Switzerland) and samples that resulted anti-HCV positive were then tested for HCV RNA (COBAS Amplicore, Roche Diagnostic Systems). An in-depth retrospective virological analysis was performed on serum samples obtained on June 2004 from all hemodialysis patients attending the unit, from the household contacts of the patients who had shown a seroconversion and from the only health care worker who was found to be anti-HCV positive.

All serum samples obtained were tested again for anti-HCV by a third generation enzyme immunoassay (Axsym HCV, version 3.0; Abbott Laboratories, Abbott Park, IL). The viral load in positive serum samples was determined by quantitative reverse transcription (RT)-PCR (HCV Amplicor Monitor, Roche Diagnostics, Milan, Italy), in accordance with the manufacturer's instructions. HCV genotyping was first performed with a commercial reverse-hybridization line probe assay (INNO-LiPA HCV II, Bayer Diagnostics, Milan, Italy) based on the 5'-noncoding region (NCR). The HCV RNA was extracted from serum samples collected in June 2004 using the QIAamp Viral RNA kit (QIAGEN, Hilden, Germany), underwent retrotranscription by random hexamer method and was used to perform molecular analysis. Two-strand direct sequencing was carried out on nested PCR products obtained from the NS5B region and from the hypervariable region 1 (HVR1) encompassing in the E2 gene, as previously reported [Faustini et al., 2005]. Sequencing was performed on ABI Prism 3100, using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Warrington, UK). Sequences of the NS5 regions obtained were aligned by using BLAST with the *National Center for Biotechnology Information* (NCBI) database (U.S. National Library of Medicine, Bethesda, MD, www.ncbi.nlm.nih.gov) and were able to attribute to the genotype 2c all the HCV from patients who seroconverted. For the amplification of the HVR1 region 2 type specific primers were chosen [Faustini et al., 2005]. The sequences were then aligned with CLUSTAL W software (version 1.5). The mean genetic distance between nucleotide sequences was calculated using a Kimura 2-parameter distance matrix with a transition/transversion ratio of 2.0. Phylogenetic trees were constructed using the neighbor-joining method, including NS5B and HVR-1 reference sequences (see figure legends) and local epidemiological unrelated HCV 1, 2b, and 2c strains. Bootstrap analysis with 1,000 replications was performed to assess the significance of the nodes; values >85% were considered to be significant. All of the algorithms used were included in MEGA software (version 2.1).

RESULTS

In October 2002, the anti-HCV seroprevalence in our HD unit was 31.2% (10/32). Between April 2003 and October 2003, four new HCV seroconversions were detected; the first (CT6) in April 2003, second and third (CT2 and CT4, respectively) in August 2003 and the fourth (CT11) in October 2003; thus, raising the total number of anti-HCV positive patients to 14 in the HD unit (Table I).

In all four patients who seroconverted the infection was asymptomatic. In the first patient who showed a seroconversion (CT6), ALT levels that were normal on the date of seroconversion and had always been normal previously, showed an important increase only in the following month. In one of the two patients (CT2) who were found to be anti-HCV on August 2003, ALT levels showed a moderate increase only at the time of seroconversion and they had always been normal previously. The other newly infected patient (CT4) who tested anti-HCV positive on August 2003 had shown an important ALT levels increase on May 2003, but he tested anti-HCV negative in that date; however, his ALT levels had always been normal until April 2003. In these two patients anti-HCV test was performed, and repeated at monthly interval for patient CT 4, as consequence of the detected ALT levels elevation. The last newly infected patient (CT11), who was found to be

TABLE I. Anti-HCV Test Results, Genotype Determination and First Detection of Elevated Alanine Aminotransferase Levels and of HCV RNA in HCV Infected Patients Receiving Dialysis in the Unit (April 2002–October 2003)

Pt	April 2002	October 2002	April 2003	May 2003	June 2003	July 2003	August 2003	September 2003	October 2003
CT 7	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 14	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 1	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 10	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 13	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 8	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 3	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 5	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 9	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 12	Pos	Pos	Pos	NT	NT	NT	NT	NT	Pos
CT 6	Neg	Neg	**Pos**	* NT ↑	NT	NT	NT	NT	Pos
CT 2	Neg	Neg	Neg	Neg	Neg	Neg	**Pos** ↑	* NT	Pos
CT 4	Neg	Neg	Neg	Neg ↑	Neg	Neg	**Pos**	* NT	Pos
CT 11	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	* **Pos**

Pos, anti-HCV test positive; Neg, anti-HCV test negative; NT, not tested for anti-HCV; ↑ denotes the first detection of elevated ALT levels; * denotes the first HCV RNA detection. Pos in bold denotes the first anti-HCV positive test.

anti-HCV positive on October 2003 (on the occasion of the anti-HCV six-monthly screening), had always shown ALT normal values before seroconversion at the monthly screening routinely performed in the unit (Table I). All patients newly infected showed a positive HCV RNA test after the seroconversion.

No further cases of new HCV infection were detected when the serum samples drawn on June 2004 from all the patients receiving hemodialysis in the unit were tested for anti-HCV and HCV RNA. Genotyping performed by INNO-LiPA revealed that all the patients who had shown a new seroconversion were infected with HCV genotype 2c. Six out of the 10 dialysis patient with chronic HCV infection were infected with genotype 1b, 2 with genotype 2c (CT1 and CT3), and in 2 patients it was not possible to determine the infecting genotype (one refused to give a blood sample and another one deceased before June 2004). Serum HCV RNA levels ranged from 7,000 to 488,000 IU/ml for the four newly infected patients and from 43,000 to >500,000 IU/ml for patients with chronic HCV infection, for which a serum sample was available. The two chronically infected patients harboring genotype 2c had serum HCV RNA titres of 171,000 UI/ml (CT1) and >500,000 IU/ml (CT3). Since in June 2004 all newly infected patients still resulted HCV RNA positive, that is more than 6 months after the detection of their seroconversion, they must be considered as having all developed a persistent HCV infection. These patients continued to be viremic while attending the unit, although their ALT levels were constantly normal, and none of them underwent antiviral therapy.

Figure 1 shows the phylogenetic tree analysis of the NS5B region sequences isolated from all HCV RNA positive patients receiving hemodialysis in the unit (newly infected and chronically infected patients). All four newly infected patients harbored very closely related viral isolates that clustered together with the 2c isolate found in one of the two 2c chronically infected patients, which is consistent with the hypothesis that the outbreak had a single epidemiologic origin (patient CT3). Phylogenetic analysis also revealed that the other patient with chronic infection harboring genotype 2c (CT1) was not associated with the outbreak. The results of the phylogenetic analysis in the HVR1 region, using only genotype 2 sequences, with the majority being 2c collected from GenBank, were consistent with the findings in the NS5B region (Fig. 2).

The results of the epidemiological investigation were also consistent with a patient-to-patient mode of transmission of the infection during the outbreak. The four newly infected patients had never received transfusion of blood or blood products. Two of them (CT2 and CT6) had no exposure to surgical or medical invasive procedures outside the hemodialysis unit, while patient CT4 had undergone dental extraction 2 months before his seroconversion, and patient CT11 had dialysed outside the unit on summer holidays (July and August). None of the newly infected patients were known to have used intravenous drug and none of their household

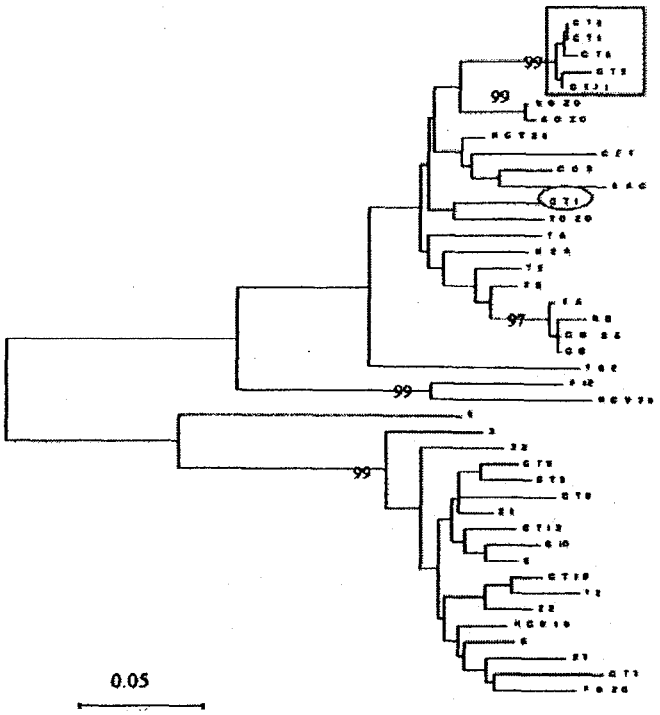

Fig. 1. Phylogenetic tree of NS5B region. The patients involved in the outbreak were indicated with the initials CT and numbered as in the table. Reference genotypes 2b, 2c, and 1b sequences from GenBank are indicated with their accession numbers (for HCV2c AJ291280; for HCV2b AB030907; for HCV 1b AY257435). Local nonrelated patient sequences, including patients with genotypes 2c, 2b, and 1b, are also included. Genetic distance is indicated by a horizontal bar. The numbers at the nodes indicate the frequency with which the node occurred in 1,000 bootstrap replicates; values greater than 95% are indicated.

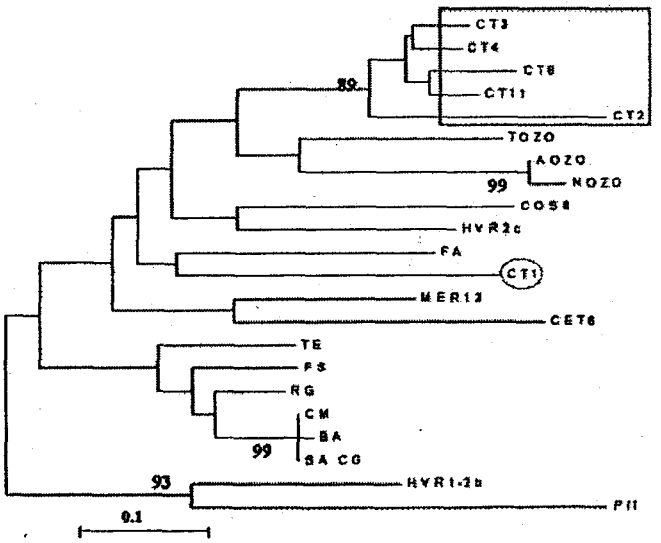

Fig. 2. Phylogenetic tree of HVR1/E2 region. Patients are indicated as in Figure 1. Reference genotypes 2c and 2b GenBank accession numbers are AF 237649 and AB030907, respectively. Local nonrelated patient sequences with genotypes 2c and 2b are also included. Genetic distance is indicated by a horizontal bar. The numbers at the nodes indicate the frequency with which the node occurred in 1,000 bootstrap replicates; values greater than 85% are indicated.

contacts were found to be anti-HCV positive. The only healthcare worker who was anti-HCV positive resulted negative when tested for HCV RNA. All patients infected by closely related genotype 2c isolates (new and old infections: patients CT2, CT3, CT4, CT6, and CT11) received dialysis on the same days (Monday-Wednesday-Friday). Patients CT2, CT3, CT4, and CT6 received dialysis on the morning shift (shift 1) and used different machines. Patients CT11 received dialysis on afternoon shifts but he had shared in several occasions the same machine with patient CT2.

The healthcare workers employed in the unit denied any violation of the standard infection control procedures. After the implementation of the infection control procedures and the use of dedicated machines for anti-HCV positive patients (but not dedicated rooms or personnel), no additional cases of new HCV infection were observed in the unit.

DISCUSSION

In this study, molecular analysis together with epidemiological investigation provided strong evidence for nosocomial patient-to-patient HCV transmission during an outbreak in a hemodialysis unit. Two potential routes of transmission were identified: horizontal transmission via healthcare workers and/or environmental contamination allowed by breaks in infection control procedures; vertical transmission via the dialysis apparatus. The occurrence of all but one cases of infection with closely related subtype 2c strains in patients who had received dialysis on the same days and the same shift (CT2, CT3, CT4, and CT6) suggests horizontal transmission during care due to breaks in infection control procedures. Several types of breaks in infection control procedures able to facilitate HCV transmission in hemodialysis setting have been suggested, such as absence of hand washing or inconstant glove use [Niu et al., 1992], failure to change gloves between patients [Fabrizi et al., 2002], sharing of multidose vials between patients [Kokubo et al., 2002], and lack of environmental disinfection (surfaces and instruments) [Delarocque-Astagneau et al., 2002; Savey et al., 2005]. Although the healthcare workers employed in the unit denied any violation of the standard infection control procedures, occasional or inadvertent mistakes might have occurred particularly during busy periods or an emergency with a patient. In an environment in which frequently performed percutaneous procedures may contribute through blood spillage to HCV contamination of surfaces and instruments [Caramelo et al., 1999], occasional mistakes could be enough to transmit the virus between patients, particularly if they are immunocompromised. Indeed, patients CT2, CT3, CT4, and CT6 had not always used the same machine, but in several occasions, two of them had shared a given machine with 36 hr of difference. However, vertical transmission via dialysis machines in this group of patients has to be considered very unlikely for two principal reasons: first, after these patients had received dialysis, each machine regularly underwent two complete cycles of disinfection during the following 36 hr (after the fist and second shift, respectively); second, none of patients receiving dialysis in the first shift on the following day had resulted to have acquired a new infection with genotype 2c or other genotypes. On the contrary, vertical transmission via dialysis apparatus between patient CT2 and CT11 could not be quite excluded. These two patients had never received dialysis on the same shift, but in several occasions have shared the same machine in the same day. Apart from patient CT2, none of the other patients infected with closely related 2c strains had had any kind of contact with patient CT11 (dialysis on the same shift or use of the same machines). Thus, even if patient CT11 had received dialysis in another unit on vacation, he had undoubtedly acquired HCV infection in the unit where the outbreak occurred, and with high probability patient CT2 had been the source of his infection. HCV transmission via the dialysis apparatus has been suggested to occur in case of dialysers reuse [dos Santos et al., 1996; Fabrizi et al., 2002] or by dialysate [Sampietro et al., 1994], when the dialysis fluid circuit was not disinfected after each session [Le Pogam et al., 1998]. Transmission has also been suggested to occur in case of potential contamination of internal components of the dialysis machine not accessible for routine disinfection. Some authors [Niu et al., 1992; Delarocque-Astagneau et al., 2002; Savey et al., 2005] have reported wetting of arterial and venous filters due to accidental blood backflow creating a potential contamination of the internal pressure sensing port, which is not accessible for routine disinfection. Thus, two successive episodes of blood backflow in the filters can contribute to the transmission of HCV to another patient. However there has been controversy in literature about the potential role of hemodialysis machines in HCV transmission: several other studies concluded that this route probably is a rare occurrence, playing a minor role or no role at all in the transmission of HCV in hemodialysis settings [Jadoul et al., 1998; Fabrizi et al., 2002; Barril and Traver, 2003]. In the unit where the outbreak occurred, dialysers and tubing sets were disposable and were never reused and disinfection of dialysis machines were performed after each shift. Furthermore episodes of accidental blood backflow into external filter were not reported on patient CT2 medical charts in the days in which he had shared the machine with patient CT11. However, it cannot be excluded that some incidents could have not been registered in the case that the nurses had considered the backflow not sufficient for concern. Alternatively, a break in infection control procedures can be supposed, such as an unsatisfactory environmental cleaning and disinfection between the first and second shift that had resulted in HCV transmission from CT2 to CT11 via contaminated environmental surfaces. A recent study suggests that HCV in dried plasma can cause infection in experimental animals when left at room temperature for $\geq$16 hr but not longer than 4 days [Krawczynski et al., 2003].

Since this investigation was retrospective it had some limitations. It was not possible to directly observe the

health care personnel employed in the unit during their day-to-day working in the period the outbreak occurred. Consequently, it was not allowed to verify if in that period there were evident breaks in universal standard precautions during tasks and procedures performed by personnel during the care of the patients and/or in environmental control procedures; thus it was necessary to rely on healthcare workers interview and medical chart review. Both two latter sources of information could have minimized the recognition and the impact of some events able to enhance the probability of HCV transmission. On the basis of the seroconversion data and epidemiological findings, assuming there were no substantial delays in the seroconversion times, the possible chain of transmission of the infection among the hemodialysis patient harboring closely related HCV 2c should have been depicted as that reported in Figure 3. Indeed, it is well known that uremic patients receiving hemodialysis may suffer a degree of immunosuppression and may have a delayed or disturbed HCV antibody response, which results in a prolonged seronegative window phase after infection [Le Pogam et al., 1998; Savey et al., 2005]. Since serum samples obtained from the patients attending the unit at various time points were not stored and thus were no longer available for HCV RNA detection, it was impossible to determine the time in which each patient involved in the outbreak could be considered actually infected and able to transmit the infection. In other words, it was impossible to establish with certainty which patient among CT2, CT4, and CT6 was infected first and consequently when patient CT2 transmitted the infection to patient CT11. Nevertheless, routine monitoring of HCV infection as was performed in the unit, that is screening ALT monthly plus anti-HCV testing upon the admission, then every 6 months and in case of ALT increase, had permitted the detection of the outbreak. However, because of the high risk of HCV transmission in hemodialysis units even through unrecognized cases, particularly in those units where the prevalence of HCV infected patients is high, and considering the high efficacy of an early anti-HCV therapy for newly infected patients [Gursoy et al., 2001], even this strategy may be not optimal. As suggested by other authors [Savey et al., 2005; Hmaied et al., 2006], it seems appropriate to test for HCV RNA any patient showing, at the monthly screening for ALT, a significant enzyme level increase (at least twice the baseline level of the patient) but a negative anti-HCV test and each new patient who enter the unit. To that end, it is necessary to archive monthly at least at −20°C, just for a brief time period, a serum sample for determination of HCV RNA in case of ALT elevation. However, for two of the newly infected patients (CT6 and CT11), monthly ALT screening did not help the detection of the infections, since their ALT levels had been normal until after seroconversion. Indeed, patient CT11 had dialysed outside the unit for 2 months on summer holidays (July and August 2003) and no data about his ALT levels were available for that period. On the contrary, for the two other newly infected patients (CT2 and CT4) the detection of increased ALT on monthly screening helped the diagnosis by inducing to perform and then to repeat at monthly interval (for patient CT4) the anti-HCV test. If on the occasion of the detection of an ALT levels increase in patient CT4 (May 2003) the detection of HCV RNA had been performed, the diagnosis of HCV infection probably could have been made 3–4 months early. While these facts underline uncertainty in depicting the chain of transmission in this outbreak, they also stress the importance of testing for HCV RNA whenever a significant ALT increase occurs.

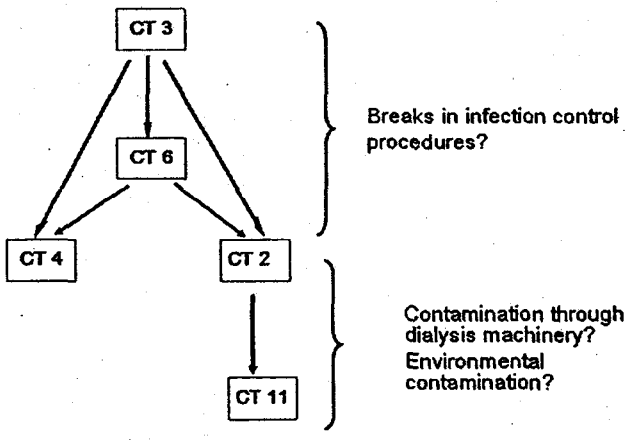

Fig. 3. Scheme of the possible chain and mechanisms of HCV transmission among genotype 2c infected patients involved in the outbreak, assuming there were no substantial delays in seroconversion times.

After the implementation of the infection control procedures and the use of dedicated machines for anti-HCV positive patients (since January 2004), no additional cases of new HCV infection were observed in the unit. The decision of using dedicate machines for anti-HCV positive patients, that became operative from January 2004, was taken by the hospital managers according to published guidelines [Barril et al., 2004]. Isolation policy of HCV infected patients on maintenance hemodialysis by rooms, machines, and personnel, is controversial. At present, The Centers for Disease Control and Prevention does not recommend the use of dedicated machines or patient isolation [Anonymous, 2001], however in some European countries, including Italy, a good proportion of the hemodialysis units, particularly those with high prevalence of infection, currently adopt isolation strategy for HCV infected patients [EBPGEGH and ERA, 2002; Fabrizi et al., 2002; Barril and Traver, 2003; Barril et al., 2004; Di Napoli et al., 2006]. Notwithstanding the high prevalence of infection, before the outbreak occurred in the unit, no isolation measures were adopted for HCV positive patients. This was due to the lack of room for patients and to the unavailability of further hospital personnel. There are convincing arguments supporting a policy of isolation of HCV infected patients. Some prospective studies have clearly showed an important

decrease in the incidence of HCV infections by a complete isolation of the infected patients [Saxena et al., 2003]. Other studies reported a reduced number of seroconversions in unit in which all patients had a dedicated machine or some machine were dedicated for HCV infected patients [Shamshirsaz et al., 2004]. The use of dedicated machine for HCV infected patients, could be useful in units with high prevalence of HCV infection and with a low patient-personnel ratio [Barril and Traver, 2003]. On the other hand, several considerations oppose to the need to isolate HCV infected patients. HCV infectivity is lower than that of HBV. An effective isolation policy would include reliable diagnostic methods to detect HCV infected patients; this means that it would be necessary to routinely test all patients for HCV RNA. Furthermore, several investigators have been able to significantly reduce the number of seroconversion by only reinforcing infection control measures [Jadoul et al., 1998]. Finally, others authors showed the HCV transmission can occur despite the use of dedicated machine because of breaks in infection control procedures [Hmaied et al., 2006]. Even if the debate over the need for isolation policy is not resolved, there is a consensus that using dedicated machines for HCV infected patients does not exclude reinforcement of universal precautions [Delarocque-Astagneau et al., 2002; Barril and Traver, 2003].

In conclusion, molecular analysis and epidemiological investigation suggested a patient-to-patient HCV transmission in this outbreak mainly due to breaks in infection control procedures even if a related-machine transmission cannot be quite excluded in one case.

Universal infection control precautions remain the key stone in the prevention of nosocomial HCV transmission in hemodialysis units. They include avoidance of sharing equipment and devices, frequently hand washing and proper gloves use, cleaning and disinfection with virucidal agents of all the unit (instruments, machine, floor, surfaces). All these measures require continuous education, written procedures and adequate patient-personnel ratio.

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医薬品 研究報告 調査報告書

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一般的名称	(製造承認書に記載なし)	研究報告の公表状況		公表国
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)		De Silva AN, Muddu AK, Iredale JP, Sheron N, Khakoo SI, Pelosi E. J Med Virol. 2008 Feb;80(2):283-8.	英国

研究報告の概要

○サウスハンプシャーにおける急性E型肝炎国内感染率の予想外の高さ:ルーチン検査を行う時期か?
先進国特有のE型肝炎とは、流行地域への渡航歴のない者に発現したE型肝炎である。近年、英国を含む経済的に発展した多くの国に新興する疾病と認識されている。しかし、E型肝炎は現在も稀な疾患と考えられており、E型肝炎のルーチンな臨床検査は実施されていない。英国サウスハンプシャーの単一施設において2005年6月から13ヶ月の期間に診断されたE型肝炎13例について報告する。これらの患者は、ルーチンのE型肝炎血清検査を導入した新規スクリーニング手順開始後に特定された。患者は中年～高齢者で、男性の方が多かった。4名(31%)は入院を要した。RT-PCR法にて確定された症例はいずれも、英国のブタに蔓延しているHEVと相同性の高いE型肝炎ウイルス(HEV)遺伝子型3を保有した。急性肝炎発症前の2ヶ月間によく加熱しない豚肉製品を食べたり、ブタと密接な接触を持った記憶のある患者はいなかった。これに対して、同一期間中、A型肝炎の診断は2例のみ、B型肝炎の診断は4例であった。これらのデータは、原因不明の急性肝疾患を発症し、関連する渡航歴のない患者全員にルーチンのE型肝炎検査を導入することの重要性を示している。ルーチン検査は、急性肝疾患患者の臨床管理を改善しながら、E型肝炎の疫学を明らかにすることができる。

報告企業の意見	今後の対応
英国サウスハンプシャーの単一施設において2005年6月から13ヶ月の期間にE型肝炎13例が発生し、同一期間中A型肝炎は2例、B型肝炎は4例であったことから、原因不明の急性肝疾患を発症し、関連する渡航歴のない患者全員にルーチンのE型肝炎検査を実施することの重要性が示されるとの報告である。	日本赤十字社では、厚生労働科学研究「E型肝炎の感染経路・宿主域・遺伝的多様性・感染防止・診断・治療に関する研究班」と共同して、献血者におけるHEV感染の疫学調査を行っている。北海道における輸血HEV感染報告を受け、試験的に北海道では研究的NATを行うなど安全対策を実施している。また、輸血による肝炎ウイルス感染防止のため、血液中のALT値61IU/L以上の血液を輸血用から排除している。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

使用上の注意記載状況・その他参考事項等

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

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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,

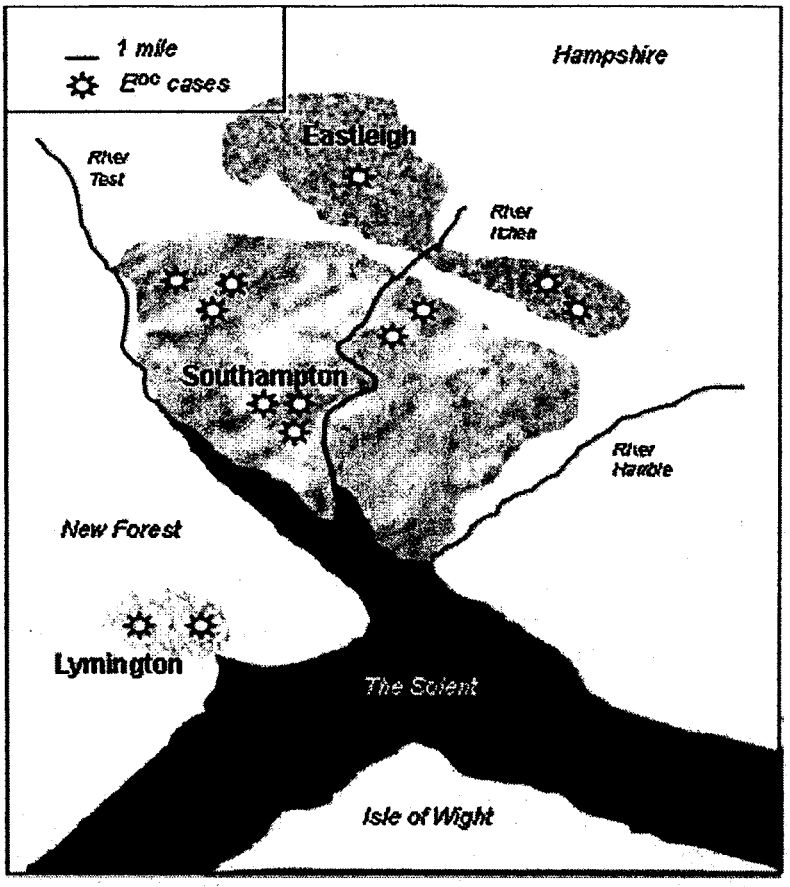

Fig. 1. South Hampshire. Proportion of people above state pension age (65 years for men and 60 years for women) according to 2001 population census data: Southampton 16.5%, Bastleigh 17.1%, Lymington New Forest 25.6%, England and Wales 18.4%).

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