program for this infection, in England and Wales.

HEV Serology

HEV IgM and IgG serology was performed using the Gene Lab ELISA assays, two immune enzymatic commercial tests based on recombinant antigens from HEV genotypes 1 and 2 (Genelabs Diagnostics, Singapore). Both laboratory test results were interpreted according to the directions given by the manufacturer: all samples with an optical density greater than the cut-off was considered positive. A positive HEV serology was confirmed by additionally testing a follow-up sample.

HEV Reverse Transcriptase-Polymerase Chain Reaction Assay (RT-PCR) and Genotyping

Samples reactive by the HEV IgM and/or IgG assays were additionally tested for HEV RT-PCR and genotyped, if HEV-RNA was detected, at the Center for Infections, HPA, London [Ijaz et al., 2005].

Clinical Features and Laboratory Results

Fifteen cases of acute hepatitis E were identified between May 2005 and June 2006. Two cases were diagnosed in patients of Indo-Pakistani origin who had traveled to the Indian subcontinent in the recent past. Thirteen patients were British of white European ethnicity, resident in three urban areas within a 10-mile radius of Southampton, Hampshire, UK (Fig. 1) and had not traveled to highly endemic areas in the 2 months prior to identification of raised serum ALT. Eight of the 13 patients (62%) returned the contact-tracing questionnaire. Two patients ate shellfish and three ate liver pate of unspecified animal origin in the 2 months prior to the detection of acute hepatitis. It is not known whether this consumption was occasional or habitual. During the same period no patient had consumed undercooked pork meat or had been in contact with jaundiced individuals or farm animals, including pigs. Five patients (38%) were dog owners, but no disease was reported in their pets.

Table I summarizes the patients' clinical details and laboratory results of hepatitis E^{IDC} cases. The median age was 71 years (range of 46–85 years) with 6 (46%) being 75 years of age or older; 11 (85%) were male. Twelve of the 13 initial samples were collected at the peak of the ALT value and the 13th was collected 2 weeks after the onset of jaundice, when the ALT value had normalized. HEV RNA genotype 3 was detected in 8/12 (67%) patients in the acute phase of the disease. Two HEV RNA positive patients had atypical serological profiles: one had only a detectable IgM response, without a measurable anti-HEV IgG response in spite of repeat analyses several weeks later, while the other patient had only detectable IgG.

The clinical presentations were similar in most cases. Typical features were a 2–3 weeks prodrome of malaise,

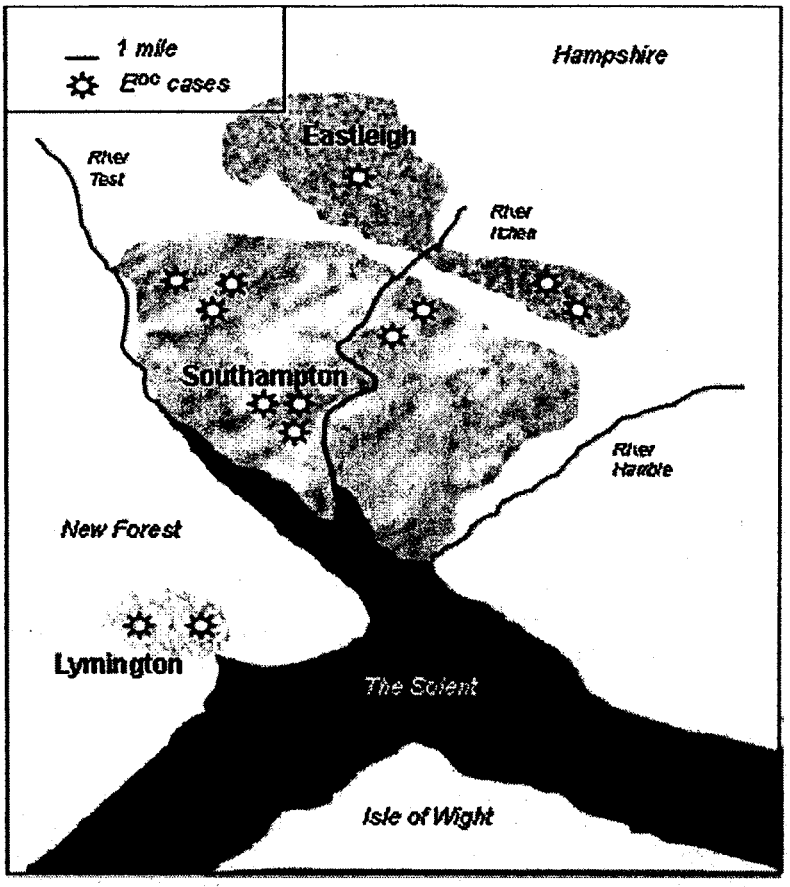

Fig. 1. South Hampshire. Proportion of people above state pension age (65 years for men and 60 years for women) according to 2001 population census data: Southampton 16.5%, Bastleigh 17.1%, Lymington New Forest 25.6%, England and Wales 18.4%).

fever, and anorexia before presenting to their general practitioner. Ten of 13 patients (77%) complained of jaundice and dark urine, suggestive of liver disease, whilst 3 (23%) had abdominal pain. Seven patients were referred to hospital and four (31%) were admitted. ALT levels varied between 300 and 6,777 IU/L (normal range of 10–40 IU/L). Liver synthetic function, as determined by international normalized ratio (INR) estimation, was impaired in two individuals (a third patient with raised INR was on concomitant warfarin therapy since the implantation of a prosthetic heart valve). The severity of the illnesses may have been contributed to by the co-morbidities that are prevalent in the elderly population. Four individuals had type II diabetes mellitus, three had hypertension, and one drank alcohol to levels above the UK Department of Health recommendations (www.dh.gov.uk/en/policyandguidance/healthandsocialcaretopics/alcoholmisus; version of 7.06.2007). Twelve out of 13 patients made a complete recovery after about 2 weeks, but one patient died 2 months after the acute illness from right lower lobe pneumonia. This death was most likely unrelated to his HEV infection.

In summary, by the use of a novel-testing algorithm, 15 cases of acute hepatitis E, of which 13 were not travel associated, have been identified in a 13-month period. By comparison, during the same period only two cases of acute hepatitis A and five cases of acute hepatitis B were identified, leading to the inference that hepatitis E^{IDC} is significantly under diagnosed.

DISCUSSION

In a 13-month period acute hepatitis E^{IDC} has been identified in 13 individuals resident in three towns of coastal Hampshire, UK, with a total population of about 340,000 inhabitants (Fig. 1). In the eight cases diagnosed during the viraemic phase of the disease, HEV genotype 3 was detected (Table I). This genotype, commonly circulating in pigs [Banks et al., 2004; Teo, 2006], has also been recognized in the other cases of hepatitis E^{IDC} reported in UK, summarized in Table II. A possible risk factor for acquiring hepatitis E^{IDC} was identified in two patients who ate shellfish [Mechnik et al., 2001; Koizumi et al., 2004; Ijaz et al., 2005] during the 2 months preceding the illness.

The majority of these patients were elderly males (85%, Table I). It is not clear if genotype 3 is attenuated in pathogenicity, thus causing preferentially overt disease in more susceptible hosts like elderly individuals, or if older people, particularly males, have a greater risk of exposure to HEV due to behavioral or environmental risk factors. This peculiar and puzzling

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TABLE I. Patients' Clinical Details and Laboratory Results of Hepatitis E^{IDC} Cases as Obtained From the First Serum Sample Tested

	Age (years)	Sex	Peak ALT[a] (IU/L)	INR	Bilirubin[b] (μmol/L)	Jaundice	Comorbid condition(s)	Hospital admission	HEV IgG	HEV IgM	HEV genotype
1	68	F	6,210	1.6	75	Yes	NIDDM and hypertension	Yes	+	+	3
2	61	M	951	1.1	28	No	None	No	+	+	3
3	71	M	1,037	1	10	No	NIDDM	No	+	+	ND
4	75	M	2,733	1	123	Yes	Bladder carcinoma, pharyngeal pouch	No	+	+	3
5	82	M	6,777	2.6	154	Yes	AF, IBS, THR	Yes	−	+	3
6	85	M	656	1	172	Yes	Aortic stenosis, hypertension	Yes	+	+	ND
7	76	M	1,705	>8[c]	320	Yes	AVR, CABG	Yes	+	+	3
8	80	F	945	1	94	Yes	Hypertension	No	+	+	3
9	47	M	630	1	28	No	None	No	+	+	ND
10	69	M	959	1.1	115	Yes	NIDDM, CABG, hypertension, hypercholesterolemia	No	+	−	3
11	83	M	3,554	1.2	115	Yes	NIDDM	No	+	+	3
12	56	M	300	NP[b]	68	Yes	Non-alcoholic fatty liver	No	+	+	NP
13	56	M	551	NP[b]	228	Yes	IHD	No	+	+	NP

NIDDM, non-insulin dependent diabetes mellitus; AF, atrial fibrillation; IBS, irritable bowel syndrome; THR, total hip replacement; AVR, aortic valve replacement; CABG, coronary artery by-pass graft; IHD, ischemic heart disease; INR, international normalized ratio; ND, not detected; NP, not performed.
[a]ALT normal value 10–40 IU/L.
[b]Bilirubin normal value 0–20 μmol/L.
[c]On warfarin since 1998.

TABLE II. Published Cases of Acute Hepatitis E^{IDC} in England and Wales

	Location	Period	Number of cases	Age (years)	Sex
Cases detected in single Centers	Southampton, Hampshire [McCrudden et al., 2000]	1999	4	41, 44, 70, 71	1 Male, 3 females
	Truro, Cornwall [Levine et al., 2000]	1999	1	61	Male
	Hull, East Yorkshire [Jary, 2005]	2005	1	54	Male
	Birmingham, Midlands [Sadler et al., 2006]	2005 (5-month period)	8[a]	Median age 60	4 Males, 3 females
	Cornwall and South-West Devon [Dalton et al., 2007]	March 1999–September 2005	21[b]	Median age 67	15 Males, 6 females
Cumulative data of England and Wales	National survey [Ijaz et al., 2005]	1997–2003	17[c]	Median age 70	13 Males, 4 females
	National survey [Lewis et al., 2006]	January–June 2005	24[d]	Median age 59	20 Males, 4 females

[a]2/8 Patients were RT-PCR positive, one patient with genotype 3 while the other with genotype 1 (the latter had been in recent contact with a jaundiced individual returning from Pakistan).
[b]HEV genotype 3 detected in 16/21 (76%) cases.
[c]HEV genotype 3 detected in 11/17 (65%) patients.
[d]10/25 (40%) cases were HEV RT-PCR positive, of which 9 were genotype 3.

demographic feature was previously documented in a nation-wide UK study [Ijaz et al., 2005] of 17 hepatitis E^{IDC} cases diagnosed between 1997 and 2003 in individuals, 14 of whom (82%) lived in coastal and estuarine areas, as are the ones found in our study in the South Hampshire region. Ijaz et al. [2005] pointed out the confounding effect of older age on the place of residence. This bias might not be relevant to this study where the elderly patients affected by acute hepatitis E belonged to a population which, on average, appears younger compared to that in the rest of England and Wales (www.statistics.gov.uk/census2001, Fig. 1).

Although documented in other European countries, Asia, and USA [van der Poel et al., 2001; Clemente-Casares et al., 2003; Widdowson et al., 2003; Buti et al., 2004; Amon et al., 2006; Peron et al., 2006], hepatitis E^{IDC} is still considered an uncommon disease. A recent report by Lewis et al. [2006] suggests that hepatitis E^{IDC} in UK is under diagnosed. However, implementation of routine serology for hepatitis E is hampered by the fact that currently available antibody assays, based on HEV genotypes 1 and 2, lack sensitivity [Lin et al., 2000; Myint et al., 2006]. This has been attributed to several factors of which the main one is likely to be that the currently available recombinant HEV proteins used in the assay systems may not include all relevant immunogenic B cell epitopes encoded within the HEV genome [Wang et al., 2001; Zhang et al., 2003; Zhou et al., 2004]. Additionally, the genetic diversity between HEV genotypes [Lu et al., 2006] warrants the inclusion of each HEV genotype in future diagnostic kits.

In spite of their limitations, currently available antibody assays have been capable of detecting a significant number of hepatitis E^{IDC} cases, leading to the recognition of this emerging disease. This consideration guided the decision to routinely include hepatitis E testing in our laboratory. Cases with a significantly deranged ALT value were tested, in order to target acute hepatitis of clinical importance. By adherence to this algorithm, a pick up rate of 9.3% was obtained.

In a situation of suboptimal performance of currently available antibody assays, RT-PCR represents a useful complementary diagnostic tool [Jothikumar et al., 2006]. Although the duration of viraemia is variable (from few days to few weeks) (1, 10) a serum sample collected at the peak of ALT values has a high chance to be RT-PCR positive thus clarifying cases of acute hepatitis E with atypical serological profiles, as found in two of our patients (Table II), including HEV seronegative cases [Lin et al., 2000; Mansuy et al., 2004].

The incidence of hepatitis E^{IDC} in our center exceeded the frequency of acute hepatitis A (two cases) and hepatitis B (five cases). In UK, where high standards of sanitation and vaccination programs have significantly reduced exposure to hepatitis A and B viruses, hepatitis E^{IDC} may emerge as a major cause of acute viral hepatitis [Lewis et al., 2006]. The high frequency observed in our uncontrolled series may in part be a reflection of a better ascertainment of hepatitis E^{IDC}, which had previously remained undiagnosed, as well as a true increase in incidence in recent time.

In conclusion, it is considered that these findings support the case for more widespread HEV testing according to clearly defined criteria and we propose an effective algorithm for this purpose. This is crucial not only for surveillance purposes and to clarify the epidemiology of HEV in UK, but also for the appropriate management of affected patients. In cases of acute hepatitis, where initial history and viral marker results are negative, autoimmune hepatitis, and idiosyncratic drug reactions are important to consider in the differential diagnosis, with implications for management and prognosis. Thus, in the absence of HEV testing, patients with unexplained raised transaminases may unnecessarily progress to liver biopsy, empirical trial of steroids, or withdrawal of presumed offending drugs. Consideration of HEV infection in individuals without travel-associated risk factors for acute hepatitis may have a major impact on clinical management.

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一般的名称	①乾燥抗HBs人免疫グロブリン ②ポリエチレングリコール処理抗HBs人免疫グロブリン	研究報告の公表状況	The New England Journal of Medicine 2008; 358: 811-817	公表国 フランス
販売名 （企業名）	①ヘブスブリン（ベネシス） ②静注用ヘブスブリンーIH（ベネシス）			

研究報告の概要

HEVは急性肝炎の原因となる病原体であって、慢性肝炎に進展することはないと考えられている。我々は、HEV急性肝炎の14症例を確認したが、3名の患者は肝臓、9名の患者は腎臓、2名は腎臓と膵臓を移植されていた。患者は全員、血清HEV RNAが陽性であった。8名の患者が慢性肝炎になり、確認はアミノトランスフェラーゼ値上昇の持続、血清HEV RNA、慢性肝炎の組織学的特徴によって行われた。移植から診断までの時間は極めて短く、リンパ球数並びにCD2、CD3及びCD4 T細胞の数は、慢性肝炎に進展した患者では著しく低かった。

使用上の注意記載状況・その他参考事項等

代表として静注用ヘブスブリンーIH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、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗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ろ過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
少なくとも免疫抑制剤を投与されている臓器移植患者においては、HEV感染が慢性肝炎に進展し得るとの報告である。 本剤からHEVが伝播したとの報告はない。万一、原料血漿にHEVが混入したとしても、EMCおよびCPVをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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BRIEF REPORT

Hepatitis E Virus and Chronic Hepatitis in Organ-Transplant Recipients

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SUMMARY

Hepatitis E virus (HEV) is considered an agent responsible for acute hepatitis that does not progress to chronic hepatitis. We identified 14 cases of acute HEV infection in three patients receiving liver transplants, nine receiving kidney transplants, and two receiving kidney and pancreas transplants. All patients were positive for serum HEV RNA. Chronic hepatitis developed in eight patients, as confirmed by persistently elevated aminotransferase levels, serum HEV RNA, and histologic features of chronic hepatitis. The time from transplantation to diagnosis was significantly shorter and the total counts of lymphocytes and of CD2, CD3, and CD4 T cells were significantly lower in patients in whom chronic disease developed.

ACUTE HEPATITIS CAUSED BY THE HEPATITIS E VIRUS (HEV) IS ENDEMIC IN developing countries and appears to be an emerging disease in industrialized countries.[1,2] Seroprevalence studies have reported anti-HEV IgG antibodies in 6 to 16% of renal-transplant recipients.[3,4] This hepatotropic RNA virus is often not fully considered or routinely sought in cases of acute hepatitis in recipients of solid-organ transplants. Only three cases of acute HEV infection have been reported in organ-transplant recipients.[5-7] Even though two cases of persistent HEV infection have been reported,[8,9] HEV is considered an agent responsible for acute hepatitis that does not become chronic.[10]

We report here 14 cases of acute hepatitis E infection in organ-transplant recipients. We suggest that HEV infection may evolve to chronic hepatitis in immunocompromised patients.

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PATIENTS AND METHODS

Between January 1, 2004, and December 31, 2006, all recipients of liver, kidney, or kidney and pancreas transplants attending our outpatient and inpatient clinics who presented with unexplained short-term elevations of liver-enzyme levels were screened for HEV infection by serologic and molecular tools. Patients chronically infected with hepatitis B, C, or D viruses were excluded from the study. Biliary-tract complications were ruled out by abdominal ultrasonography. Toxin- and drug-related causes of abnormal liver-function test results were ruled out by patient history. Fourteen of 217 patients (6.5%) tested positive for serum HEV RNA.

Anti-HEV status was determined by an enzyme immunoassay (HEV EIA, Abbott). HEV RNA in serum and stool was detected by real-time polymerase-chain-reaction (PCR) amplification (TaqMan, Applied Biosystems) of a 189-bp product located in the ORF2 region.[11] Strains were sequenced and compared with reference HEV strains (GenBank). The grades and stages of chronic hepatitis were assessed according to the Metavir classification.[12]

Proportions were compared by the chi-square test or Fisher's exact test. Quantitative variables were compared by the nonparametric Mann–Whitney, Friedman, and Wilcoxon tests. A P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

PREVALENCE OF ANTI-HEV IgG

All patients who received a kidney transplant (241 recipients) or a liver transplant (86 recipients) between January 1, 2004, and December 31, 2006, in the department of nephrology, dialysis, and multiorgan transplantation were screened for HEV infection at the time of transplantation. The prevalence of anti-HEV IgG was 13.5% for all recipients, 14.5% for kidney recipients, and 10.4% for liver recipients.

CLINICAL AND BIOLOGIC PRESENTATION

We identified 14 patients with a solid-organ transplant (3 liver recipients, 9 kidney recipients, and

Table 1. Demographic Features of Transplant Recipients at Diagnosis of Acute HEV Infection.*

Patient No.	Organ Transplanted	HEV Infection†	Donor‡	Years of Age	Sex	Mo since Transplantation	Initial Organ Disease	Induction Therapy	Immunosuppressive Therapy
1	Liver	Chronic	Cadaver	57	M	6	Alcoholic cirrhosis	None	Tacrolimus/mycophenolate mofetil/steroid
2	Liver	Chronic	Cadaver	67	M	53	Alcoholic cirrhosis	Basiliximab	Tacrolimus/mycophenolate mofetil/steroid
3	Liver	Chronic	Cadaver	28	F	10	Wilson's disease	None	Tacrolimus/mycophenolate mofetil/steroid
4	Kidney	Chronic	Cadaver	49	M	10	Thrombotic microangiopathy	Basiliximab	Mycophenolate mofetil/steroid
5	Kidney	Resolving	Cadaver	34	M	90	Malformative uropathy	Rabbit antithymocyte globulins	Everolimus/mycophenolate mofetil/steroid
6	Kidney	Resolving	Living	33	M	57	Interstitial nephropathy	Basiliximab	Sirolimus/mycophenolate sodium/steroid
7	Kidney	Chronic	Cadaver	52	M	63	IgA nephropathy	None	Sirolimus/steroid
8	Kidney	Resolving	Cadaver	42	M	168	Crescentic glomerulonephritis	Rabbit antithymocyte globulins	Cyclosporin A/mycophenolate mofetil
9	Kidney	Chronic	Cadaver	30	M	48	Alport's disease	Rabbit antithymocyte globulins	Sirolimus/steroid
10	Kidney	Resolving	Cadaver	51	M	67	Interstitial nephropathy	Rabbit antithymocyte globulins	Cyclosporin A/mycophenolate mofetil/steroid
11	Kidney	Resolving	Cadaver	62	F	108	Chronic glomerulonephritis	Rabbit antithymocyte globulins	Cyclosporin A/steroid
12	Kidney	Resolving	Cadaver	28	M	25	IgA nephropathy	Rabbit antithymocyte globulins	Tacrolimus/mycophenolate mofetil/steroid
13	Kidney and pancreas	Chronic	Cadaver	55	F	60	Diabetes mellitus	Rabbit antithymocyte globulins	Tacrolimus/azathioprine/steroid
14	Kidney and pancreas	Chronic	Cadaver	58	M	27	Diabetes mellitus	Rabbit antithymocyte globulins	Tacrolimus/mycophenolate mofetil

* All patients were born in France. HEV denotes hepatitis E virus.
† Resolving indicates clearance of HEV RNA from serum and stools, and chronic indicates persisting elevated liver-enzyme levels and detectable RNA in the serum or stools at least 6 months after the acute phase.
‡ Cadaveric donors had a heartbeat.

2 kidney and pancreas recipients) in whom acute HEV infection developed (Table 1). The acute hepatitis episode was asymptomatic in 7 of the 14 patients; these 7 patients were tested for HEV after liver-enzyme abnormalities were detected during routine biologic examinations that are performed every 3 to 4 months after organ transplantation. The seven other patients presented with fatigue, diffuse arthralgias, and myalgias that had evolved over a period of 1 to 2 weeks. One of the symptomatic patients also had marked weight loss (approximately 8 kg [18 lb] during the month before the presenting symptoms appeared) and was icteric. The symptoms disappeared within 2 weeks after diagnosis. No abnormalities were detected during physical examination of any other patient. No patients were febrile, and none had traveled outside France during the year before their hepatitis episode. Only two patients reported having been in contact with animals: one patient with chickens and rabbits and the other with birds. No patients had had an acute rejection episode after undergoing transplantation. Immunosuppressive therapy had remained unchanged in all patients for at least 6 months before their acute episode. Liver-enzyme levels were significantly higher than the levels 3 to 4 months before the diagnosis of HEV infection (Table 2).

DIAGNOSIS OF HEV INFECTION

At admission, classic causes of hepatitis were ruled out (Table 1 of the Supplementary Appendix, available with the full text of this article at www.nejm.org). The ferritin level was 567 ng per milliliter (range, 110 to 2007; normal range, 30 to 380), and the ceruloplasmin level was 0.35 ng per milliliter (range, 0.24 to 0.47; normal range, 0.20 to 0.45). At diagnosis, HEV RNA was detected in the serum of all patients and in the stool of the three patients whose stool was examined. PCR-amplification products of the serum HEV of 12 patients were sequenced and analyzed. Phylogenetic analysis revealed that all the strains belonged to genotype 3 (GenBank accession numbers, EU220992 to EU221003) (Fig. 1 of the Supplementary Appendix). We tried but failed to sequence the strains of the remaining two patients. No correlation was found between HEV RNA concentration and either liver-enzyme levels or liver-activity scores at diagnosis.

LIVER HISTOLOGIC FINDINGS DURING THE ACUTE PHASE

In the acute phase, 9 of the 14 patients underwent a liver biopsy to evaluate the severity of the acute episode of hepatitis; the remaining 5 patients declined biopsy. In liver-transplant recipients, liver biopsy also was performed to detect acute rejection. The mean (±SD) Metavir activity and fibrosis scores were 1.3±1.0 and 0.9±0.6, respectively (for assessment of disease activity, a Metavir score of 0 indicates no activity, 1 mild activity, 2 moderate activity, and 3 severe activity; for assessment of fibrosis, a Metavir score of 0 indicates no fibrosis, 1 portal fibrosis without septa, 2 a few septa, 3 numerous septa without cirrhosis, and 4 cirrhosis). The dominant lesions were lobular, with inflammation but no ballooning, and with spotty necrosis that included acidophilic bodies. The portal tract was mildly or moderately expanded and included an inflammatory infiltrate composed mainly of lymphocytes. Mild piecemeal necrosis was observed in six patients.

COURSE OF HEV INFECTION

Immunosuppressive therapy and target immunosuppressive trough levels were not modified after the diagnosis of HEV infection (data not shown). HEV infection resolved in six patients (43%); serum and stool HEV RNA in these patients became undetectable within 6 months after diagnosis and remained undetectable until the last follow-up at a mean of 12 months (range, 5 to 36) (Table 2). However, in the eight other patients (57%), HEV infection evolved to chronic hepatitis, as indicated by persistently elevated liver-enzyme levels and detectable HEV RNA in the serum or stool for a mean of 15 months (range, 10 to 24) after the acute phase.

Among the patients with resolving HEV infection, the levels of aspartate aminotransferase and alanine aminotransferase returned to preinfection values within 1 month (five patients) or 3 months (one patient) after diagnosis. The levels of γ-glutamyltransferase and alkaline phosphate returned to baseline levels within 3 months after diagnosis. Among those with chronic HEV infection, liver-enzyme levels remained above the upper limit of normal at the last follow-up. In both groups, the total bilirubin levels rapidly returned to preinfection levels. In both groups, hematologic and re-

Table 2. Liver Function in Patients with HEV Infection.

Patient No.	Time of Measurement	Alanine Aminotransferase*	Aspartate Aminotransferase†	γ-Glutamyl-transferase‡	Bilirubin§	Liver Biopsy	
			units/liter		*mg/dl*	*Metavir activity score*¶	*Metavir fibrosis score*‖
1	Baseline	10	16	18	584		
	Diagnosis	69	37	40	584	0	1
	15-Mo follow-up	59	41	30	409	3	2
2**	Baseline	102	95	1164	584		
	Diagnosis	248	229	3482	2339		
	16-Mo follow-up	59	54	173	701	1	3
3	Baseline	49	23	35	584		
	Diagnosis	169	76	76	994	1	1
	17-Mo follow-up	85	47	35	701	1	1
4	Baseline	26	12	19	701		
	Diagnosis	166	47	167	760	1	1
	15-Mo follow-up	135	57	146	760	3	1
5	Baseline	41	26	73	584		
	Diagnosis	66	47	118	526	0	1
	5-Mo follow-up	52	35	148	584		
6	Baseline	26	25	26	608		
	Diagnosis	245	104	118	468		
	12-Mo follow-up	30	32	18	584		
7	Baseline	26	18	55	397		
	Diagnosis	874	436	669	701	1	0
	10-Mo follow-up	158	89	156	584		
8	Baseline	32	24	32	1286		
	Diagnosis	770	340	373	2514		
	36-Mo follow-up	22	22	19	1169		
9	Baseline	42	39	26	584		
	Diagnosis	310	160	92	643	2	2
	24-Mo follow-up	90	39	42	760	2	2
10	Baseline	37	30	26	584		
	Diagnosis	518	235	459	1286		
	36-Mo follow-up	28	27	109	1286		
11	Baseline	23	18	42	351		
	Diagnosis	255	154	1055	3041	3	1
	12-Mo follow-up	13	7	80	351		
12	Baseline	12	14	19	368		
	Diagnosis	298	71	216	818	2	1
	5-Mo follow-up	15	24	51	877		
13	Baseline	13	22	8	643		
	Diagnosis	156	115	47	935	2	0
	15-Mo follow-up	298	238	79	760		

Table 2. (Continued.)

Patient No.	Time of Measurement	Alanine Aminotransferase*	Aspartate Aminotransferase†	γ-Glutamyl-transferase‡	Bilirubin§	Liver Biopsy	
			units/liter		*mg/dl*	*Metavir activity score*¶	*Metavir fibrosis score*‖
14	Baseline	14	23	30	1169		
	Diagnosis	143	106	132	877		
	13-Mo follow-up	126	118	585	994	1	3
Median							
	Baseline	26	23	32	584		
	Diagnosis††	248	115	167	818		
	Follow-up‡‡	59	40	79.5	731		

* Normal values for alanine aminotransferase range from 5 to 34 units per liter.
† Normal values for aspartate aminotransferase range from 3 to 30 units per liter.
‡ Normal values for γ-glutamyltransferase range from 7 to 38 units per liter.
§ To convert values for bilirubin to micromoles per liter, multiply by 17.1. Normal values range from 2 to 21 mg per deciliter.
¶ For assessment of disease activity, a Metavir score of 0 indicates no activity, 1 mild activity, 2 moderate activity, and 3 severe activity.
‖ For assessment of fibrosis, a Metavir score of 0 indicates no fibrosis, 1 portal fibrosis without septa, 2 a few septa, 3 numerous septa without cirrhosis, and 4 cirrhosis.
** Patient 2 had substantial alcohol consumption before the acute phase.
†† The differences between values at baseline and at diagnosis are significant for alanine aminotransferase, aspartate aminotransferase, and γ-glutamyltransferase (P=0.001) and for bilirubin (P=0.02).
‡‡ The differences between values at diagnosis and at last follow-up (median, 15 months) are significant for alanine aminotransferase (P=0.003), aspartate aminotransferase (P=0.02), and γ-glutamyltransferase (P=0.03).

nal measurements remained unchanged during the follow-up as compared with preinfection levels (data not shown). HEV seroconversion was observed in four patients with resolving HEV infection (two at 1 month and one each at 3 and 6 months after diagnosis) and seven patients with chronic infection (one at 3 months, two at 6 months, two at 12 months, and one each at 13 and 15 months after diagnosis).

Only six of the eight patients with chronic infection underwent a second liver biopsy (one at 10 months, two at 12 months, and one each at 13, 15, and 18 months after the diagnosis of acute HEV infection). The two remaining patients declined liver biopsy. The mean Metavir activity and fibrosis scores of the six patients who underwent biopsy were 2.0±1.0 and 1.8±0.8, respectively. All biopsy specimens showed features of chronic viral hepatitis, characterized by fibrosis and portal hepatitis, with dense lymphocytic infiltrate and variable degrees of piecemeal necrosis. Lobular hepatitis was mild to moderate in all cases. In the four patients who underwent a liver biopsy during both the acute phase and the chronic phase, the Metavir activity scores progressed from 1.0±0.8 to 2.2±0.9 and the fibrosis scores from 1.2±0.5 to 1.5±0.5.

RESOLVING VERSUS CHRONIC HEV INFECTION

During the acute phase, there were no significant differences between the patients with resolving HEV infection and those with chronic infection in median serum HEV RNA concentrations (5.97 $\log_{10}$ copies of RNA per milliliter [range, 5.79 to 6.44] and 6.18 $\log_{10}$ copies per milliliter [range, 4.92 to 7.28], respectively). There also were no significant differences between the groups in peak liver-enzyme levels. Hepatitis developed later after transplantation in patients with resolving HEV infection than in those in whom the infection progressed. Patients in whom chronic hepatitis developed had significantly lower serum creatinine levels at baseline and significantly lower counts of leukocytes, total lymphocytes, platelets, and CD2, CD3, and CD4 lymphocytes (Table 3). The percentages of patients who received induction therapy at transplantation or who received calcineurin inhibitors, mycophenolate mofetil or sodium, or inhibitors of the mammalian target of rapamycin (mTOR) were similar in the two groups. The dosage and trough levels of immunosuppressive drugs, as well as the proportions of patients with anti–hepatitis A virus, anticytomegalovirus, or IgG antibodies to Epstein–Barr virus, were similar in the two groups (data not shown).

Table 3. Patients with Resolving HEV Infection and Those in Whom the Infection Evolved to Chronic Hepatitis.

Variable	Patients with Resolving Infection (N=6)	Patients with Chronic Infection (N=8)	P Value
	median (range)		
At diagnosis			
Time since transplantation — mo	78.5 (25–168)	37.5 (6.0–63.0)	0.03
Leukocyte count — $\times 10^{-3}/mm^3$	8.85 (6–9.66)	4.31 (2.19–7.20)	0.004
Lymphocyte count — $\times 10^{-3}/mm^3$			
Total	1.73 (1.12–2.33)	0.75 (0.63–1.04)	0.004
CD2+	1.59 (0.84–2.25)	0.66 (0.58–0.92)	<0.001
CD3+	1.54 (0.70–1.88)	0.61 (0.49–0.79)	0.01
CD4+	0.93 (0.49–1.07)	0.22 (0.16–0.40)	0.004
Platelet count — $\times 10^{-3}/mm^3$	261 (190–285)	155.5 (75.0–250.0)	0.01
Serum creatinine — mg/dl*	2.15 (1.31–2.84)	1.33 (1.08–1.89)	0.01
At last follow-up			
Aspartate aminotransferase — IU/liter	25.5 (7–35)	55.5 (39.0–238.0)	0.002
Alanine aminotransferase — IU/liter	25 (13–45)	108.0 (59.0–298.0)	0.002

* To convert values for creatinine to micromoles per liter, multiply by 88.4.

DISCUSSION

HEV infection is transmitted by the fecal–oral route and may be a zoonosis in industrialized countries. It has a mortality rate of about 1% in the general population and 30% in pregnant women.[13] HEV-induced acute hepatitis may be fulminant,[14] but we are not aware that any cases of chronic hepatitis have previously been reported. Recently, the diagnosis of many cases of acute HEV hepatitis in nonimmunocompromised patients in southwest France[15] prompted us to look systematically for HEV in recipients of solid-organ transplants who had unexplained hepatitis. Of the 14 patients with acute HEV infection whom we report on here, 8 underwent progression to chronic hepatitis. In addition, in this issue of the *Journal*, Gérolami et al. report a case of HEV-related cirrhosis in a kidney-transplant recipient.[16]

After all other causes of hepatitis had been ruled out, the serum of 14 patients, none of whom had traveled outside France in the previous year, was found to be positive for HEV RNA. We did not identify any source of contamination. The peak aminotransferase levels were lower than in nonimmunocompromised patients.[17,18] Histologic lesions (mainly spotty lobular necrosis) that are characteristic of classic acute viral hepatitis were seen; these lesions were less severe than those typically seen in nonimmunocompromised patients. These findings could be related to the immunosuppressive therapy in transplant recipients.

HEV infection resolved in 6 of the 14 patients within 6 months after the end of the acute phase. In contrast, HEV infection in eight patients evolved to chronic hepatitis, as indicated by persistently elevated liver-enzyme levels and detectable serum HEV RNA at a median of 15 months (range, 10 to 24) after the end of the acute phase. Liver biopsies performed at a median of 12.5 months (range, 10 to 18) after the acute phase revealed signs of chronic viral hepatitis. The histologic lesions — dense lymphocytic portal infiltrate with constant piecemeal necrosis — were similar to those observed in patients chronically infected with hepatitis C virus. None of the patients received any specific therapy; in particular, none received antiviral therapy. Immunosuppressive therapy was not modified after the diagnosis of HEV. In the absence of available therapeutic recommendations for patients infected with HEV, we only performed close monitoring of liver-enzyme levels.

There were no significant differences between patients with resolving HEV infection and those with chronic HEV infection in demographic or clinical features, including treatment with immunosuppressive agents before the acute phase. However, the immunologic status of the patients may have had a role in the evolution to chronic dis-

ease. In patients in whom the infection became chronic, the time from transplantation to the development of infection was significantly shorter — and consequently, the total lymphocyte counts and the CD2, CD3, and CD4 lymphocyte counts were significantly lower — than in patients in whom HEV infection resolved. Hence, the T-cell response seems to have a role in HEV clearance, as does the B-cell response.

HEV seroconversion occurred later in patients with chronic infection than in those with resolving infection. This difference may be related to the reduction in the humoral immune response caused by treatment with mycophenolate, inhibitors of mTOR, or both. These drugs are known to decrease the synthesis of antibodies[19,20] and to inhibit the cell-cycle progression and differentiation of human B lymphocytes.[21] The humoral immune response is necessary to clear HEV and to prevent hepatitis. Bryan et al. have shown that antibodies to the HEV capsid can be protective against hepatitis E.[22] Passive immunoprophylaxis studies in cynomolgus monkeys have confirmed that the antibody to the HEV capsid may prevent HEV infection in humans.[23] Recently an HEV recombinant protein vaccine was found to be effective in preventing HEV infection.[24]

Further studies are required to determine the incidence of chronic HEV infection in transplant recipients who live in areas where the disease is not endemic. Vaccination against HEV could be proposed to patients before or after organ transplantation. However, the efficacy of vaccination in these populations should be addressed.

In conclusion, our data suggest that HEV should be considered an etiologic agent of hepatitis in organ-transplant recipients. We have demonstrated that HEV infection can evolve to chronic hepatitis, at least in organ-transplant recipients. A longer follow-up is required to assess the outcome of HEV infection in organ-transplant recipients.

No potential conflict of interest relevant to this article was reported.

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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	機構処理欄
				2008. 3. 18	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Barin F, Cazein F, Lot F, Pillonel J, Brunet S, Thierry D, Damond F, Brun-Vézinet F, Desenclos JC, Semaille C. AIDS. 2007 Nov 12;21(17):2351-3.	公表国 フランス
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			

研究報告の概要

○フランスの新規HIV診断例におけるHIV-2、HIV-1のグループO型の感染率:2003～2006年
新規HIV診断例のHIV血清型を同定するため、フランス国内で調査用に採取された乾燥血清spotsを用いた。2003年1月～2006年6月に、10,184の新規診断例が報告された。HIV-2、HIV-1のグループO型感染の割合は、それぞれ1.8、0.1%であった。これら症例のほとんどは、異性との接触により感染した流行地域出身の患者であった。HIV-2感染の3症例は、男性との性交渉を持つ非アフリカ系男性で報告された。

報告企業の意見	今後の対応
2003年1月～2006年6月に、フランスにおいて10,184の新規HIV診断例が報告され、HIV-2、HIV-1のグループO型感染の割合は、それぞれ1.8、0.1%であったとの報告である。	日本赤十字社では、HIV抗体検査に加えて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。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)の導入を順次進めている。さらに、HIV-2及びHIVグループOの検出が可能な次世代NATの導入に向けた準備を進めている。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

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

Prevalence of HIV-2 and HIV-1 group O infections among new HIV diagnoses in France: 2003–2006

Francis Barin[a], Françoise Cazein[b], Florence Lot[b], Josiane Pillonel[b], Sylvie Brunet[a], Damien Thierry[a], Florence Damond[c], Françoise Brun-Vézinet[c], Jean-Claude Desenclos[b] and Caroline Semaille[b]

French national surveillance of new HIV diagnoses included the collection of dried serum spots to identify HIV serotypes. Between January 2003 and June 2006, 10 184 new diagnoses were reported. The proportions of HIV-2 and HIV-1 group O infections were 1.8 and 0.1%, respectively. Most of these cases occurred in patients infected through heterosexual contact and originated from the corresponding endemic areas. Three cases of HIV-2 infections were reported in non-African men having sex with men.

HIV-2, first suspected by serological findings in west African residents, was isolated from patients with AIDS originating from Cape Verde and Guinea Bissau [1,2]. Although HIV-2 causes AIDS, it is clearly less pathogenic than HIV-1 [3,4]. The viral load is significantly lower in HIV-2-infected patients, and consequently HIV-2 is less transmissible [5,6]. The precise diagnosis of HIV-2 has implications, particularly for monitoring RNA levels, as no specifically dedicated commercial assays are currently available, and for the choice of antiretroviral treatment, because HIV-2 strains are naturally resistant to non-nucleoside reverse transcriptase inhibitors and fusion inhibitors, and are less sensitive *in vitro* to some protease inhibitors [7,8]. HIV-2 is endemic in west Africa. Most cases described outside Africa have been traced to contacts with individuals from this endemic region. This has been particularly observed in European countries with historical links with west Africa such as France, the United Kingdom and Portugal [9–11]. No extensive epidemiological surveys have, however, allowed the determination of the exact prevalence of HIV-2 in these European countries. Similarly, HIV-1 group O variants are restricted geographically, mainly to Cameroon and the surrounding areas [12]. Rare cases have been reported in industrialized countries, but the exact prevalence of these variants among HIV-1-infected patients is unknown. Similar to HIV-2, most of the commercially available assays for the quantification of HIV-1 RNA do not detect viral sequences from HIV-1 group O variants [13], and non-nucleoside reverse transcriptase inhibitors are inefficient at controlling HIV-1 group O replication [14].

Mandatory anonymous HIV case reporting was implemented in France in 2003, with which virological monitoring using dried serum spots was associated. The procedures and the first results of this surveillance system have been described elsewhere [15]. In brief, any HIV-positive serology confirmed for the first time by a clinical laboratory must be reported, with a unique anonymous code for each patient. Clinical and epidemiological details are supplied by the physicians in charge of the patients. For each case, the laboratory is asked to send dried serum spots collected on filter papers from the serum sample obtained for the original diagnosis to the National Reference Centre (NRC). Although HIV notification is mandatory, virological surveillance is based on volunteer participation by both microbiologists and patients. The patient's consent for virological surveillance is obtained by the reporting clinician through the HIV notification form. Serological identification of the type and group of HIV is performed by enzyme-linked immunosorbent assay at the NRC, as described [16]. Results from the NRC are then linked to the epidemiological data in the HIV national database using the patient's anonymous code. Any specific diagnosis of infection by either HIV-2 or HIV-1 group O implies transmission of the information to the clinical laboratory of origin in order to adapt the clinical, biological and therapeutic management of the patient.

Here we report the results of the HIV-2 and HIV-1 group O infections that were identified among new HIV diagnoses during the past 3 years. Between January 2003 and June 2006, 10 184 new diagnoses with participation in the virological surveillance were reported. Among these, 186 were from patients infected by HIV-2 [1.8%; 95% confidence interval (CI) 1.6–2.1], of which 164 (1.6%; 95% CI 1.4–1.9) were HIV-2 only and 22 (0.2%; 95% CI 0.1–0.3) were probable dual infections. The serological diagnosis of dual infection was based on similar high antibody binding to both the immunodominant epitope of gp41 and the V3 region of both HIV-1 and HIV-2 [16,17]. Such a stringent criteria was validated earlier [17], and more recently on a panel of samples for which single or dual infections were diagnosed by type-specific polymerase chain reaction (data not shown). Patients infected with HIV-2 were mostly citizens of a west African country (65%; $n=121$), mainly Côte d'Ivoire ($n=64$), Mali ($n=19$) and Senegal ($n=12$), but there were also 22 European individuals, 20 from France and two from Portugal (Fig. 1). The majority of cases was observed in women (63%; $n=118$). Although the risk factor was unknown for 26% ($n=48$) of cases, 72% ($n=134$) of HIV-2 infections were caused by heterosexual transmission. HIV-2 was, however, identified in three men who have sex with men (MSM), one from France and two from the Americas.

Twelve patients (0.1%; 95% CI 0.1–0.2) were infected with HIV-1 group O variants. Most of them originated from the sub-Saharan endemic area: nine from Cameroon and one from Chad (Fig. 1). Two of those patients had dual M/O infection; those two cases have been described in detail earlier [18]. The two other cases were French

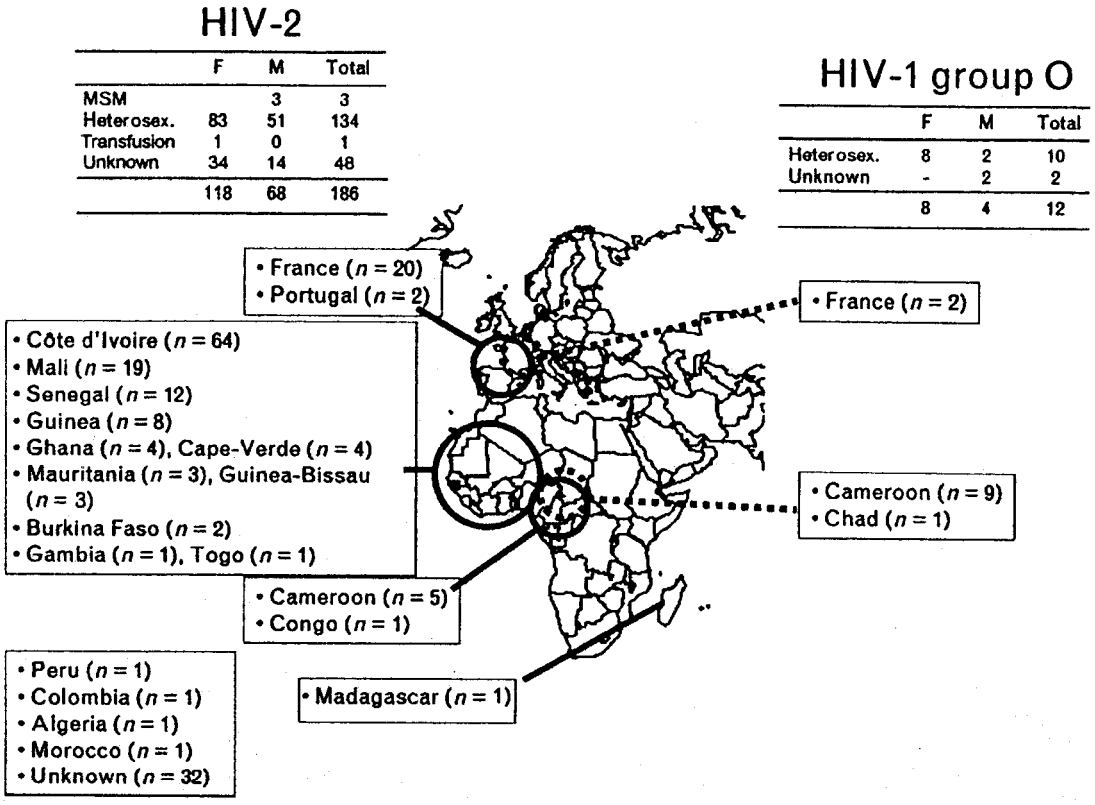

Fig. 1. Nationality and mode of transmission of patients identified as infected by HIV-2 (left) and HIV-1 group O (right) in France, 2003–2006. F, Female; Heterosex., heterosexual transmission; MSM, men who have sex with men; M, male.

citizens who had probably been infected through heterosexual intercourse.

A specific serological diagnosis of HIV-2 infection may be missed if adapted confirmation tools are not routinely used in clinical laboratories, a situation that is frequent in non-endemic areas. There is a frequent use of HIV-1 Western blots for confirmatory diagnosis, on which serum samples positive for antibodies to HIV-2 may cross-react, even on envelope glycoproteins, leading to a misclassification as anti-HIV-1 positives [19]. Similarly, HIV-1 group O infections are not systematically diagnosed as such, except if there are dissociations between clinical and biological findings in an HIV-1-positive patient; for example, AIDS stage with undetectable viral load. This is because there is no commercially available specific serological tool for this purpose. Therefore, there are no data that would provide estimates of the prevalence of these rare variants in western countries. The French national surveillance of new HIV diagnoses included the collection of dried serum spots to identify HIV serotypes with dedicated peptide immunoassays [16,17]. This allowed, for the first time, the provision of reliable estimates of the proportion of these rare variants in a European country. The results indicate that most of the cases diagnosed during this 3-year period still occurred in patients originating from the endemic areas, west Africa and Cameroon, for HIV-2 and HIV-1 group O, respectively. Three cases of HIV-2 infections were, however, reported in MSM, an observation that should deserve further attention because of the persistent high-risk behaviours in some individuals in the gay community.

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別紙様式第2-1

No. 42

医薬品 研究報告 調査報告書

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販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			

研究報告の概要

○1999～2006年の長崎における日本人献血者ヒトT細胞向性ウイルス1型 (HTLV-1) 感染率の傾向

HTLV-1の流行地域である日本の長崎では、1986年から献血者のルーチンの血清スクリーニング検査、1987年からウイルスキャリアの母親からの乳汁媒介伝播予防のために長崎県ATL ウイルス母子感染防止研究協力事業(APP)が実施されている。本試験では、1999年1月～2006年12月に献血を行った初回献血者の年齢別、出生年別、および期間別HTLV-1血清陽性率の傾向分析を行った。初回献血者55,668名(献血時年齢16～65歳、出生年1934～1990)のうち、718名はHTLV-1検査陽性であり、全体的な血清陽性率は1.29% (95%CI, 1.20-1.39)であった。陽性率は男性よりも女性の方が高かった(1.53%vs.1.13%; OR; 1.36, 95%CI; 1.17-1.57)。血清陽性率は、年齢が高くなるにつれ有意に上昇し、献血時年齢16～25歳では0.70%、献血時年齢56歳以上では7.34% (χ2乗検定、P<0.0001)であった。年間感染率は1999年が1.32、2002年が1.31、2006年が1.37であり、期間中に有意な経年傾向がないことが示された(P for trend=0.99)。献血時年齢の解析では、陽性率は56歳以上(P for trend=0.02) と16～25歳 (P for trend=0.0007)では有意に減少したが、出生年別解析では、1981～90年出生群で1999年の1.22%から2006年の0.44%へ減少(P for trend<0.0001)したことを除き、陽性率の経時的変化は見られなかった。1985～90年出生群の解析では、血清陽性率は1985～86年出生群の0.75% 、1987～88年出生群の0.31%から1989～90年出生群の0%に減少した(P for trend=0.0002)。

HTLV-1血清陽性率は、APP開始後の1987～90年に生まれた献血者では、1985～86年に生まれた献血者と比較して有意に低かった。以上の結果は、ほとんどのウイルス伝播が幼児期に起こるため、HTLV-1陽性率の出生年解析が経年傾向の評価に適切であると考えられることを示し、ウイルスキャリアの母親の授乳を避けることを指導した県をあげての対応が、当該地域のHTLV-1血清陽性率の減少に貢献していることを示している。

報告企業の意見	今後の対応
1999～2006年の長崎における献血者のヒトT細胞向性ウイルス1型感染率は、1987～90年に生まれた献血者では1985～86年に生まれた献血者と比較して有意に低く、ウイルスキャリアの母親の授乳を避けることを指導した県をあげての対応が陽性率の低下に貢献していることが示されたとの報告である。	日本赤十字社では、HTLV-1のスクリーニング検査を行っている。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

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

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

(二)

Basic Science and Clinical Practice in Blood Transfusion

Basic Science and Clinical Practice in Blood Transfusion

Trend in Prevalence of Human T-Lymphotropic Virus Type-1 (HTLV-1) Infection in Japanese Blood Donors, Nagasaki, 1999 to 2006.

Masako Iwanaga, MD, MPH[1,*], Shin Chiyoda, MD[2,*], Eisuke Kusaba, MD[3,*], Shimeru Kamihira, MD[4,*] (Intr. by Yasuaki Yamada)

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Abstract

To aluate time-trend of HTLV-1 prevalence and the effect of preventative measure against the viral transmission are important in the virus endemic regions. In Nagasaki, Japan, an endemic area of HTLV-1, a routine serological virus screening for blood donors and a prefecture-wide intervention project (the ATL Prevention Program; APP) to prevent milk-borne transmission for the virus carrier mothers have been conducted since 1986 and 1987, respectively. However, the effects of both projects on the virus seroprevalence have not been well evaluated. In this study, we conducted trend analyses of age-specific, birth-year-specific, and period-specific seroprevalence of HTLV-1 for first-time blood donors who donated between January 1999 and December 2006. Among 55668 first-time donors (age at donation; 16–65 years, birth year; 1934–1990), 718 were test positive for HTLV-1, indicating that the overall seroprevalence was1.29% (95%CI, 1.20–1.39). Prevalence was significantly higher in women than men (1.53% vs. 1.13%; OR; 1.36, 95%CI; 1.17–1.57). Seroprevalence increased significantly with increasing age at donation from 0.70% at 16–25 years to 7.34% at over 56 years (Chi-square test, $P < 0.0001$). The annual prevalence was 1.32 in 1999, 1.31 in 2002, and 1.37 in 2006, indicating that there was no significant secular trend during 1999–2006 (P for trend=0.99). In analyses by age at donation, trends of HTLV-1 prevalence significantly de ied among age over 56 years (P for trend=0.02) and age 16–25 years (P for trend=0.0007), whereas in birth-year-specific analyses, there was no apparent change of the prevalence over time, except in birth year 1981–90 group in which the prevalence declined from 1.22% in 1999 to 0.44% in 2006 (P for trend < 0.0001). In analyses for limited birth year from 1985 to 1990, the seroprevalence declined from 0.75% in birth year 1985–86 group, 0.31% in 1987–88 group, to zero% in 1989–90 group (P for trend =0.0002). HTLV-1 seroprevalence was significantly lower among donors born in 1987–90 (after APP) than 1985–86 (before APP). These results indicate that a birth-year-specific analysis for HTLV-1 prevalence may be appropriate to evaluate secular trend since the virus mostly transmit during infancy, and that a prefecture-wide intervention, the refraining from breast-feeding by the virus carrier mothers, contributes a declining HTLV-1 seroprevalence in our region.

Footnotes

Disclosure: No relevant conflicts of interest to declare.

別紙様式第2-1

No. 28

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	機構処理欄
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一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		AABB Weekly Report. 2008 Feb 29.	米国

研究報告の概要

○インフルエンザパンデミックと血液供給に関するAABBの組織横断作業部会がパンデミック時に献血間隔の例外的な取り扱いを認めるようFDAに要求

インフルエンザパンデミックと血液供給に関するAABBの組織横断作業部会が、2月14日付で米国食品医薬品局に送付した文書である。

パンデミック時には、供血者が発症したり家族を看病したりするために、基準に合致する供血者の数が少なくなり、血液の安定供給に影響するという懸念が広がっている。

作業部会は、全血と赤血球採血の献血間隔を半分に短縮(8週間のところを4週間、16週間のところを8週間)することを提案した。これはパンデミック時の血液供給の問題を最小限にするためにFDAが取りうる手段としては最も効果的であると主張している。

また、作業部会の前回のミーティングでは、過去6ヵ月以内に血液が使用された供血者については、感染症検査の前に供給するという方法が紹介された。これに関してのFDAの意見を求めている。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

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
インフルエンザパンデミックと血液供給に関するAABBの組織横断作業部会はパンデミック時に献血間隔の例外的な取り扱いを認めるようFDAに求めているとの報告である。	日本赤十字社では家禽に高病原性トリインフルエンザの流行が認められた場合、当該飼養農場の関係者や防疫作業従事者の献血制限を行っている。新型インフルエンザが流行した場合、献血者減少につながることも予想される。今後も引き続き情報の収集に努める。

(2)

February 29, 2008 | Vol. 14 | No. 8

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IN THIS ISSUE

Advance Registration for 2008 Spring Conference Ends March 7

Only one week remains to register in advance for the 2008 AABB Spring Conference. After March 7, individuals who have not signed up for the conference can still attend but must register on-site. The conference, which will be held March 28-29 in Orlando, Fla., features educational sessions in four tracks — Blood Inventory Management, Cellular Therapy, Perioperative Blood Management and Tissue Management. Registration includes entrance to educational sessions in any of the four tracks, course materials, continental breakfast and access to the exhibits.

AABB Task Force Asks FDA for Exception to Interdonation Interval During Influenza Pandemic

The AABB Interorganizational Task Force on Influenza Pandemics and the Blood Supply sent a letter to the Food and Drug Administration on Feb. 14 asking the agency to review a template to be used by blood facilities to request an exception to the interdonation interval requirements in the event of an influenza pandemic. According to the letter, there is widespread concern that a pandemic would severely impact the availability of blood products by limiting the number of eligible donors. The task force stated that shortening the interdonation interval for whole blood and red blood cell collection is the most significant step the agency can take to minimize supply issues during a pandemic.

Report Finds Alternatives for Blood Irradiators, Other Radiation Sources to Improve National Security

EventCalendar

March 3-6 – The AIIM International Exposition & Conference read more »

March 4-5 – International Plasma Protein Congress read more »

March 5 – AABB Audioconference: Hematopoietic Cell Donation: Ensuring Safety for the Donor, Product and Recipient CT read more »

March 5-6 – Southeastern Area Blood Bankers Annual Meeting read more »

March 6-7 – President's Council on Bioethics Meeting read more »

March 19 – AABB Audioconference: Serological to Molecular Testing: Points to Consider for Successful Conversion read more »

Full Calendar read more »

New Web Resources

Updates to the Variances for Collection of Blood and Blood

Letter to the FDA on a Draft Template for a Shorter Inter-donation Interval During an Influenza Pandemic – 2/14/08

Alan Williams, PhD
Associate Director for Regulatory Affairs (HFM-300)
Food and Drug Administration
Center for Biologics Research and Review
Woodmont Office Center, Suite 400N
1401 Rockville Pike
Rockville, MD 20852

Dear Dr. Williams:

The AABB Interorganizational Task Force on Influenza Pandemics and the Blood Supply appreciates the Food and Drug Administration's (FDA) assistance in working to ensure that patients' blood needs can be met in the event of a severe influenza pandemic. As we have discussed, there is widespread concern that a severe pandemic will severely impact the availability of blood products as individuals who are sick or caring for sick loved ones will not be able to donate.

In an effort to prevent critical blood shortages, the Task Force has developed a template to request an exception for a shorter inter-donation interval for Whole Blood and Red Blood Cell donations in the event of a pandemic. Experience with donors with hemochromatosis has sho that donors can tolerate phlebotomy much more frequently than the standard eight-week requirement, so long as they have acceptable hemoglobin levels. The Task Force believes that allowance for a shorter inter-donation interval is the most significant step that FDA can take to alleviate shortages during a pandemic. We are asking FDA to acknowledge that the language proposed in the attached draft template is appropriate so that the Task Force can distribute it to blood collection facilities working to prepare for a severe pandemic.

In addition, during the last meeting of the Task Force, the concept of a "walking donor" pool – i.e., allowing blood collected from individuals who had successfully donated in the previous six months to be distributed prior to infectious disease testing, which would be completed subsequently – was introduced. The Task Force would welcome your expansion on how you see such an approach being applied. Would the donor examination or history be amended or truncated? Would testing requirements be relaxed? Please let us know your thoughts.

Thank you for your efforts to address blood supply issues relating to a possible influenza pandemic. The Task Force looks forward to your response to the above requests.

Sincerely,

Louis Katz, MD
Chair

Attachment

Cc: Elizabeth Callaghan

aft Request for Exception to Inter-Donation Interval in the Event of an Influenza Pandemic

Director, Division of Blood Applications (HFM-375)
Food and Drug Administration
Center for Biologics Research and Review
C/O Document Control Center (HFM-99)
Woodmont Office Center, Suite 200N
1401 Rockville Pike
Rockville, MD 20852-1448

n: (Blood Center/Bank Name)
(Mailing Address)
(License Number, if licensed, or Registration Number, if registered)

Request for Exception to Inter-Donation Interval in the Event of an Influenza Pandemic

uest: Our establishment requests an exception under 21 CFR 640.120 to allow, in the event of an influenza pandemic:

1. Single unit collection of Whole Blood and Red Blood Cells (RBCs) from donors who had donated a single unit less than eight weeks but at least four weeks previously, or a double unit of RBCs less than 16 weeks but at least eight weeks previously, provided the donor meets the other donor eligibility criteria in 21 CFR 640.3(b) and 640.3(c).
2. Double unit collection of Whole Blood and Red Blood Cells from donors who had donated less than sixteen weeks but at least eight weeks previously, provided the donor meets the other donor eligibility criteria in 21 CFR 640.3(b) and 640.3(c).

tification: There is widespread concern in the transfusion medicine community that a pandemic could severely impact the availability of od products in the United States since individuals who are sick or caring for sick loved ones will not be able to donate. This exception uld be granted to enhance the adequacy of the blood supply in the event of an influenza pandemic in the United States that the AABB rorganizational Task Force on Domestic Disasters and Acts of Terrorism, with the concurrence of FDA, declares has or will negatively ct the blood supply, either at a regional or national level.

re is substantial evidence that donors can tolerate a more frequent inter-donation frequency, as evidenced by the FDA guidance, *idance for Industry: Variance for Blood Collection from Individuals with Hereditary Hemochromatosis*", August 2001.

cerely,

sponsible Person

別紙様式第2-1

No. 16

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	機構処理欄
				2008. 2. 18	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	Robinson JL, Lee BE, Patel J, Bastien N, Grimsrud K, Seal RF, King R, Marshall F, Li Y. Emerg Infect Dis. 2007 Dec;13(12):1865-70.	公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)			カナダ

研究報告の概要

○幼児におけるブタインフルエンザ(H3N2)感染および市中伝播の可能性:カナダ
ブタ起源と思われるインフルエンザ(H3N2)ウイルス(A/Canada/1158/2006)が、カナダの共同農場に居住する、7ヶ月齢の入院患児より分離された。当該患児は無事に回復した。A/Canada/1158/2006の赤血球凝集抑制反応を利用した血清学的検査を当該農場の農民90名のうち54名に実施した。初発患者およびその家族7名中4名、家族以外の農民46名中4名に血清陽性が示された;この前年に呼吸器疾患による入院歴を持つ者はいなかった。当該ブタインフルエンザ株の血清エビデンスは、当該農場のブタ10頭(12週齢～6ヶ月齢)中1頭に認められた。カナダではブタインフルエンザウイルスのヒトへの感染は、過少に評価されており、ウイルス株は農場に順応または再集合が可能であり、その結果、効率的なヒト-ヒト伝播にいたることから、パンデミックインフルエンザ対策の一環として、養豚業従事者の定期的な調査を検討すべきである。

報告企業の意見	今後の対応
ブタ起源と思われるインフルエンザH3N2ウイルスが、カナダの共同農場に居住する7ヶ月齢の入院患児より分離され、患児の家族7名中4名、家族以外の農民46名中4名と、当該農場のブタ10頭中1頭に血清陽性が認められたとの報告である。	新型インフルエンザが流行した場合、献血者減少につながることも予想される。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「日赤」
新鮮凍結血漿-LR「日赤」

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

(13)

Swine Influenza (H3N2) Infection in a Child and Possible Community Transmission, Canada

Joan L. Robinson,*† Bonita E. Lee,* Jagdish Patel,‡ Nathalie Bastien,§ Karen Grimsrud,¶ Robert F. Seal,† Robin King,‡ Frank Marshall,# and Yan Li†§

An influenza A virus (H3N2) of probable swine origin, designated A/Canada/1158/2006, was isolated from a 7-month-old hospitalized child who lived on a communal farm in Canada. The child recovered uneventfully. A serosurvey that used a hemagglutination-inhibition assay for A/Canada/1158/2006 was conducted on 54 of the 90 members of the farm. Seropositivity was demonstrated in the index patient, 4 of 7 household members, and 4 of 46 nonhousehold members; none had a history of hospital admission for respiratory illness in the preceding year. Serologic evidence for this strain of swine influenza was also found in 1 of 10 pigs (12 weeks–6 months of age) on the farm. Human infection with swine influenza virus is underrecognized in Canada, and because viral strains could adapt or reassort into a form that results in efficient human-to-human transmission, routine surveillance of swine workers should be considered as part of pandemic influenza preparedness.

Influenza A is endemic in a broad range of species, with avian and swine strains having the greatest potential for transmission to humans. Pandemics of influenza A occur when a major change occurs in the proteins of circulating strains of the virus. During the pandemics of the past century, this antigenic shift resulted from reassortment of human and avian strains or adaptation of avian viruses to facilitate person-to-person transmission (*1*). Avian influenza preferentially binds to sialic acid–galactose receptors with an α-2,3 linkage that is abundant on duck intestinal epithelium; human influenza preferentially binds to sialic acid–galactose receptors with an α-2,6 linkage that is abundant on human respiratory epithelium. The respiratory epithelium of swine contains both types of receptors and can potentially be simultaneously infected with avian and human influenza (*2*). Human infection with avian influenza subtype H5N1 is of great concern, with 194 deaths of 321 cases reported worldwide through August 16, 2007 (*3*). Swine infected with avian subtype H5N1 have been identified in Vietnam (*4*), raising the possibility that swine could act as the "mixing vessel" that allows avian influenza (H5N1) to reassort with a human influenza strain, resulting in a virus with high pathogenicity and a high potential for person-to-person spread.

Another theoretical mechanism for the origin of an influenza pandemic would be the adaptation of a swine strain that results in efficient person-to-person transmission, although cross-protection by antibodies to recently circulating human strains may prevent this from occurring with swine influenza virus (SIV) H1 and H3 strains. Infection of humans with SIV was first recognized in 1974 with an H1N1 strain (*5*); the solitary outbreak occurred in military recruits at Fort Dix, New Jersey, USA, in 1976 (*6*). Human infection with SIV subtype H3N2 was first described in Europe in 1993 (*7*). The first reported case of probable infection of a person in North America with a non-H1N1 subtype of SIV occurred in Ontario, Canada, in 2005 with an H3N2 strain detected in the respiratory tract of an adult with no serologic evidence of infection (*8*). We describe a case of SIV (H3N2) infection in a Canadian infant, confirmed by viral isolation and serologic testing.

*Public Health and Provincial Laboratory (Microbiology), Edmonton, Alberta, Canada; †University of Alberta, Edmonton, Alberta, Canada; ‡Alberta Agriculture and Food, Edmonton, Alberta, Canada; §National Medical Laboratory, Winnipeg, Manitoba, Canada; ¶Alberta Health and Wellness, Edmonton, Alberta, Canada; and #Marshall Swine Health Service, Camrose, Alberta, Canada

Case Report

A 7-month-old boy was admitted to the hospital on September 10, 2006, with a 3-day history of fever, rhinitis, and cough. He had had no previous contact with ill persons. The child was born at term and was hospitalized for 21 days at 5 weeks of age when he received ventilation for 6 days for pneumonia due to respiratory syncytial virus. He lived on a communal farm (90 occupants) with horses, cows, swine, sheep, dogs, cats, turkeys, geese, ducks, and chickens but had no direct contact with the animals. The swine were contained in barns and did not mix with the other animals. His household contacts did not work directly with animals, but his father occasionally spent time in the barns, and his uncle, who lived next door, worked in the swine barns.

On admission, the child was afebrile with a heart rate of 120 beats/min, respiratory rate 56/min, and oxygen saturation of 85% on room air. Diffuse wheeze was noted. Chest radiograph results were unremarkable. Direct fluorescent antibody testing on a nasopharyngeal aspirate was positive for influenza A, and the virus was isolated in rhesus monkey cell culture. The isolate was sent to the National Microbiology Laboratory for influenza subtyping as a requirement of the Canadian influenza surveillance program, where it was subsequently designated A/Canada/1158/2006. The child stayed in the hospital for 2 days and then made an uneventful recovery at home. A cough and rhinitis developed in his 19-month-old brother on the day the index patient was admitted to the hospital, but the brother was not assessed by a physician.

Methods

Antigenic Analysis

For the antigenic characterization of A/Canada/1158/2006, hemagglutination-inhibition (HI) assay was performed by using 4 hemagglutination units of virus, 0.7% v/v guinea pig erythrocytes, and postinfection fowl serum specimens for the currently circulating human strains (A/New Caledonia/20/99 [H1N1], A/Wisconsin/67/2005 [H3N2]), past circulating human strains (A/Panama/2007/99 and A/Nanchang/933/95), and swine serum for A/Swine/Texas/4199–2/98 (H3N2) treated with receptor-destroying enzyme (*9*).

Molecular Characterization

All 8 RNA segments of A/Canada/1158/2006 were amplified by reverse transcriptase–PCR (RT-PCR) and sequenced. A universal primer set for the full-length amplification of all influenza A viruses was used for the RT-PCR (*10*). Viral RNA was extracted from 100 μL of tissue culture fluid with the RNeasy Mini Kit (QIAGEN, Mississauga, Ontario, Canada). Viral RNA was amplified in a OneStep RT-PCR reaction (QIAGEN) following the manufacturer's recommendations. Briefly, 5 μL RNA was added to the RT-PCR mixture containing 2 μL QIAGEN OneStep RT-PCR enzyme mix, 10 μL 5× QIAGEN OneStep RT-PCR buffer, 400 μmol/L dNTP, 0.6 μmol/L of each primer, and 10 μL Q-solution in a final volume of 50 μL. The conditions used for the Gene Amp 97700 (Applied Biosystems, Streetsville, Ontario, Canada) thermocycler were as follows: 50°C for 30 min for reverse transcription, 95°C for 15 min for the activation of the HotStart DNA polymerase; then 35 cycles of 94°C for 20 s, 58°C for 30 s, 72°C for 4 min, followed by an extension of 10 min at 72°C. The PCR products were purified by using QIAquick PCR purification kit (QIAGEN) and sequenced on an ABI 377 Sequencer, using a fluorescent dye-terminator kit (Applied Biosystems). The DNA sequences were assembled and analyzed with SEQMAN, EDITSEQ, and MEGALIGN programs in Lasergene (DNASTAR, Madison, WI, USA). Phylogenetic trees were generated by the neighbor-joining method using the MEGA program (*11*).

Serologic Testing

Once it became evident that A/Canada/1158/2006 was closely related to swine influenza viruses, HI was performed on serum specimens collected from the index patient, the symptomatic sibling, and both parents 29 days after the hospitalization. To further investigate the spread of SIV to humans, approval was then granted by the Health Research Ethics Board of the University of Alberta to obtain information and serum specimens from other members of the communal farm. The study team visited the farm 3 months after the hospitalization of the index patient and explained the study to the occupants. Serum specimens were then collected from the other 4 siblings of the index patient and 46 other occupants who lived in a total of 17 households. Participants provided the following data: age, exposure to swine (none, <1 hour/week, or ≥1 hour/week), and history of influenza-like illnesses (ILI; defined as cough and fever) in the preceding year. Serum samples were tested by using an HI assay against the currently circulating human strains A/New Caledonia/20/99 (H1N1), A/Wisconsin/67/2005 (H3N2), and the isolate from the index patient, A/Canada/1158/2006. HI titers were defined as the reciprocal of the highest dilution of serum that completely inhibited hemagglutination of a 0.7% solution of guinea pig erythrocytes. Specimens were considered seropositive for influenza virus at a titer of ≥32.

Swine Investigation

The purpose of these investigations was to determine the extent of recent swine influenza in swine on the farm and to look for evidence of infection with the SIV strain isolated from the index child. The history of influenza or unexpected respiratory illness in the swine on the farm was

obtained. Nasal swabs were obtained from grower pigs (4 to 16 weeks of age) and processed by RT-PCR for influenza A matrix gene. Serologic testing for influenza, using an ELISA for H1N1 and H3N2 strains and HI for A/Canada/1158/06, was performed on samples from grower-finisher pigs (12 weeks to 6 months of age). Five grower pigs that were doing poorly were killed and pulmonary autopsies were performed. All swine used in these investigations were on the farm at the time the index child was ill.

Results

Antigenic and Molecular Characterization of A/Canada/1158/06

Initial HI testing showed that the isolate was not inhibited by antiserum against recent (A/Wisconsin/77/2005 and A/New Caledonia/20/99) and past (A/Panama/2007/99 and A/Nanchang/933/95) human influenza A strains but was inhibited by antiserum against A/swine/Texas/4199–2/98 (H3N2) virus with HI titer of 128. These findings indicate that the A/Canada/1158/06 virus was antigenically related to SIV (Table 1). The results also indicate that the assay is specific because no cross-reactivity was observed between the human reference strain antiserum and the swine influenza viruses (Table 1). Nucleotide sequences of the full-length coding regions of all 8 RNA segments of the isolate further determined that it was most closely related to A/swine/Ontario/33853/2005 (H3N2) virus, which shares the same human/classic swine/avian triple reassortant genotype as the H3N2 subtype viruses that emerged in swine in the United States in 1998 (*8*). Sequence analysis showed that nucleic acid homology between A/Canada/1158/2006 and A/swine/Ontario/33853/2005 ranges from 98.4% (HA) to 100% (M1), and that amino acid (aa) identities range from 97.9% (HA) to 100% (NP, NS2, M1). A deletion of 4 aa at position 156–159 was observed in the HA1 region of the A/Canada/1158/2006 HA protein. Amino acid substitutions were found in the HA (HA1 domain: G7, K142, S162; HA2 domain: T77, Q139, M149, E150, N160), neuraminadase (NA) protein (P45, K74, N150, M349, L354), NS1 (M112), PB1 (K211, D738), PB2 (K368, S661, T722), and PA (V44, R99, I42) proteins. Phylogenetic analysis showed that all of the genes of A/Canada/1158/06 clustered with Canadian swine isolates from 2005 (*9*) (data not shown). Nucleic acid identity between the HA and NA genes of A/Canada/1158/06 and the current vaccine strain A/Wisconsin/67/05 was 90.9% and 94.6%, and the aa identities were 90.2% and 94.5%, respectively.

Serologic Testing

Seropositivity (HI titer ≥32) to A/Canada/1158/2006 was demonstrated in the index patient, the symptomatic sibling, 1 asymptomatic sibling, and both parents (Table 2, household A). Three other siblings were seronegative. Four children from 2 other households were also seropositive (Table 2, households B and C); the father from household B, 1 other child from household B, and the mother from household C were seronegative. The father from household C worked in the swine barn but was unavailable for testing. History of ILI within the preceding 12 months in seropositive participants was reported only for the index patient and for a 3-year-old girl from household C who was not hospitalized or tested for influenza virus during her illness. Seronegative results were obtained from another 20 adults (14 women and 6 men) and 19 children (8 girls and 11 boys) from 14 different households. For these households, swine exposure was reported as none for 9 adults and 7 children, <1 hour/week for 11 adults and 8 children, and ≥1 hour/week for 4 children including 3 teenagers who worked in the swine barns. When serum samples from the 54 participants in the study were tested for HA-specific antibodies to the current human influenza A virus H3N2 and H1N1 subtypes, one of the patients who was seropositive for SIV at a titer of 32 had an identical titer for A/Wisconsin/67/2005 (H3N2) (Table 2), and one of the adults who was seronegative for SIV had a titer of 32 for A/New Caledonia/20/99 (H1N1) (data not shown). All other persons tested were seronegative for the 2 human strains of influenza.

Table 1. Hemagglutination-inhibition reaction of A/Canada/1158/2006 isolates with reference antiserum against currently circulating human and swine viruses

	Antiserum (titers)				
Antigen	A/New Caledonia/20/99 (human H1N1)	A/Wisconsin/67/2005 (human H3N2)	A/Panama/2007/99 (human H3N2)	A/Nanchang/933/95 (human H3N2)	A/Swine/Texas/4199–2/98 (swine H3N2)
Control					
A/New Caledonia/20/99 (human H1N1)	320	<4	<4	<4	<4
A/Wisconsin/67/2005 (human H3N2)	<4	320	64	8	8
A/Ontario/RV1273/2005 (swine H3N2)	<4	<4	<4	<4	256
Patient					
A/Canada/1158/2006	<4	<4	<4	<4	128

Table 2. Clinical features and hemagglutination-inhibition reaction of positive antiserum from 9 members of 3 different households of a communal farm with recently circulating swine influenza (H3N2) virus A/Canada/1158/2006*

Household	Age, y	Sex	A/Wisconsin/ 67/2005 titer	A/New Caledonia/ 20/99 titer	A/Canada/ 1158/2006 titer	Swine exposure	Clinical features
A (index patient)	0.6	M	<4	<4	256	None	Hospitalization with ILI and isolation of swine influenza
A†	1	M	<4	<4	256	None	None (URI coincident with ILI in index case)
A	35	F	<4	<4	32	None	None
A	38	M	8	<4	32	<1 h/wk	None
A	8	M	<4	<4	64	<1 h/week	None
B	7	M	32	<4	32	<1 h/wk	None
C	8	M	4	<4	64	≥1 h/week	None
C	5	M	<4	<4	128	<1 h/week	
C	3	F	<4	<4	128	None	ILI 1 mo before index case

*URI, upper respiratory illness; ILI, influenza-like illness.
†Symptomatic sibling.

Swine Investigation

Influenza (H3N2) was last documented in the swine herd in September 2005. The herd received breeding animals from a Manitoba herd, where swine influenza of an unknown subtype had recently been documented. Nasal swabs collected from 25 grower pigs ≈3 weeks after the index child was ill were negative for SIV. Serum specimens obtained from 10 grower-finisher pigs were all negative by ELISA for swine influenza (H1N1), but 4 were positive for swine influenza (H3N2) strains, with 1 of these 4 strains being seropositive for A/Canada/1158/2006 by HI assay (HI titer 32). Results of the lung autopsies all showed evidence of subacute bronchointerstitial pneumonia, varying from mild to moderate. Lesions typical for swine influenza were not noted, but an initial insult due to SIV could not be excluded.

Discussion

We describe an infant with virologic and serologic evidence of infection with SIV (H3N2) and an ILI. Serologic evidence of infection with the same strain was found in 4 of 7 household members and in 3 of 46 nonhousehold contacts, with only 1 of the seropositive patients having a history of an ILI within the preceding year, which demonstrated unrecognized human infection with SIV. This relatively high seroprevalence is in contrast to a recent outbreak of avian influenza (H7N3) in which seropositivity was not documented in 91 persons exposed to infected poultry, including 2 poultry workers from whom the virus was isolated (*12*). The difference in the apparent incidence of infection may be explained in part by the fact that culling of infected poultry occurred immediately; in our study, infection of swine was not recognized and long-term human exposure may have occurred.

Infection of swine with human influenza viruses has been recognized for decades (*2*); in a recent US study, 22.8% of pigs were seropositive for human influenza viruses, although some may have had vaccine-induced immunity (*13*). Swine influenza (H3N2) emerged in 1998 in the United States, where subtype H1N1 viruses had predominated for 60 years (*2*). The isolate from this current study is closely related to triple reassorting genotype viruses that spread rapidly throughout the US swine population and have HA, NA, and RNA polymerase (PB1) genes of human influenza virus lineage; nucleoprotein, matrix, and nonstructural genes of classic swine influenza (H1N1) lineage; and RNA polymerase (PA and PB2) genes of North American avian virus lineage (*8*). However, triple reassortant SIV was not documented in swine in Canada until 2005 (*8*), which makes it unlikely that human cases occurred before that year and that seroreversion had occurred in any of the persons in the current serosurvey.

A previous study showed cross-reactivity in HI assay between the vaccine strain A/Panama/2007/99 reference antiserum and the triple reassortant A/swine/Minnesota/593/99, which is not unexpected since the HA gene of the triple reassortant viruses is a descendant of human viruses that circulated in 1995 (*14,15*). However, no cross-reactivity was observed between the reference human strain antiserum and the isolate from this study, which suggests that the seroconversion observed was indeed due to infection with swine influenza (H3N2) and not to cross-reactive antibody to human influenza (H3N2) infection. The low rate of seropositivity to recently circulating strains of human influenza in the study is likely explained by the fact that the farm is a relatively closed community. The child who was seropositive for both human and swine influenza viruses was likely exposed to both viruses. The HA protein of A/Canada/1158/2006 diverges significantly from the one of A/Wisconsin/67/2005, and antiserum against A/Wisconsin/67/2005 does not inhibit A/Canada/1158/2006 in HI assay.

Swine influenza (H3N2) has recently reassorted with H1N1 strains to produce H1N2 subtypes and has spread to turkeys in the United States (*16*) and Canada (*8*). A 4-aa deletion was found in the HA protein of A/Canada/1158/2006 when compared with similar swine influenza (H3N2)

strains currently circulating in North America. This region of the protein has been assigned to antigenic sites (*17*) and has been associated with adaptation to growth in eggs (*18*). Phylogenetic analysis showed that each of the 8 viral genes of A/Canada/1158/2006 clustered with A/swine/Ontario/33853/2005 (H3N2) and other swine/turkey Canadian isolates from 2005. Although the HA gene of these isolates were shown to be closely related to American viruses that were first isolated from pigs in 1999, they represent a new distinct cluster (*2*). The NA genes are phylogenetically distinct from the US swine isolates and are represented by human influenza (H3N2) isolates from Asuncion, Paraguay (2001), and New York (2003) (*2*).

A recent review described 50 cases of symptomatic human infection with SIV, documented in the literature through April 2006; 46 cases were infected with subtype H1N1 and 4 were infected with subtype H3N2 (*19*). The spectrum of pathogenicity of SIV infection ranges from asymptomatic infection (*6*) to death; 7 of these 50 patients died (*5,20–24*). Laboratory-confirmed swine influenza in humans may be "the tip of the iceberg." Diagnosis of the current case was serendipitous because typing was performed only because the case occurred outside of influenza season.

The mode of spread of SIV in humans is not established. Because of his young age, the index patient was not likely to have had unrecognized direct contact with swine. That aerosolization of influenza virus occurs is increasingly recognized (*25*), but the child was reportedly never in the barns that housed the swine. However, other members of the farm reported that infants were sometimes taken for walks through the barn. The child also may have acquired the virus from person-to-person spread or from fomites. All 13 patients in the Fort Dix outbreak and 15 of 37 previously reported civilian case-patients also had no swine contact (*19,20*).

The Fort Dix outbreak of SIV in humans lasted only 21 days and never spread outside the military base. The calculated basic reproductive rate (R_0) was only 1.1 to 1.2. This suggests that person-to-person spread of the implicated H1N1 strain was not efficient enough to produce a major epidemic (*26*). However, future strains of SIV could have a higher R_0, and documentation of a case of swine influenza (H3N2) in a child with unrecognized transmission within the community adds another possible mechanism by which major epidemics of influenza could arise. Swine influenza infection in humans most commonly results in either no symptoms or a self-limited illness (*6*). However, routine surveillance for cases among swine workers may enable early detection of a strain with the potential for person-to-person transmission, prompting institution of infection control measures and vaccine development.

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別紙様式第2

別紙(3)-2

医薬品　研究報告　調査報告書

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一般的名称			研究報告の公表状況	Clinical and virological characterization of persistent human infection with simian foamy viruses. AIDS research and human retroviruses, Nov 2007, 23	公表国 米国	
販売名（企業名）	タココンプ（CSL ベーリング株式会社）					

研究報告の概要91

問題点（SFV 感染者は輸血や移植）

HIV と HTLV の病原性のレトロウイルスは、非ヒト霊長類から SIV と STLV のヒトへの感染が定着し、人から人への感染が拡大した。simian foamy viruses (SFV) 感染は、捕獲された非ヒト霊長類間でかなり伝播している。職業上非ヒト霊長類やその体液と接触したり、咬まれたり、引掻かれた人達に SFV 感染が確認されてきた。遺伝子配列を解析すると、人への SFV 感染はチンパンジー、ヒヒ、アフリカサバンナモンキーなど多様な非ヒト霊長類を起源としていることが分かっている。

筆者らは、SFV 感染した人 13 名の中で 7 名を長期追跡調査した。遺伝子配列を解析すると、SFV は参加者 2 名がヒヒ由来であり、他の 5 名はチンパンジー由来であった。

参加者 7 名は、非ヒト霊長類やその唾液、尿などの体液に接する機会があり、体液が参加者の皮膚と粘膜と暴露したり、皮膚損傷があると報告している。

全ての参加者の抹消血単核球（PBMC）から SFV DNA が検出され、口腔の検体、尿、精液からも検出された。

自己申告による健康状態を検討したが、SFV 感染と関連がある共通した臨床的な症候群は示唆できなかった。

臨床検査を調べ最も興味ある事は、参加者 3 名で好酸球減少、血小板減少の軽度の異常があったことである。しかし臨床上重要ではない。

参加者 3 名の妻を SAV 感染者との接触者として、ウエスタンブロット法と PCR 法で調べたが陰性であった。少人数での限られた追跡調査期間での観察のため、SFV 感染に関連する病状や人の二次的 SFV 感染は特定できなかった。

筆者らは、人における感染は医学的に重要性が解明されていないので、SFV 感染者は輸血や移植を控えるべきであると警告している。

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
SFV 感染の人での病態や二次感染について明確になっていない。SFV はレトロウイルスであることから、本剤の製造工程で不活化できると考えられる。	今後とも情報収集に努める所存である。

(4)

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Clinical and Virological Characterization of Persistent Human Infection with Simian Foamy Viruses

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ABSTRACT

Persons occupationally exposed to nonhuman primates (NHPs) can be persistently infected with simian foamy virus (SFV). The clinical significance and person-to-person transmissibility of zoonotic SFV infection is unclear. Seven SFV-infected men responded to annual structured interviews and provided whole blood, oral, and urogenital specimens for study. Wives were tested for SFV infection. Proviral DNA was consistently detected by PCR in PBMCs of infected men and inconsistently in oral or urogenital samples. SFV was infrequently cultured from their PBMCs and throat swabs. Despite this and a long period of intimate exposure (median 20 years), wives were SFV negative. Most participants reported nonspecific symptoms and diseases common to aging. However, one of two persons with mild thrombocytopenia had clinically asymptomatic nonprogressive, monoclonal natural killer cell lymphocytosis of unclear relationship to SFV. All participants worked with NHPs before 1988 using mucocutaneous protection inconsistently; 57% described percutaneous injuries involving the infecting NHP species. SFV likely transmits to humans through both percutaneous and mucocutaneous exposures to NHP body fluids. Limited follow-up has not identified SFV-associated illness and secondary transmission among humans.

INTRODUCTION

TWO PATHOGENIC RETROVIRUSES, human immunodeficiency virus (HIV) and human T-lymphotropic virus (HTLV), established endemicity in human populations following infection of individual humans with simian immunodeficiency viruses (SIV) and simian T cell lymphotropic viruses (STLV) from nonhuman primate (NHP) reservoirs, respectively, and viral adaptation facilitating human-to-human spread.[1] Continued direct contact between human and NHPs in occupational and other settings provides an ongoing opportunity for introduction of additional simian retroviruses across species into human populations.

Foamy viruses (FVs), retroviruses in the *Spumavirus* genus,[2,3] establish persistent infections endemic to many mammalian species. Simian FV (SFV) infection is highly prevalent among captive NHPs.[4–6] Despite SFV coevolving with primates for at least 30 million years,[7] endemically infected human populations have not been identified.[6] A prototype FV (PFV), previously termed "human" FV (HFV) because it was isolated from a nasopharyngeal carcinoma (NPC) from a Kenyan man in 1971,[8] is now known to be of chimpanzee origin.[9,10] SFV infections have been identified in persons exposed directly to NHPs and their body fluids occupationally or through hunting, butchering, or habitat sharing.[10–16] Sequence analysis suggests

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that these human SFV infections originated from multiple NHP species, including chimpanzee, baboon, African green monkey, macaque, De Brazza's guenon, mandrill, and gorilla.[4,6,10] Putative associations between FV infection and various human diseases, including NPC, have not been supported in well-designed studies.[6,17,18]

Unlike HIV and HTLV, the clinical significance and secondary transmissibility of human infections with SFV are unknown. SFV is strongly cytopathic in human and NHP cells *in vitro*,[6] but is not recognized to be associated with disease in any natural host, though this has not been systematically evaluated. SFVs are easily transmitted between NHPs, mostly by contact with oral secretions during grooming or biting.[5,6]

Surveillance for simian retrovirus infection in persons occupationally exposed to NHPs at research centers and zoos in North America identified 14 persons with serological evidence of SFV infection.[10,12] Using archived sera, most workers were shown to have long-standing zoonotic infection with unclear exposure risks or public health significance [10,12] We report preliminary results from the first prospective cohort study of persons persistently infected with SFV. We characterize the potential for secondary transmission by determining the presence of virus in various body fluids and by longitudinal testing of intimate contacts, evaluate the health status of humans persistently infected with SFV, and assess possible occupational risk exposures leading to infection.

MATERIALS AND METHODS

Study design and enrollment

Persons with documented SFV infection identified through surveillance of occupationally exposed workers were eligible to provide informed consent and enroll as primary participants in a prospective cohort study approved by the Centers for Disease Control and Prevention (CDC) Institutional Review Board.[10,12] Documented SFV infection was defined as seroreactivity to SFV antigens by Western blot (WB) combined with evidence of proviral DNA sequences in peripheral blood mononuclear cells (PBMCs) by polymerase chain reaction (PCR) and/or isolation of SFV from PBMCs.[10,12]

Spouses/partners and children of SFV-infected humans or other persons living in the same household were also eligible to enroll as contact participants. Informed consent was obtained from all participants before enrollment. All enrollees were offered follow-up for a minimum of 5 years.

Participant interviews

Participants were interviewed by telephone at enrollment and annually for 5 years using a standard questionnaire. Information was collected on demographics and health status at the time of enrollment. Participants were asked about their general health status including personal health observations and conditions diagnosed by physicians and about symptoms known to be associated with retroviral infections such as malignancies or lymphoproliferative, inflammatory, and neurological diseases.[3] To evaluate exposure opportunity, information was collected on the duration of occupational exposure to specific NHP species, work activities and practices including use of protective equipment, past injuries, and specific exposures to NHP blood or body fluids. To identify opportunities for secondary transmission, participants were questioned about sexual contacts, practices, and other activities that may result in intimate exchange of body fluids, including donation of blood or other living biological material. Participants were counseled regarding current knowledge about human SFV infection and provided the opportunity to ask questions.

Specimen collection and preparation

Whole blood, parotid saliva, swabs of saliva and the posterior oropharynx ("throat swabs"), urine, and semen (all primary participants were male) were requested from participants annually for clinical, virological and immunological testing. Parotid saliva was collected in intraoral (Schaefer) cups and immediately transferred with a pipette to cryovials.[19] Throat and saliva swabs were collected with viral culturettes (Becton/Dickinson). Nonblood specimens were shipped to the CDC immediately after collection on wet ice; whole blood was shipped at room temperature.

Upon arrival at the CDC parotid saliva was centrifuged for 2 min at $1000 \times g$ and cell pellets and supernatant were aliquoted and frozen at $-80°C$ until tested. Throat and saliva swabs were placed in 2 ml phosphate-buffered saline (PBS), vortexed, and then centrifuged for 5 min at $1000 \times g$ to pellet any cells present. The cell pellet was washed twice with PBS, then divided equally for PCR testing and for tissue culture for some participants. Urine and, when available, semen samples were centrifuged for 10 min at $800 \times g$ to pellet any cells present and washed twice with PBS and stored at $-80°C$. Urine and semen supernatants were aliquoted and stored at $-80°C$ for mucosal immunity studies.[19]

Clinical laboratory testing

Clinical testing was performed by a commercial diagnostic laboratory. Clinical testing included complete blood counts (CBC) with differential analysis of white blood cells, testing of serum for electrolytes, glucose, creatinine, blood urea nitrogen, uric acid, total protein, albumin and globulin, total bilirubin, adenosine phosphatase, lactate dehydrogenase (LDH), serum aspartate aminotransferase (AST), and serum alanine aminotransferase (ALT).

Virological and immunological analysis

DNA lysates were prepared from PBMCs and from pelleted cells from parotid saliva, mixed saliva, throat swabs, and urine and tested for SFV polymerase (*pol*) proviral sequences using nested PCR.[10,12] DNA quality was confirmed by β-actin PCR as previously described.[10,12] Virus isolation was attempted from selected participant's PBMCs, throat, and saliva samples. Specimens were cultured on canine thymocytes and/or *Mus dunni* fibroblasts and monitored biweekly for up to 40 days for cytopathic effect, reverse transcriptase (RT) activity, and proviral *pol* sequences.[10,12]

Plasma was tested for SFV antibodies using a WB assay that can detect both monkey and ape SFV as described in detail elsewhere.[4,10,19,20] Saliva and urine were tested for the presence of anti-SFV IgG and IgA by WB analysis.[19]

To perform mononuclear cell phenotyping, 20 μl of well-mixed MultiTEST four-color reagent (CD3/CD8/CD45/CD4) (Becton Dickinson Biosciences, San Jose, CA) and 50-μl aliquots of EDTA-anticoagulated whole blood were added to a TruCOUNT tube (Becton Dickinson Biosciences, San Jose, CA) containing a known concentration of beads. The mixture was incubated for 20 min at room temperature in the dark before 450 μl of FACS lysing solution (Becton Dickinson Biosciences, San Jose, CA) was added. After 15 min of incubation, the lyse/no-wash-stained samples were analyzed with the FACSort flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). Data were analyzed using MultiSET software (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Contact enrollments

Contacts of SFV-infected participants were evaluated annually for evidence of SFV infection by WB testing of serum or plasma and PCR testing of PBMC DNA, but are not interviewed.

RESULTS

Primary participants

Seven of 13 persons eligible to participate enrolled for long-term follow-up. We refer to these participants as cases 2, 3, 6, 7, 9, 10, and 12 to be consistent with previous reports and the chronological order in which they were identified as SFV-infected.[10,12] Sequence analysis indicates that the virus infecting cases 2 and 3 originated from baboons while virus infecting the remaining five participants all originated from chimpanzees.[10,12] Case 11 dropped out of the initial surveillance study and was not available for further study.[10]

Table 1 summarizes demographic and exposure data of SFV-infected persons by enrollment status. Participants are not entirely representative of the eligible population, limiting interpretation of results. Male gender, higher level job status, and longer duration of both occupational exposure and SFV seropositivity are overrepresented among study participants.

All participants are male compared to four/six (67%) eligible persons who did not enroll ("nonparticipants"). Two of five (40%) animal caretakers, both animal care supervisors, the sole research associate, and two/four (50%) veterinarians participated. At the time SFV infection was confirmed, the median age of participants (median 56, range 41–62 years) was similar to that of three nonparticipants for whom age was available (median 57, range 49–58). Participants enrolled a median of 2 years (range 1–5) after infection was confirmed. Participants were exposed to NHPs longer prior to confirmation of infection than were five nonparticipants for whom adequate data were available (median 26, range 8–37 years versus median 19, range 10–29 years, respectively).

The availability of stored serum allowed determination of a minimal duration of seropositivity for six/seven participants and four/six nonparticipants. Prior to documentation of SFV infection,

TABLE 1. DEMOGRAPHIC AND EXPOSURE CHARACTERISTICS OF ELIGIBLE AND ENROLLED PARTICIPANTS

Case	*Sex*[a]	*SFV species origin*	*Occupation*	*Age when SFV infection documented (years)*	*Year SFV infection documented*	*Year first positive sera archived*	*Minimal duration (years) SFV infection when identified*	*Duration of NHP exposure (years) when SFV infection identified*
				Enrolled participants				
2	M	Baboon	Research associate	56	1996	1978	18	29
3	M	Baboon	Animal care supervisor	57	1996	1988	8	37
6	M	Chimpanzee	Veterinarian	60	1998	1981	17	19
7	M	Chimpanzee	Veterinarian	41	1999	1990	9	23
9	M	Chimpanzee	Animal caretaker	41	1999	1980	19	8
10	M	Chimpanzee	Animal care supervisor	50	1999	1976	23	26
12	M	Chimpanzee	Animal caretaker	62	2001	NA[b]	NA	32
				Eligible nonparticipants				
1	M	AGM[c]	Animal caretaker	57	1996	1995	1	19
4	M	Baboon	Veterinarian	49	1997	1994	3	20
5	M	Baboon	Veterinarian	58	1998	1979	19	29
8	M	Chimpanzee	Animal caretaker	NA	1999	1985	14	>14
13	F[d]	Chimpanzee	Veterinary technician	NA	2002	NA	NA	11
14	F	Chimpanzee	Animal caretaker	NA	2002	NA	NA	10
				Ineligible nonparticipants				
11	M	Chimpanzee-like[d]	Research technician	NA	1999	NA	NA	10

[a]M, male; F, female.
[b]NA, not available.
[c]AGM, African green monkey.
[d]Based on SFV-type-specific WB.

participants with available stored sera were seropositive a median 9 years longer than were nonparticipants (median 17.5, range 8–23 years versus median 8.5, range 1–19 years, respectively). Initial clinical testing of participants under this protocol occurred after a median of 19 years (range 2–24) of documented infection.

Exposure history and use of protective equipment

All seven participants reported direct, frequent, and intimate opportunity for exposure to NHPs and their biological fluids including animal saliva, urine, feces, and blood. All seven workers reported histories of both mucocutaneous exposures to NHP body fluids and skin-penetrating injuries. Five of seven participants (71%) described NHP bite wounds, five/seven (71%) described scratch wounds, and six/seven (86%) described percutaneous exposure to NHP body fluids via skin penetrating sharp injuries. However, only four/seven (57%) participants (cases 3, 6, 7, and 9) described a percutaneous injury that was associated with the NHP species from which sequence analysis suggests their infecting virus arose.

All participants reported currently wearing leather or latex gloves when handling NHPs. However, these seven participants worked with NHPs for a median of 16 years (range 5–29) prior to 1988 when universal precaution guidelines were established. Five workers consistently and two inconsistently wore gloves prior to 1988. All reported historically inconsistent use of face shields and goggles transitioning to more regular use in recent years. Two participants denied use of goggles or face shields for mucocutaneous protection; four reported wearing them 25–75% of the time for specific tasks such as working with chemicals or infected animals or cleaning cages. One wore face shields consistently "when required." The use of protective equipment was rare prior to 1988, and workers noted that current use of face shields did not always protect them from mucocutaneous exposure to NHP saliva and other body fluids.

Body distribution of SFV

The distribution of SFV in human body fluids is summarized in Table 2. SFV DNA sequences were found in PBMCs from all seven persons and in all of their 19 serial samples tested. Virus culture was attempted at least once on PBMCs from six/seven participants. SFV was isolated from only one/two PBMC samples from each of cases 6 and 10, representing 33% of persons and 20% of specimens from which virus culture was attempted.

SFV DNA was detected in oral cavity specimens from three/seven (43%) participants; overall, 6/16 (38%) throat swab samples from these seven participants were SFV DNA positive. Saliva specimens from two/seven (29%) persons were positive for SFV DNA; 6/23 (26%) serial samples were positive. Interestingly, SFV was isolated from throat swab samples from case 6, for whom six/seven (86%) oral cavity specimens were positive for SFV DNA. SFV was not isolated from saliva available from the other five participants (seven samples).

Although all subjects provided urine specimens, most (13/18, 72%) samples had insufficient cellular DNA present to support SFV PCR testing. Of five samples from four persons with sufficient cellular DNA, two persons (cases 3 and 6) were positive for SFV DNA. Two participants (cases 2 and 12) each provided two semen samples; a third (case 6) provided a single specimen. Specimens from case 2 (who had received a vasectomy more than 20 years ago) and case 12 tested negative for B-actin sequences, indicating the absence of cellular DNA or the presence of PCR inhibitors. Thus these semen specimens were not suitable for further PCR testing. The semen specimen from case 6, who had benign hemospermia, was positive for SFV DNA. Insufficient material was available to attempt virus isolation from semen or urine specimens.

SFV-specific immunoglobulin G (IgG) antibodies against structural (Gag) and accessory (Bet) proteins were detected by WB in plasma, saliva, and urine from participants. Immunoglobulin A antibodies were not detected in any specimens, as previously reported.[19]

Clinical status

Self-reported medical histories identified chronic conditions, nonspecific symptoms, and diseases of aging common in the U.S. population. In addition, case 9 reported congenital heart disease and mild thrombocytopenia since 1997 and case 3 underwent aortic valve replacement during this observational pe-

TABLE 2. PRESENCE OF SFV IN BODY COMPARTMENTS

	Number of specimens positive/number of specimens tested (%)					
	Peripheral blood mononuclear cells		*Oral cavity*[a]			
Enrollee	*SFV DNA (%)*	*SFV isolation (%)*	*SFV DNA (%)*	*SFV isolation (%)*	*Urine*[b] *SFV DNA (%)*	*Semen*[b] *SFV DNA (%)*
Case 2	2/2 (100)	0/1 (0)	0/6 (0)	0/2 (0)	0/1 (0)	NT[c]
Case 3	4/4 (100)	0/2 (0)	0/4 (0)	0/4 (0)	1/1 (100)	NT
Case 6	4/4 (100)	1/2 (50)	6/7 (86)	1/4 (25)	1/2 (50)	1/1 (100)
Case 7	4/4 (100)	0/2 (0)	0/9 (0)	0/4 (0)	0/1 (0)	NT
Case 9	1/1 (100)	0/1 (0)	1/2 (50)	0/2 (0)	NT[c]	NT
Case 10	3/3 (100)	1/2 (50)	0/6 (0)	0/2 (0)	NT	NT
Case 12	1/1 (100)	NT	3/5 (60)	NT	NT	NT
Total	19/19 (100)	2/10 (20)	10/39 (26)	1/18 (6)	2/5 (40)	1/1 (100)

[a]Includes throat and saliva swabs and parotid saliva.
[b]Specimen quantity insufficient for SFV isolation.
[c]NT, not tested.

riod. No symptom or diagnostic patterns suggested a common clinical syndrome associated with SFV infection.

Results of clinical laboratory testing for each participant are summarized in Table 3. Repeated clinical laboratory testing was within normal limits for cases 2, 10, and 12. Testing identified unremarkable patterns of mildly abnormal glucose and renal function tests compatible with a three decade history of diabetes (case 3) and of fluctuating mild liver transaminase elevations (case 7).

Clinical laboratory testing identified hematological abnormalities for three participants. Three times on annual testing case 6 had eosinophil counts at the lower limit of or below normal range; his eosinophil count was within normal limits on subsequent testing in May 2002. Mild thrombocytopenia without other blood count abnormalities was confirmed in case 9. In addition to mild laboratory abnormalities expected to accompany long standing diabetes, case 3 had the unexpected findings of intermittent mild thrombocytopenia accompanied by natural killer (NK) cell lymphocytosis (NKCL). NK cells [$CD3^-CD16^+56^+$ cells] at three time points were 44% (780/μl), 39% (854/μl), and 38% (840/μl), respectively (upper normal limit 25% or 480/μl). Additional hematological tests performed by a specialized laboratory on specimens collected 9 months apart (personal communication, W.G. Morice, II and A. Tefferi, Mayo Clinic) repeatedly showed that 40% of lymphocytes were CD16-positive, CD3-negative NK cells, expressing CD56 and CD57, and CD161 with partial loss of CD94. These findings along with other tested markers indicated monoclonal NKCL consistent with a chronic NK-large granular lymphocytosis (indolent large granular lymphocytosis) of unclear clinical significance. The patient has no related clinical symptoms.

Secondary transmission

Wives of cases 2, 3, and 9 were tested for SFV infections as contact participants. All remained WB and PCR negative despite a collective minimum of 57 person-years of intimate exposure to an SFV-infected partner (median 20, range 13–24 years). The wives of three additional nonparticipants also tested SFV negative.[10,12] All participants reported intimate marriages that provided frequent opportunity for exposure to saliva and other body fluids. All denied the routine use of barrier precautions or other practices documented to minimize sexual transmission of infections.

Case 2 ceased regular blood donation in 1969. A serum collected in 1967 was WB negative; the next available specimen collected in 1978 was WB seroreactive. Cases 6, 7, and 10 ceased regular blood donation when notified of SFV infection; retrospective testing of archived sera confirmed earlier seropositivity. As previously reported elsewhere, four recipients of leuko-reduced blood products from one SFVcpz-infected donor (case 6) showed no evidence of SFV infection.[20]

DISCUSSION

This is the first longitudinal description of the clinical, immunological, and virological status of humans infected with SFV. After a minimum median of 26 years (range 6–31) of documented infection as of 2007, participants continue to demonstrate strong antibody response by WB testing. Viral DNA can be consistently detected in all subjects' PBMCs but inconsistently from other body sites. SFV was successfully cultured from the throat swab and PBMCs of one participant and from the PBMCs of a second. SFV DNA was also detected in the urogenital tract of two persons. Despite this, no secondary transmission among humans via intimate exposure or blood product transfusion from one SFV-infected donor was identified.[20] Our data support a persistent infection in humans consistent with the demonstrated nature of endemic infections in NHPs, but suggest that the presence of detectable viral DNA in human body fluids does not correlate with transmissibility.

Repeated exposure to SFV DNA in body fluids may be insufficient for human-to-human transmission due to a limited viral load, the persistent presence of virus in a latent noninfectious state, or other mechanisms. SFV isolations were most frequent from case 6, the only participant whose specimens were received and processed within 8 h of collection. This may suggest that time elapsed from collection to processing influenced virus recovery, or may reflect higher viral loads in case 6 than other participants. Quantitative PCR testing for viral DNA and RNA levels in blood and other body compartments might provide further insight on the relationship between variations in viral load and inconsistencies between detection of SFV DNA in, and isolation of SFV from, body fluids. Fluctuations in viral load may also explain the variable detection of SFV in some body fluids.

Six of seven retrovirus genera are associated with hematological, neurological, dermatological, arthritic, or oncogenic diseases affecting nearly all vertebrate species, typically after long incubation periods.[3] Despite being highly cytopathic in cell culture, FVs have not been associated with any *in vivo* disease.[6] Our preliminary observations on humans with prolonged infection, while limited, are reassuring. Most clinical laboratory results were within normal limits or explained by the presence of conditions common in human populations of comparable age.

The most intriguing clinical observations were mild hematological abnormalities in three participants. The fluctuating, inconsistent and mild eosinopenia of case 6 appears to be clinically insignificant. The mild thrombocytopenia in two/seven participants deserves attention, but also does not appear to be clinically significant.

Although the total lymphocyte counts in all participants were normal, the NKCL in one participant with minimal thrombocytopenia is a notable finding. NKCL is a rare condition of unknown etiology, accompanied by thrombocytopenia in 12% of subjects in one series.[21] Persistent viral infection (e.g., Epstein–Barr, hepatitis B or C viruses)[22] or persistent immunological stimulation[23] has been hypothesized to play an etiological role. With the caveat that we do not know whether case 3 has other persistent infections, we may speculate that persistent SFV infection and, possibly, the presence of long-term diabetes mellitus[24] might contribute to case 3's NKCL. Future studies quantifying SFV integration in specific blood cells or cell lines may cast light on whether SFV plays a role in the observed hematological abnormalities.

SFV transmits naturally among NHPs via casual exposure to oral and respiratory secretions, and has been experimentally

TABLE 3. ABNORMAL CLINICAL LABORATORY TEST RESULTS OVER THE PERIOD OF FOLLOW-UP

Participant	*Years of age, Year 1*	*Known duration of infection (years), Year 1*	*Year 1*	*Year 2*	*Year 3*	*Year 4*	*Year 5*
Case 2	62	23	WNL[a]	WNL	WNL	NA[b]	NA
Case 3	62	13	Glucose 111 mg/dl (65–109)[c]; creatinine 1.7 mg/dl (0.5–1.4); BUN[d] 33 mg/dl (7–25); NK[e] cells 780/μl, 44% (68–482, 4–25%)	NK cells 854/μl, 39%; uric acid 8.6 (1.7–8.2); platelets 138,000 (140,000–400,000)	Glucose 131 mg/dl; creatinine 1.4; ALT[f] 116 U/liter (5–35); NK cells 38%	NA	NA
Case 6	62	19	Eosinophils 34/mcl (50–500)	Eosinophils 17/mcl	Eosinophils 56; platelets 122,000	Eosinophils 33 (15–500)	WNL
Case 7	42	10	AST[g] 48 IU (0–42); ALT 70 IU (0–48)	ALT 67	WNL	NA	WNL
Case 9	41	20	Platelets 107,000	NA	NA	NA	NA
Case 10	50	24	WNL	WNL	WNL	NA	NA
Case 12	62	2	WNL	NA	NA	NA	NA

[a]WNL, within normal limits.
[b]NA, not available.
[c]Limits of normal are shown in parentheses.
[d]BUN, blood urea nitrogen.
[e]NK, natural killer.
[f]ALT, alanine aminotransferase.
[g]AST, aspartate aminotransferase.

transmitted among NHP through fresh whole blood.[5,6,25,26] Previous reports have stressed the role of skin penetrating injuries in human acquisition of infection.[10–12] The absence of a discernible history of percutaneous injury associated with the species from which the infecting virus strain arose for 43% of participants raises the possibility that human infection with SFV may be acquired through mucocutaneous exposure to SFV-containing NHP body fluids without injury, similar to the routes of transmission of simian herpes viruses.[27] Thus, it is prudent for persons occupationally exposed to NHPs to take precautions to avoid exposure to primate saliva and other body fluids through either percutaneous injuries or mucocutaneous exposures.

Limited observations have not identified infection-associated pathology or secondary SFV transmission among humans through either intimate contact or transfusion of blood products. However, the small number of observed individuals and the limited duration of follow-up restrict our ability to draw definitive conclusions about the clinical significance of human infection with SFV and the ability of SFV to transmit secondarily. Like HTLV, the incidence of disease may be low or may follow long latency periods. It is also unknown what effect, if any, immunosuppression may have on clinical outcomes of human infection with SFV. For example, SFV replication was recently shown to expand to the small intestinal jejunum of SIV-immunosuppressed macaques, a site for significant $CD4^+$ T cell depletion and inflammation in these animals, suggesting that SFV may play a role in the gut-associated pathology observed during progression to simian AIDS.[28] We caution SFV-infected persons to refrain from donation of biological materials for transfusion or transplantation pending a better understanding of the significance of human infection.[12,29] Additional observations will be necessary to further define the public health significance of zoonotic SFV infection.

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販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)		Centers for Disease Control and Prevention (CDC). Morb Mortal Wkly Rep. 2007 Nov 16;56(45):1181-4.	米国

研究報告の概要

○アデノウイルス血清型14に関連した米国4州の急性呼吸器疾患(2006～2007年の報告)
アデノウイルス血清型14型(Ad14)は、希にしか報告されないが新興しているアデノウイルスの血清型株で、健常若年成人を含め全ての年齢層の患者に、重症で時に致死性の呼吸器疾患を惹起する可能性がある。2006年5月に、ニューヨーク州で生後12日目の乳児が、Ad14が原因の呼吸器疾患により死亡した。2007年3月～6月の間に、オレゴン州、ワシントン州の介護施設、およびテキサス州の空軍基地で発生した小集積事例において、合計で140名のAd14感染患者が確認された。このうち53名(38%)が入院し、24名(17%)はICUで治療を受け、9名(5%)が死亡した。全4州の患者から分離されたAd14株は、hexonおよびfiber遺伝子全長の塩基配列データは同一であったが、1955年以来のAd14レファレンス株とは区別された。このことから、米国で新たなAd14変異株が新興し感染拡大したことが示唆される。州および各地公衆衛生当局は、Ad14が原因の集団感染発生可能性に警戒すべきである。
アデノウイルスは1950年代に初めて記録され、結膜炎、発熱性上気道疾患、肺炎および胃腸疾患などの広範囲な臨床症状に関連している。新生児や高齢患者、基礎疾患のある患者では重症化の可能性があるが、健常成人では一般的に致死性感染とはならない。本報告は、米国内に感染拡大した新規病原性Ad14変異株の新興を示唆している点で異例である。Ad14感染は1955年に初めて記録され、1969年にはヨーロッパの新兵での流行性急性呼吸器疾患と関連したが、それ以降はあまり検出されていなかった。Ad14のより広域での感染循環は数年前から発生している可能性もある。

報告企業の意見	今後の対応
2006～2007年に、米国ニューヨーク州、オレゴン州、ワシントン州、テキサス州で合計140名のアデノウイルス血清型14感染患者が確認され、新たなAd14変異株が新興し感染拡大した可能性が示唆されるとの報告である。	日本赤十字社は、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、問診で呼吸器疾患などの体調不良者を献血不可としている。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

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MMWR
Weekly
November 16, 2007 / 56(45);1181-1184

Acute Respiratory Disease Associated with Adenovirus Serotype 14 --- Four States, 2006--2007

Adenovirus serotype 14 (Ad14) is a rarely reported but emerging serotype of adenovirus that can cause severe and sometimes fatal respiratory illness in patients of all ages, including healthy young adults. In May 2006, an infant in New York aged 12 days died from respiratory illness caused by Ad14. During March--June 2007, a total of 140 additional cases of confirmed Ad14 respiratory illness were identified in clusters of patients in Oregon, Washington, and Texas. Fifty-three (38%) of these patients were hospitalized, including 24 (17%) who were admitted to intensive care units (ICUs); nine (5%) patients died. Ad14 isolates from all four states were identical by sequence data from the full hexon and fiber genes. However, the isolates were distinct from the Ad14 reference strain from 1955, suggesting the emergence and spread of a new Ad14 variant in the United States. No epidemiologic evidence of direct transmission linking the New York case or any of the clusters was identified. This report summarizes the investigation of these Ad14 cases by state and city health authorities, the U.S. Air Force, and CDC. State and local public health departments should be alert to the possibility of outbreaks caused by Ad14.

New York

In May 2006, a fatal case of Ad14 illness occurred in New York City in an infant girl aged 12 days. The infant was born after a full-term pregnancy and uncomplicated delivery. She was found dead in bed, where she had been sleeping. The infant had been examined 3 days after birth and noted to have lost weight but was otherwise healthy. The next week she had decreased tears with crying, suggesting early dehydration. Physical activity and feeding progressively decreased during the week before her death.

Postmortem tracheal and gastric swabs from the infant were sent to the Wadsworth Center laboratory of the New York State Department of Health, where adenovirus was detected by polymerase chain reaction (PCR). Adenovirus also was isolated by culture, confirmed by immunofluorescence assay (IFA), and typed as Ad14 by antibody neutralization assay. Analysis at CDC identified the same unique genetic sequences in this isolate as were later identified in the Ad14 isolates from the three 2007 clusters.

Autopsy and histologic findings at the Office of the Chief Medical Examiner in New York City included presence in the lung of chronic inflammatory cells with intranuclear inclusions, consistent with adenoviral bronchiolitis and acute respiratory distress syndrome. Investigation by the New York City Department of Health and Mental Hygiene has not identified any other local cases of Ad14 illness.

Oregon

In early April 2007, a clinician alerted the Oregon Public Health Division (OPHD) regarding multiple patients at a single hospital who had been admitted with a diagnosis of severe pneumonia during March 3--April 6. A total of 17 specimens were obtained from patients; 15 (88%) yielded isolates that were identified by CDC as Ad14. Through retrospective examination of laboratory reports from the three clinical laboratories in the state that have virology capacity and the Oregon State Public Health Laboratory (OSPHL), OPHD identified 68 persons who tested positive (by culture, PCR, or IFA) for adenovirus during November 1, 2006--April 30, 2007. Isolates from 50 (74%) of these patients were available for further adenovirus typing at either CDC or OSPHL. Of the 50 patient isolates, 31 (62%) were identified as Ad14, and 15 (30%) were identified as another adenovirus type

'igure); four (8%) did not test positive for adenovirus.

ımong 30 Ad14 patients (i.e., all but one) whose medical charts were reviewed, 22 (73%) were male; median age 'as 53.4 years (range: 2 weeks--82 years). Five cases (17%) occurred in patients aged <5 years, and the remaining) (83%) occurred in patients aged >18 years. Twenty-two patients (73%) required hospitalization, sixteen (53%) :quired intensive care, and seven (23%) died, all from severe pneumonia. Median age of the patients who died 'as 63.6 years; five (71%) were male. One death occurred in an infant aged 1 month. Of the 30 Ad14 cases with atient residence information available, 28 (93%) occurred in residents of seven Oregon counties, and two cases ccurred in residents of two Washington counties. No link was identified in hospitals or the community to explain ansmission of Ad14 from one patient to another.

ı comparison with the Ad14 patients, among the 12 adenovirus non-type 14 patients (i.e., all but three) whose ıedical charts were reviewed, nine (75%) were male. Median age was 1.1 years, and 11 (92%) patients were aged 5 years. Two (17%) adenovirus non-type14 patients required hospitalization; no ICU admissions or deaths were :ported in this group.

Vashington

ın May 16, 2007, the Tacoma-Pierce County Health Department notified the Washington State Department of lealth (WADOH) of four residents housed in one unit of a residential-care facility who had been hospitalized :cently for pneumonia of unknown etiology. The patients were aged 40--62 years; three of the four were female. ıne patient had acquired immunodeficiency syndrome (AIDS); the three others had chronic obstructive ulmonary disease. All four were smokers.

he patients had initial symptoms of cough, fever, or shortness of breath during April 22--May 8, 2007. Three atients required intensive care and mechanical ventilation for severe pneumonia. After 8 days of hospitalization, ıe patient with AIDS died; the other patients recovered. Respiratory specimens from all four patients tested ositive for adenovirus by PCR at the WADOH laboratory; isolates were available from three patients, and all ıree isolates were identified as Ad14 by CDC. Ad14 had last been identified in an isolate from a patient from Vashington in May 2006, marking the first identification of Ad14 in the state since 2004. Active surveillance mong facility residents and staff did not identify any other cases of Ad14 illness.

'exas

ince February 2007, an outbreak of cases of febrile respiratory infection* associated with adenovirus infection as been reported among basic military trainees at Lackland Air Force Base (LAFB). During an initial ıvestigation, conducted from February 3 to June 23, out of 423 respiratory specimens collected and tested, 268 53%) tested positive for adenovirus; 118 (44%) of the 268 were serotyped, and 106 (90%) of those serotyped /ere Ad14. Before this outbreak, the only identification of an Ad14 isolate at LAFB occurred in May 2006 (*1*).

)uring February 3--June 23, 2007, a total of 27 patients were hospitalized with pneumonia (median ospitalization: 3 days), including five who required admission to the ICU. One ICU patient required xtracorporeal membrane oxygenation for approximately 3 weeks and ultimately died. All 16 hospitalized patients :om whom throat swabs were collected, including the five patients admitted to the ICU, tested positive for Ad14. ifteen of these hospitalized patients tested negative for other respiratory pathogens, and one patient had a sputum ulture that was positive for *Haemophilus influenzae*.

ıll health-care workers from hospital units where trainees had been admitted were offered testing for Ad14, :gardless of history of respiratory illness. Of 218 health-care workers tested by PCR, six (3%) were positive for ıd14; five of the six reported direct contact with hospitalized Ad14 patients.

revention measures implemented during the outbreak included increasing the number of hand-sanitizing stations, ridespread sanitizing of surfaces and equipment with appropriate disinfectants, increasing awareness of Ad14 mong trainees and staff members, and taking contact and droplet precautions for hospitalized patients with Ad14. :eginning on May 26, trainees with febrile respiratory illness were confined to one dormitory and both patients nd staff members were required to wear surgical masks.

Cases reported postinvestigation. Since the investigation, new cases of febrile respiratory illness have continued to occur at LAFB, but the weekly incidence has declined from a peak of 74 cases with onset during the week of May 27--June 2, to 55 cases with onset during the week of September 23--29 (the most recent period for which data were available). In addition, during March--September 2007, three other military bases in Texas that received trainees from LAFB reported a total of 220 cases of Ad14 illness (Air Force Institute for Operational Health, personal communication, 2007). However, whether Ad14 spread from LAFB to these three bases has not been determined. Ad14 also was detected in April in an eye culture from an outpatient in the surrounding community who had respiratory symptoms and conjunctivitis. No link between this case and the LAFB cases was identified.

Reported by: *Oregon Dept of Human Svcs. Washington State Dept of Health Communicable Diseases. 37th Training Wing, 59th Hospital Wing, Air Force Institute for Operational Health, Epidemic and Outbreak Surveillance, US Air Force. Naval Health Research Center, US Navy. Texas Dept of State Health Svcs. New York City Dept of Health and Mental Hygiene. Div of Viral Diseases, National Center for Immunization and Respiratory Diseases; Div of Healthcare Quality Promotion, National Center for Preparedness, Detection, and Control of Infectious Diseases; Career Development Div, Office of Workforce and Career Development, CDC.*

Editorial Note:

Adenoviruses were first described in the 1950s and are associated with a broad spectrum of clinical illness, including conjunctivitis, febrile upper respiratory illness, pneumonia, and gastrointestinal disease. Severe illness c occur in newborn or elderly patients or in patients with underlying medical conditions but is generally not life-threatening in otherwise healthy adults. Adenoviruses are known to cause outbreaks of disease, including keratoconjunctivitis, and tracheobronchitis and other respiratory diseases among military recruits (*2,3*). Although adenovirus outbreaks in military recruits are well-recognized (*3*), infection usually does not require hospitalization and rarely requires admission to an ICU. Beyond the neonatal period, deaths associated with community-acquired adenovirus infection in persons who are not immunodeficient are uncommon and usually sporadic.

Fifty-one adenovirus serotypes have been identified (*4*). The cases described in this report are unusual because they suggest the emergence of a new and virulent Ad14 variant that has spread within the United States. Ad14 infection was described initially in 1955 (*5*) and was associated with epidemic acute respiratory disease in military recruits in Europe in 1969 (*6*) but has since been detected infrequently. For example, during 2001--2002, Ad14 was associated with approximately 8% of respiratory adenoviral infections in the pediatric ward of a Taiwan hospital, with approximately 40% of Ad14 cases in children aged 4--8 years manifesting as lower airway disease (*7*).

The National Surveillance for Emerging Adenovirus Infections system includes military and civilian laboratories at 15 sites. During 2004--2007, this surveillance system detected 17 isolates of Ad14 from seven sites (*8*). Ten of t 17 isolates (60%) were collected from three military bases (*8*). Despite this surveillance, adenovirus infections often go undetected, because few laboratories routinely test for adenovirus and even fewer do serotyping. Wider circulation of Ad14 might have occurred in recent years and might still be occurring.

Further work is needed to understand the natural history of Ad14, risk factors for severe Ad14 disease, and how Ad14 transmission can be prevented effectively. Vaccines against adenovirus serotypes four and seven (i.e., Ad4 and Ad7) were used among military recruits during 1971--1999, before vaccines were no longer available. Adenoviral disease among U.S. military recruits subsequently increased (*9*). Ad4 and Ad7 oral vaccines have been redeveloped and are being evaluated in clinical trials. Work is ongoing to determine whether the new Ad4 and Ad7 vaccines will protect against Ad14 infection. Management of adenoviral infections is largely supportive. A number of antiviral drugs, including ribavirin, vidarabine, and cidofovir, have been used to treat adenoviral infections such as Ad14, but none have shown definitive efficacy against adenoviruses (*2*).

Control of adenovirus outbreaks can be challenging because these viruses can be shed in both respiratory secretions and feces and can persist for weeks on environmental surfaces. Guidelines for the care of patients with pneumonia (*10*) should be followed in cases of suspected adenoviral pneumonia.

Clinicians with questions related to testing of patients for adenovirus or Ad14 infection should contact their state health departments, which can provide assistance. State health departments and military facilities should contact CDC to report unusual clusters of severe adenoviral disease or cases of Ad14 or to obtain additional information

:garding laboratory testing.

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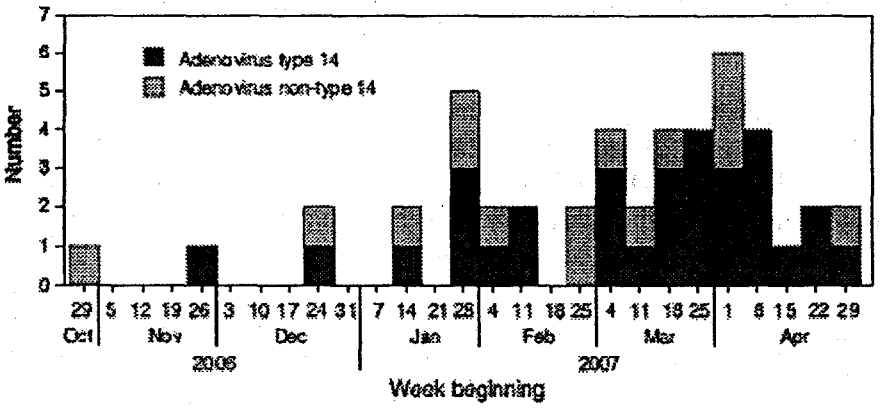
Defined as 1) fever ≥100.5°F (≥38.1°C) plus at least one other sign or symptom of respiratory illness or 2) diagnosis of pneumonia.

'igure

FIGURE. Number of cases of laboratory-confirmed adenovirus (type 14 and non-type 14*), by week of illness onset — Oregon, November 1, 2006–April 30, 2007



*Confirmatory typing performed at Oregon State Public Health Laboratory or 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 2008年1月29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	①～③人血清ｱﾙﾌﾞﾐﾝ④人血液凝固第ＸⅢ因子 ⑤⑥ﾌｨﾌﾞﾘﾉｹﾞﾝ加第ＸⅢ因子		研究報告の公表状況	Clinical outcome of frequent exposure to Torque Teno virus (TTV) through blood transfusion in thalassemia patients with or without hepatitis C virus (HCV) infection Journal of Medical Virology 1, 2008, 80/2 (365-371)	公表国 米国	
販売名（企業名）	①アルブミンーベーリング②アルブミナー5% ③アルブミナー25%④フィブロガミン P⑤ベリプラスト⑥ベリプラスト P コンビセット（CSL ベーリング株式会社）					

研究報告の概要

問題点（サラセミア患者の輸血による TTV 感染）

サラセミア患者は、頻繁にウイルスに暴露された輸血に依存していて肝不全や肝障害を頻繁に合併している。HBV と HCV 検査によるスクリーニング導入以来、この患者グループでの輸血関連の肝炎は激減した。しかしながら既知の肝炎に感染していないサラセミア患者の 37%がいまだに ALT 異常値を示しているが、原因は特定できていない。

TTV は一般の人々の間で高率で広く分布しているが、特にサラセミア患者などの頻繁に輸血を受ける患者では 80%以上が複数の TTV の遺伝子型を保有している。

著者らはアラブ首長国連邦で定期的に輸血を受ける（年間 13-18 回）サラセミア患者 197 名の TTV の遺伝子型およびフェリチン、AST、ALT レベルを検査した。

フェリチン、AST、ALT レベルは TTV 陽性患者群が、陰性患者群より有意に高かったが、HCV と TTV 共に感染した患者群は、TTV 単独感染患者群に比べ ALT が有意に高かった。

TTV 陽性群において ALT 異常値率は、年齢による差はなく、年間の投与間隔や投与数に起因していないことが示唆された。

TTV ウイルスは 27 遺伝子型から成る少なくとも 5 グループに分類される。TTV DNA を RD プライマー、TT6/7/8/9 プライマーおよび NG プライマーの 3 種を用いて増幅して、遺伝子型を解析したが、TTV 感染患者のほとんどが複数の遺伝子型を保持しているので、フェリチン、AST、ALT を上昇に関与する遺伝子を特定できなかった。

筆者らは、TTV 感染が HCV 感染患者の肝疾患を重篤にさせるとは結論できないとしている。重篤な肝疾患の進展には TTV よりも HCV の方が重要な役割を果たしている。

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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
現在まで TTV の病原性は十分解明されていないので、当社製品の原料血漿の段階で TTV に対しての検査は実施されていない。 TTV と ALT に関する CPMP の見解(Plasma-derived medicinal products : position paper on ALT testing : CPMP/BWP/385/99）は、TTV DNA 値と ALT 値との間には明確な相関関係はないことを示唆しているとしている。 TTV 検査は実施していないが、本剤の製造工程（60℃10 時間の液状加熱）で不活化されると考えられる。 今後とも情報収集に努める。	今後とも情報収集に努める所存である。

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Journal of Medical Virology 80:365–371 (2008)

Clinical Outcome of Frequent Exposure to Torque Teno Virus (TTV) Through Blood Transfusion in Thalassemia Patients With or Without Hepatitis C Virus (HCV) Infection

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As a consequence of the high prevalence of TorqueTeno virus (TTV) in blood donors, thalassemia patients frequently acquire various genotypes of this virus through therapeutic blood transfusions. At present, the clinical consequences of TTV infection remain indeterminate for these patients. Here, several hundred thalassemia patients were tested for the presence of TTV and its genotypes using a combination of PCR and clone-based DNA sequencing. Approximately 10% (12/118) of the patients aged 2–20 years remained negative for TTV including eight genotypes of SENV. Ferritin, aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) levels were invariably lower in TTV-negative patients ($P=0.02$, <0.01, and 0.06, respectively) than in TTV-positive patients. Patients with TTV–HCV co-infection showed elevated ferritin and ALT levels compared with patients with TTV infection alone ($P<0.02$ and $P<0.01$). AST and ALT levels were within the normal range for all TTV-negative patients, whereas abnormal levels of AST and ALT were seen in a significant proportion of TTV-positive patients (30.7% and 33.6%, respectively) and patients with TTV–HCV co-infections (70.0% and 56.6%, respectively). Only TTV-positive patients (28.0%) and patients with TTV–HCV co-infections (36.3%) had hyper-ferritin levels (≥3,000 ng/ml). The genotype(s) of TTV responsible for the liver dysfunction could not be determined. However, high levels of AST and ALT were found to be correlated with detection of a higher number of TTV genotypes in the patients. The data suggests that frequent and persistent TTV infection through blood transfusion is associated with hepatic dysfunction and/or damage in transfusion dependent thalassemia patients. ***J. Med. Virol. 80:365–371, 2008.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: TTV; HCV thalassemia patient; liver disease

INTRODUCTION

Hepatic dysfunction and/or damage are frequent complications in thalassemia patients who depend on blood transfusions that are associated with a high frequency of exposure to viruses. Accordingly, the incidence of transfusion related hepatitis for this group of patients has been markedly reduced since the implementation of blood screening for hepatitis B virus (HBV) and hepatitis C virus (HCV) nucleic acid and antibodies. However, over one third (37%) of thalassemia patients without infection by known hepatitis viruses still have an abnormal alanine-aminotransferase (ALT) pattern [Chen et al., 1999]. The exact cause of the ALT abnormality in those patients remains unknown [Okamoto and Mayumi, 2001].

A virus with a small single-stranded DNA genome was identified by Nishizawa et al. [1997] in Japan from patients with non-A-E transfusion acquired hepatitis in

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1997. With reference to the index patient, the virus was originally named TT virus however it is currently renamed as Torque Teno virus (TTV) the type species of the genus Anellovirus, in an unassigned family that is most closely related to the Circoviridae [Hino, 2002]. TTV is widely distributed geographically with a high rate of viremia within the general population [Simmonds, 1998; Cossart, 2000]. Mixed genotype infections are therefore common, particularly in frequently transfused patients such as thalassemia patients where 80% of them carry more than one genotype of TTV [Chen et al., 1999; Okamoto et al., 1999b]. This is presumably due to the high frequency of viral transmission through blood transfusion and the persistent nature of TTV viral infections [Chen et al., 1999; Gallian et al., 1999; Kanda et al., 1999; Kobayashi et al., 1999; Matsumoto et al., 1999; Oguchi et al., 1999; Prati et al., 1999]. TTV viruses have been classified into at least five groups consisting of more than 27 genotypes as a result of the extremely wide range of sequence divergence observed among TTV isolates [Okamoto et al., 1998; Tanaka et al., 1998; Takayama et al., 1999; Worobey, 2000; Okamoto and Mayumi, 2001]. Early studies indicate that TTV might cause some forms of cryptogenetic hepatitis, post transfusion hepatitis and/or other diseases, however these observations have not been confirmed in most subsequent studies although it has been suggested that certain TTV groups or genotypes (e.g., group 4, genotype 1 and genotype 21) might be especially pathogenic and associated with liver or other diseases [Simons et al., 1995; Okamura et al., 2000; Sugiyama et al., 2000; Bendinelli et al., 2001; Maggi et al., 2003; Pifferi et al., 2005; Szladek et al., 2005]. The clinical significance of TTV infection thus remains controversial. [Okamura et al., 2000; Sugiyama et al., 2000; Maggi et al., 2003; Pifferi et al., 2005; Szladek et al., 2005]. Considering that thalassemia patients frequently acquire multiple genotypes of TTV through repeated blood transfusion administered throughout their lifetime, the role of this virus in the development of clinical disease in this group of patients cannot be excluded [Chen et al., 1999]. In addition to TTV, a substantial proportion of thalassemia patients have acquired HCV infection through blood transfusion. It remains uncertain if HCV–TTV co-infections result in more severe biochemical and histological changes compared to TTV infection alone [Charlton et al., 1998; Watanabe et al., 1999; Yuki et al., 1999; Zein et al., 1999; Cleavinger et al., 2000; Meng et al., 2001; Tokita et al., 2002].

Since TTV cannot be cultivated in vitro, PCR is the only available tool for detection of TTV. It has been difficult to determine the clinical significance of TTV infections because diagnostic systems using one or two sets primers for PCR are unable to detect the entire spectrum of TTV genotypes and their variants that exist in individuals [Okamoto et al., 1999b, 2000; Maggi et al., 2003]. Obviously, this has impeded a proper assessment of TTV viral pathogenesis. We recently found that all TTV genotypes (except genotype 21), and all SENV genotypes (A–H) can be detected using three TTV primer sets [Hu et al., 2005]. This finding has provided a more accurate and efficient tool for TTV diagnosis. In this study, we used this efficient primer system combined with clone-based DNA sequencing to investigate the prevalence of various genotypes of TTV with respect to clinical outcome in various age groups of blood transfusion dependent thalassemia patients with or without HCV co-infection.

MATERIALS AND METHODS

Study Groups

A total of 197 thalassemia patients from the United Arab Emirates (UAE) who had received regular blood transfusions (13–18/year) were enrolled in this study, including 54 with hepatitis C virus (HCV) infection. Among these patients a younger group of 118 thalassemia patients (49 female and 69 male) ranging in age from 2 to 20 years (with a median age of 10.8 years) was tested for blood ferritin, AST and ALT levels to study the clinical outcome of TTV and HCV infections. The remaining thalassemia patients ($n = 79$: aged 21–53 years) were only tested for ALT in this study. All samples were negative in standard donor-screening tests including HIV, Human T Cell Leukemia Viruses, and hepatitis A, B and G.

Isolation of Viral DNA

Plasma (100 μl) was used for isolation of viral DNA with a silica gel based membrane while using microspin technology as described in the QIAamp blood kit (QIAGEN, Inc., Mississauga, Ontario).

Amplification and Detection of TTV DNA

Purified TTV DNA was amplified using three sets of nested primers derived from the conserved regions in 5′UTR and ORFs of the TTV genome following the procedure as previously described [Okamoto et al., 1999b; Hu et al., 2005]. They include RD037-038 plus RD051-052 [Okamoto et al., 1998], TT6-7 plus TT8-9 [Hohne et al., 1998], and NG 5′UTR based nested primers (NG054-147 plus NG133-132) [Okamoto et al., 1999a]. TTMV (TTV-like mini virus) was also tested in the thalassemia patients using two sets of TTMV specific primers as described previously [Hu et al., 2005]. The sensitivity of PCR used in this study had previously been evaluated by both DNA dilution and real-time PCR methods. The sensitivity was determined to be ≤ 10 copies/ml. To confirm that thalassemia patients testing negative for TTV were truly negative, the 12 TTV negative samples were subjected to additional PCR reaction conditions including different and lower stringency primer annealing temperatures and additional reaction cycles. PCR amplified TTV DNA was detected on a 1.5% agarose gel using ethidium bromide staining.

Direct DNA Sequencing

Direct DNA sequencing was performed using an automated DNA sequencer (Visible Genetics). A 5′

Cy5.5 labeled sense primer, RD051, was used to produce a sequence of approximately 150 bases from RD primer set PCR fragments. For the PCR products amplified using TT primers, a 5′ Cy5.5 labeled sense primer, TT8, was used to produce a sequence of approximately 150 bases in length. 5′UTR based primers NG133F and NG132R were used for sequencing the PCR products amplified by 5′UTR based primers.

Cloning Based DNA Sequencing

Clonal based DNA sequencing was performed using TOPO- TA™ vector (Amersham Pharmacia Biotech, USA) for cloning of purified PCR products. The TTV sequence was amplified from colonies of *Escherichia coli* using M13 primers (Amersham Pharmacia Biotech, USA). Fifteen clones from each individual were sequenced. Re-amplified PCR products were sequenced using the same procedure as for direct DNA sequencing.

Genotyping and Computer Analysis of Nucleotide Sequences

DNA sequences derived from DNA sequencing were compared using an on line database for the best possible match using the Basic Local Alignment Search Tool (BLAST) program at the National Center for Biotechnology Information home page (http://www.ncbi.nlm.nih.gov/). All further sequence analyses and comparisons were performed using the DNA-STAR Lasergene '99 software package (DNAStar, Madison, WI).

Statistical Analysis

The mean differences in AST, ALT and ferritin between three clinical groups were compared using Student's *t*-tests. Since the sample size for both TTV-negative and TTV + HCV positive groups is small compared to that of the TTV positive group, we performed a matching comparison to increase the comparison power. Each case in the TTV-negative group (or TTV + HCV positive group) was matched to a case of the same age and gender in the TTV-positive group. Paired *t*-test was performed for the matching comparison. In addition, we categorized the AST, ALT and ferritin into normal and abnormal groups using the clinical normal range as the cut-off value, and compared the abnormal rates in the three clinical groups. Chi-square tests were used in the comparison of rates. The SAS software package was used for the statistical analyses.

RESULTS

Clinical Significance of TTV Infection

Ferritin, AST and ALT levels. For a thorough analysis of the clinical data, we first investigated the impact of age and gender on the levels of clinical parameters (Table I). Ferritin, AST and ALT levels were found to be higher in patients aged 11–20 years than in younger patients aged 2–10 years. However, the difference did not reach statistical significance ($P = 0.13$, 0.10 and 0.10 for ferritin, AST and ALT, respectively). All three parameters were slightly increased in male relative to female patients, but again no significant difference was found between different genders ($P = 0.46$, 0.19 and 0.43 for ferritin, AST and ALT, respectively). The data suggest that age and gender are not major factors that significantly contributed to liver disease in thalassemia patients aged 2–20 years. However, since all three parameters were slightly higher in older patients and male patients in comparison with younger patients and female patients, a minor impact of age and gender on liver dysfunction could not be completely excluded. To improve the accuracy of data analysis, we therefore decided to use both age-sex matching and non-age-sex matching methods for evaluating the clinical significance of TTV infection in thalassemia patients.

The results in Table II indicate that by using an exact age-sex matching method, the ferritin, AST and ALT levels were found to be invariably lower in TTV-negative patients compared to the TTV-positive patients ($P = 0.02$, $P < 0.01$ and $P < 0.06$, respectively). The trends of the results generated by non-age-sex matching were, in general, consistent with those derived using the age-sex matching approach, where ferritin, AST and ALT levels were shown to be higher in TTV-positive patients than in TTV-negative patients ($P < 0.02$, $P > 0.05$ and $P > 0.05$, respectively), but in this case only ferritin was significantly elevated. The clinical data regarding TTMV (TTV-like mini virus) negative samples (8.5%, 10/118) was also analyzed and compared to the TTMV positive samples by age-sex matching method. The levels of ALT, AST and ferritin were not significantly different between TTMV-negative and TTMV-positive patients (data not shown). Of the TTV negative patients, 91.7% (11/12) were positive for TTMV. These data further support the observation that TTV, but not TTMV causes liver dysfunction and/or damage. Using age-sex matched groups, the levels of both ferritin and

TABLE I. Ferritin, AST and ALT Levels in Different Age and Gender Groups of TTV Infected Thalassemia Patients

Patients (no.)	Ferritin (ng/ml) (*P*)	AST (IU/L) (*P*)	ALT (IU/L) (*P*)
Age			
2–10 (45)	2290.9 ± 1331.2	35.1 ± 20.7	37.3 ± 47.2
11–20 (47)	2706.3 ± 1274.5 (0.13)	42.9 ± 24.2 (0.10)	53.2 ± 44.8 (0.10)
Gender			
Male (52)	2566.4 ± 131318.5	42.2 ± 24.4	48.8 ± 47.4
Female (40)	2364.1 ± 1296.5 (0.46)	35.9 ± 21.4 (0.19)	41.2. ± 43.8 (0.43)

TABLE II. Comparison of Ferritin, AST and ALT Levels Between TTV Negative Patients, Patients With TTV Infection Alone (TTV+) and With TTV–HCV Co-Infections (TTV + HCV+)

Patients (no.)	Age	Ferritin (ng/ml) (*P*)	AST (U/L) (*P*)	ALT (U/L) (*P*)
Age-sex matching				
TTV– (12)	9.5 ± 5.6[a]	1470.3 ± 514.7	27.1 ± 7.1	22.1 ± 12.2
TTV+ (12)	9.5 ± 5.6	2340.0 ± 733.5 (0.02)	38.8 ± 18.1 (<0.01)	49.3 ± 45.3 (0.06)
No age-sex matching				
TTV– (12)	9.5 ± 5.6	1470.3 ± 514.7	27.1 ± 7.1	22.1 ± 12.2
TTV+ (92)	10.8 ± 4.6	2477.7 ± 1305.6 (0.02)	39.5 ± 23.3 (0.08)	45.5 ± 45.8 (0.11)
Age-sex matching				
TTV+ alone (11)	13.2 ± 4.4	2340.0 ± 733.5	36.2 ± 22.0	27.6 ± 17.0
TTV + HCV+ (11)	13.2 ± 4.4	3112.9 ± 1477.8 (0.02)	53.1 ± 27.6 (0.13)	55.4 ± 27.6 (<0.01)
No age-sex matching				
TTV+ alone (92)	10.8 ± 4.6	2477.7 ± 1305.6	39.5 ± 23.3	45.5 ± 45.8
TTV + HCV+ (11)	13.2 ± 4.4	3112.9 ± 1477.8 (0.08)	53.1 ± 27.6 (>0.05)	55.4 ± 27.6 (>0.05)

[a]Mean ± SD; significant differences are in bold.

ALT were found to be higher in patients with HCV–TTV co-infections than in patients with TTV infection alone ($P = 0.02$ and $P < 0.01$). It was expected that patients with TTV–HCV co-infections would have more severe liver disease than TTV alone as HCV is a serious liver pathogen. Non-age-sex matched analysis showed that all three clinical parameters were higher in patients with TTV–HCV co-infections than in patients with TTV infection alone, but no significant difference was found between the two groups of patients. This suggests that the use of age-sex matching approach is necessary for the clinical data analysis.

Abnormality rate of ferritin, AST and ALT. We found that all 12 TTV-negative patients aged 2–20 years had normal AST (≤40 IU/L ranging from 14 to 40 IU/L) and ALT (≤50 IU/L, ranging from 10 to 48 IU/L) levels. However, AST and ALT levels were elevated in nearly one-third of TTV-positive patients aged 2–20 years (30.7%, 27/88 for AST; and 33.6%, 48/143 for ALT; Fig. 1). Abnormal levels of AST (70%, 7/10) and ALT (56.6%, 30/53) was even higher in patients with TTV–HCV co-infections than patients with TTV infection alone. Data resulting from analysis of abnormalities in three clinical parameters agreed completely with those obtained from the assessment of actual differences in ferritin, AST and ALT levels. Although the ferritin levels were significantly higher ($P = 0.02$) with respect to TTV and HCV positivity for matched patient groups, the ferritin baseline level of the patient group is high due to transfusion treatments. The ferritin level in TTV-negative thalassemia patients was five times higher (1470.3 ± 514 ng/ml; Table II) than normal (10–300 ng/ml). Therefore, we used the hyper-ferritin level value, which is ten times the normal limit (3,000 ng/ml) to calculate the abnormality rate in thalassemia patients. None of the TTV-negative patients had ferritin levels over 3,000 ng/ml with the highest level being 2,440 ng/ml. However, about one third of patients with TTV infection alone (28.0%, 26/93) and patients with HCV–TTV co-infections (36.4%, 4/11) had hyper-ferritin levels (3,000 ng/ml ranging up to 6,250 ng/ml).

One might expect that the ALT abnormality rate should be highest in older patients due to the greater duration of transfusion treatment. In fact, the ALT abnormality rate was the highest (33.6%, 48/143) in young patients (11–20 years) compared to the youngest patients aged 2–10 years (21.2%, 14/66) and the older patient group, between the ages of 21 and 53 (20.9%, 9/43; Fig. 2). This indicates that ALT elevation in thalassemia patients was not dependent or directly attributable to the time span or number of transfusions alone.

TTV Prevalence and Pathogenesis of Various Genotypes

As reported previously [Hu et al., 2005], RD primers are known to specifically amplify genotype 1 (1a and 1b) and TT6/7/8/9 primers detect genotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 15, and 16; mainly in genogroups 1 and 3. TT6/7 primers specifically detect TTV genotypes 2 and 3. NG primers can detect almost all known TTV genotypes except genotype 21, including eight genotypes (A–H) of SENV. Use of the three nested sets of TTV primers

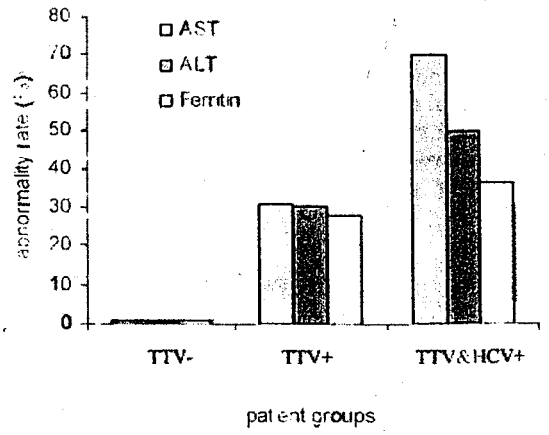

Fig. 1. Abnormality rate of ferritin, aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) levels in TTV-negative patients (TTV–), TTV-positive patients (TTV+) and patients with TTV–HCV co-infections (TTV and HCV+).

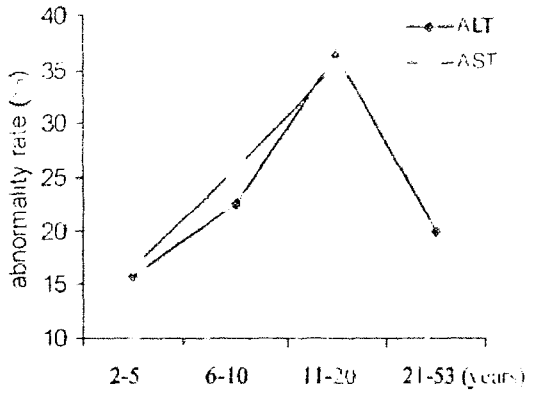

Fig. 2. Abnormality rate of AST (■) and ALT (◆) at different ages in TTV-positive thalassemia patients.

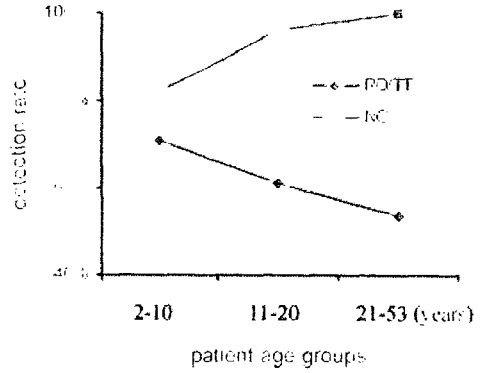

Fig. 3. TTV-positive rates of various genotypes in different age groups of thalassemia patients. The graphs indicate the viremia rates (%) of NG primer-specific genotypes (■), RD/TT6/7/8/9-specific genotypes (◆). The ages of patient groups (2–10, 11–20 and 21–53 years) are plotted against rate of infection.

allowed us to assess the prevalence of various genotypes in different age groups of thalassemia patients. Figure 3 shows that the positivity rate for the genotypes detected by NG primers increased significantly with age from younger (2–10 years) to older groups of patients (ages 11–20 years as well as 21–53 years) with detection rates of 81.8%, 45/55; 96.2%, 50/52; and 100.0%, 30/30, respectively (trend test $P < 0.0001$). In contrast, the detection frequency for the most prevalent genotypes that are detectable by RD/TT6/7/8/9 primers (i.e., genotypes 1–10 and 14–16) declined significantly from younger to older groups. The TTV-positive rate was 71.0%, 27/38; 61.5%, 32/52; and 53.6%, 30/56; for the younger and the two older groups of patients, respectively (trend test $P < 0.001$). The genotypes of amplified TTV viruses were confirmed by clone-based sequencing (see Materials and Methods Section, data not shown). The shifting of predominant TTV genotypes over a patient's lifetime suggests that younger patients have a greater susceptibility to the most prevalent genotypes relative to older patients. Presumably this is due to the fact that some of the older patients may become immune due to prior exposure and subsequent clearance of infection. As a result, these patients are protected from re-infection by some genotypes such as the genotypes detectable by RD/TT6/7/8/9 primers. However, in addition to being exposed to more TTV genotypes, the older patients have also been exposed to less prevalent TTV genotypes over time because of increased exposure due to repeated transfusions. Overall, the TTV viremia rate is higher in older patients. The genotype shifting during a patient's life-time provides strong evidence to indicate that both self-limited and chronic TTV infections exist and that persistence of the virus (at least certain genotypes) may not be life-long in thalassemia patients. The declining prevalence of some genotypes in older patients was paralleled by a decrease in the ALT abnormality rate (see Figs. 2 and 3). This suggests that the most prevalent genotypes such as 1, 2 and 3 may play a more important role in the development of liver disease in thalassemia patients. However, the exact genotype(s) responsible for the elevation of ferritin, AST and ALT levels could not be determined because the majority of TTV infected patients carried more than one genotype.

DISCUSSION

The role of TTV infection in liver disease has been the subject of much debate since the first TT virus was identified in 1997. In our present study, the data suggest that frequent and persistent TTV infection through blood transfusion is associated with abnormal AST, ALT and ferritin levels (seen in about one third of blood-dependent thalassemia patients). This supports the hypothesis that certain genotypes or variants of TTV cause disease when individuals are exposed to these genotypes or their variants for the first time and/or re-exposed to partially cross-reactive or non-cross reactive TTV strains [Bendinelli et al., 2001]. To further assess whether a specific genotype or group of TTV is associated with elevated ALT levels in thalassemiacs, we compared the genotypes infecting patients with abnormal ALT levels (>100 IU/L) and patients with normal ALT levels (<50 IU/L). A total of 480 clones from 32 samples (15 clones for each sample) were genotyped by DNA sequencing. We found that the majority (78.1%, 25/32) of the thalassemia patients were infected with more than one genotype. It was therefore difficult to judge which genotype was responsible for the elevation of ALT levels in patients with mixed genotype infections (data not shown). However, the number of mixed genotypes was found to be significantly increased in patients with abnormal ALT levels (three vs. two genotypes per patient, $P = 0.01$). It appears that ALT elevation is associated with a higher frequency of TTV mixed genotype infections, including transient and persistent infections through blood transfusion.

It is reasonable to propose that multiply transfused thalassemia patients are at a much greater risk of being infected by new and more pathogenic genotypes or strains than blood donors. In the UAE thalassemia patients average over 15 transfusion per year, where over one third of blood donors carry the virus. It would be expected that transfusion dependent thalassemia